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FURTHER STUDIES IN ANAPHYLAXIS.*

II. *On Recurrent Anaphylaxis and Repeated Intoxication in Guinea-pigs by Means of Horse Serum.*

F. P. GAY AND E. E. SOUTHARD.

(*From the Pathological Laboratories of the Harvard Medical School and the Danvers Insane Hospital.*)

In a previous article[1] (1907) we have shown that the stage of "immunity" (Rosenau and Anderson, and Otto) or "anti-anaphylaxis" (Besredka and Steinhardt) which follows several moderate-sized injections or one large initial injection without intoxication, or which is present in animals after the intoxication caused by a second spaced dose of horse serum, is in reality not a state of immunity, properly speaking, but a refractory condition. The animals in this refractory condition contain no elements in their blood capable of neutralizing the toxic effect of horse serum, although their blood does contain, at times, other reaction bodies, such as precipitins. So far as they themselves are concerned as regards intoxication by horse serum, they are essentially in a condition similar to guinea-pigs which have just received a small initial dose of serum, for they eventually become sensitive to the toxic action of this serum; they differ from guinea-pigs sensitized by the small dose only in undergoing a longer period of incubation. The fact that animals who had received a single large initial dose, or several doses, showed an incubation period proportionate to the amount of serum injected was of importance in the evolution of our hypothesis, which insists on the

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presence of two substances in horse serum: the unassimilable anaphylactin which sensitizes and the assimilable elements which are gradually eliminated, and the subsequent ingress of which to properly prepared cells give rise to the intoxication. It is the presence of a large amount of assimilable substances, which naturally require time for their elimination, that, in the case of the large initial dosage, requires a longer period of incubation. A condition in the refractory animal of complete or nearly complete assimilation but of little or beginning elimination of the toxic element would account for the nonreaction of these animals to horse serum and explain why, at the same time, their blood on transference is more sensitizing for normal guinea-pigs than is the blood of sensitive guinea-pigs — since they have obviously much more of the anaphylactin, but little or none of the toxic substance of horse serum remaining unassimilated.

The point which we wish to emphasize in the present section is not that animals receiving a large dose of serum eventually become sensitive, but the fact that a sensitive animal intoxicated with a large dose, and recovering and thereby passing into a refractory state (antianaphylaxis of Besredka and Steinhardt), by the same mechanism again becomes sensitive to the toxic effect of horse serum. This fact has indeed already been evidenced in our own protocols and in those of other workers. We previously stated — "As may have been deduced from our observation of the refractory guinea-pigs and their eventual sensitivity, animals which have once suffered severe symptoms and recovered, if reinjected after a sufficient period, may be made to undergo again the typical serum intoxication. By a proper dosage the disease may be repeated at relatively short intervals and as frequently as desired."

It might be possible to draw an analogy between this form of recurrent hypersusceptibility, due indirectly to an intoxication, and the evidence of repeated intoxication in some diseases characterized by "crises" or repeated attacks, such as general paresis, epilepsy, and purpura, the only point of

analogy which would be difficult to explain being the re-intoxication — which in this experimental disease of guinea-pigs is artificially induced. It is of advantage, however, to present concretely the protocols of certain animals that for the purpose of histological study have been intoxicated with serum two or three times.

I. Guinea-pigs showing secondary intoxication:

G.P. No. 46.	Jan. 2, 1907.	Given three cubic centimeters of horse serum subcutaneously.
	Jan. 17, 1907.	Given five cubic centimeters horse serum intraperitoneally. Slight symptoms. Recovered.
	April 9, 1907.	Given three cubic centimeters of horse serum intraperitoneally. Dead sixty minutes. Gastric and pulmonary hemorrhages.
G.P. No. 50.	Jan. 9, 1907.	Given 1.5 cubic centimeters serum of refractory guinea-pig "B."
	Jan. 24, 1907.	Five cubic centimeters horse serum intraperitoneally. Severe symptoms. Recovered.
	April 25, 1907.	Two cubic centimeters horse serum intraperitoneally. Dead in twenty minutes. Hemorrhages of heart, lungs, and stomach.
G.P. No. 47.	Jan. 2, 1907.	Given .01 of a cubic centimeter of horse serum subcutaneously.
	Jan. 17, 1907.	Five cubic centimeters of horse serum intraperitoneally. Severe symptoms. Recovered.
	April 9, 1907.	Three cubic centimeters of horse serum intraperitoneally. Dead one hour. Hemorrhages of lungs.

II. Guinea-pigs showing tertiary intoxication:

G.P. No. 37.	Dec. 24, 1906.	Given .01 of a cubic centimeter of horse serum.
	Jan. 10, 1907.	Given five cubic centimeters of horse serum intraperitoneally. Severe symptoms. Recovered
	March 28, 1907.	Given one cubic centimeter of horse serum intraperitoneally. Slight symptoms.

G.P. No. 37.	April 25, 1907.	Given seven cubic centimeters of horse serum intraperitoneally. Distinct but moderate symptoms. Chloroformed four hours. Gastric and pulmonary hemorrhages.
G.P. No. 30.	Dec. 26, 1906.	Given .01 centimeter of horse serum subcutaneously.
	Jan. 8, 1907.	Given five cubic centimeters horse serum intraperitoneally. Severe symptoms. Recovered.
	March 28, 1907.	Given one cubic centimeter horse serum intraperitoneally. Slight symptoms. Recovered.
	April 25, 1907.	Given ten cubic centimeters horse serum intraperitoneally. Severe symptoms. Chloroformed four hours. Hemorrhages of stomach, lungs, kidney, liver.

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FURTHER STUDIES IN ANAPHYLAXIS.*

III. *The Relative Specificity of Anaphylaxis.*

F. P. GAY AND E. E. SOUTHARD.

(*From the Pathological Laboratories of the Harvard Medical School and the Danvers Insane Hospital.*)

Rosenau and Anderson,[1] in their first article on the anaphylaxis in guinea-pigs produced by horse serum, considered the relative specificity of the reaction. They found that guinea-pigs which had been sensitized by a small dose of horse serum could be intoxicated after an interval of ten days by a subsequent dose not only of horse serum, but also to a less extent by serum from other animals; conversely, animals sensitized by other sera became more or less susceptible to horse serum. In each combination, however, a relative ("quantitative") specificity was to be noted, for in each instance the maximum reaction was obtained if the serum used for first and second injections was the same. These authors later[2] apparently showed that when protein substances of a more divergent origin are used the specificity becomes more apparent. Thus they showed that such substances as egg white, milk, and extract of peas, when given to guinea-pigs in successive spaced doses will cause intoxication on the second dose. According to the protocols of Rosenau and Anderson these different instances of anaphylaxis showed absolute specificity; that is, sensitization by milk, egg white, or horse serum was followed by intoxication only when the same substance which had sensitized was used for a second dose.

In a recent and most interesting article,[3] the same writers have continued their observations on this subject of the specificity of anaphylaxis. They have shown that if guinea-pigs be given a mixture of horse serum, egg white, and milk they become, after the proper period of incubation, susceptible to intoxication by each of these substances in turn, when

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administered on following days or even on the same day. It is well known that if a guinea-pig sensitive to horse serum recovers from a second dose of the serum, it immediately passes into a refractory stage and is unaffected by another dose of horse serum, for at least several weeks. This fact renders still more striking this serial intoxication with different substances after mixed sensitization with the same substances, and makes it seem that each reaction is, indeed, absolutely specific.

We have recently considered this question of the specificity of anaphylaxis, and our results, although agreeing in the main with those of Rosenau and Anderson, differ in essential particulars. In our hands the matter of specificity has been proved to be by no means absolute. It is clearly shown in the tables that follow that although an animal sensitized by a given proteid will react most violently to a second injection of the same proteid, it will frequently show distinct intoxication on injection of a proteid of different origin.

TABLE 1.

These animals were given each one cubic centimeter of egg white (1 egg to 10 cc. NaCl sol.) subcutaneously as a first injection and at a subsequent injection were given the substances noted below.

G.P. No.	Interval.	Second Injection.	Resulting Symptoms.
154.....	16 days.	4 cc. egg white subcutaneously.	Moderate.
151.....	16 days.	5 cc. egg white intraperitoneally.	Dead 10 minutes.
112.....	35 days.	5 cc. egg white intraperitoneally.	Dead 20 minutes.
152.....	16 days.	4 cc. horse serum subcutaneously.	Slight.
150.....	16 days.	10 cc. horse serum intraperitoneally.	Severe.
113.....	35 days.	5 cc. horse serum intraperitoneally.	Dead 1 hour.
153.....	16 days.	4 cc. milk subcutaneously.	Suggestive.
149.....	16 days.	10 cc. milk intraperitoneally.	Doubtful.
114.....	35 days.	5 cc. milk intraperitoneally.	Distinct.

From Table 1 it appears that guinea-pigs sensitized with egg white react not only to egg white but very markedly to horse serum and less distinctly to milk. Under more favorable conditions, such as a longer period before intoxication, it seems indicated that the reaction to milk would be more notable.

TABLE 2.

These animals were sensitized with one cubic centimeter of whole milk subcutaneously.

G.P. No.	Interval.	Second Injection.	Resulting Symptoms.
80.....	13 days.	5 cc. milk subcutaneously.	Distinct.
107.....	35 days.	6 cc. milk intraperitoneally.	Very severe.
81.....	13 days.	5 cc. horse serum subcutaneously.	Marked.
110.....	35 days.	6 cc. horse serum intraperitoneally.	Very severe.
155.....	16 days.	10 cc. egg albumin intraperitoneally.	Doubtful symptoms.
108.....	35 days.	5 cc. egg albumin intraperitoneally.	Slight.

From Table 2 it is evident that guinea-pigs sensitized to milk will react on second injection not only to milk but quite as distinctly to horse serum; the reaction to egg white after milk is only slight.

TABLE 3 *a*.

These animals had received .01 of a cubic centimeter of horse serum subcutaneously as a first injection.

G.P. No.	Interval.	Second Injection.	Resulting Symptoms.
106	35 days.	5 cc. egg albumin.	Distinct but slight symptoms.
104	35 days.	5 cc. milk.	Doubtful symptoms.

TABLE 3 *b*.

These animals had received five cubic centimeters of horse serum subcutaneously as a first injection.

G.P. No.	Interval.	Second Injection.	Resulting Symptoms.
24	44 days.	7 cc. egg albumin intraperitoneally.	No symptoms.
48	28 days.	8 cc. egg albumin intraperitoneally.	No symptoms.
26	41 days.	7 cc. egg albumin intraperitoneally.	Doubtful.
29	28 days.	6 cc. milk intraperitoneally.	No symptoms.

Only the slightest indication of symptoms can be elicited by egg albumin or milk after sensitization by horse serum in the doses used. It is probable that, although under the conditions of sensitivity here given the animals are known to be highly sensitive to horse serum, the intoxication by the other proteids, which is only suggested, might be made more clear by a longer anaphylactic period or different initial dose.

A study of these tables shows that animals sensitized with either egg white or milk will react more or less characteristically to horse serum. It may also be seen that after sensitization with egg white guinea-pigs will react faintly to milk; and after sensitization with milk will react slightly to egg white. The reaction to egg white after sensitization with horse serum was noted as distinct in only one instance; the reaction to milk after horse serum was not to be noted in one instance and was doubtful in the other.*

A comparison of these different tables indicates in the first place that anaphylaxis is only relatively specific; and the fact that, in certain combinations (*e.g.*, sensitization with horse serum followed by milk or egg), an absolute specificity

*Since our results apparently refute the observations of Rosenau and Anderson on the specificity of these reactions we have accepted them only after repeated corroboration. As is indicated in the tables the animals tested for cross intoxication were put through in several separate lots. It is perhaps not gratuitous, in view of the small amount of horse serum which will produce anaphylaxis, to insist that the animals used not only had never themselves been previously used, but were the offspring of normal animals; the syringes used for the sensitizing dose, although they had been repeatedly washed, were boiled for from fifteen to twenty minutes before using.

would seem to be present leads to correlative conclusions of interest. It is obvious that after sensitization by a given substance the maximum intoxication is caused by the same substance; we might refer to this as the "complete" intoxication; in addition there may or may not be "partial" intoxication by the other protein substances. It has been shown by our previous studies[4] that in the case of horse serum anaphylaxis, the substance of the serum which sensitizes (anaphylactin) is not identical with the substance which intoxicates. The distinction in these substances is brought out more clearly when we consider these instances of "crossed" intoxication. Horse serum has little or no power to sensitize against the other two proteids, while milk and egg have a more "general" anaphylactizing power; on the other hand, horse serum intoxicates readily after sensitization with one of the other substances, egg less readily, and milk still less so.

When we consider the serial intoxication by the different proteids after sensitization by a single substance, either milk or egg, the relation of a "partial" to a "complete" intoxication is brought out more clearly.

TABLE 4.

These guinea-pigs had been sensitized by previous injection of one cubic centimeter of egg white sixteen days previously.

G.P. No.	Date.	Given	Result.
153	March 20, 1908.	4 cc. milk subcutaneously.	Suggestive symptoms.
	March 21, 1908.	5 cc. horse serum intraperitoneally.	Marked symptoms.
	March 23, 1908.	5 cc. egg albumin intraperitoneally.	Marked symptoms.
150	March 20, 1908.	10 cc. horse serum intraperitoneally.	Severe symptoms.
	March 21, 1908.	5 cc. egg albumin.	Severe symptoms.
152	March 20, 1908.	4 cc. horse serum subcutaneously.	Slight symptoms.
	March 21, 1908.	5 cc. egg albumin intraperitoneally.	Dead 30 minutes.
154	March 20, 1908.	4 cc. egg albumin subcutaneously.	Moderate symptoms.
	March 21, 1908.	5 cc. horse serum intraperitoneally.	No symptoms.

This table shows that even after "partial" intoxication has been caused in animals sensitive to egg white, by one or even by both of the other proteids in turn (G.P. No. 153) a succeeding set of symptoms may be elicited by egg white. When, however, egg white is used for the first (complete intoxication) further intoxication is not elicited by the partial intoxicating substance, horse serum (G.P. No. 154). Similar results are obtained after sensitization with milk.

TABLE 5.

These guinea-pigs had been given one cubic centimeter of milk thirteen days previously.

G.P. No.	Date.	Given	Results.
81	March 30, 1908.	5 cc. horse serum subcutaneously.	Marked symptoms.
	March 31, 1908.	5 cc. milk intraperitoneally.	Marked symptoms.
80	March 30, 1908.	5 cc. milk subcutaneously.	Distinct symptoms.
	March 31, 1908.	5 cc. horse serum intraperitoneally.	Doubtful symptoms.

It is evident from these two tables that if the protein substances are given in a properly chosen order, namely, partially intoxicating followed by completely intoxicating, that a series of intoxications may be produced after sensitization by a single protein substance as well as after combined sensitization with several protein substances.

When we come to consider the results of successive intoxications after the combined anaphylaxis due to injection of a mixture of several proteids, we find that they are no more absolutely specific than are the intoxications just detailed, obtained after sensitization by a single proteid. A careful study of the results obtained both by Rosenau and Anderson and by ourselves shows distinctly that, in the case of simple anaphylaxis followed by intoxication with the same substance, the combination egg followed by egg is most toxic, and serum followed by serum the next most toxic, while the combination milk followed by milk is least toxic of all. When we consider the resultant intoxication by a given proteid we must consider, first, the relation of the intoxicating substance to the anaphylactizing substance, that is, whether homologous or not; and secondly, we must consider what the absolute intoxicating power of each proteid might be if an anaphylactin which was common to all the proteids used could be imagined. Such a "common" anaphylactin is, of course, purely hypothetical, but we may approach a condition such as

might be caused by such a substance, by means of the "mixed" sensitization of Rosenau and Anderson. If we sensitize guinea-pigs with a mixture of horse serum, egg white, and milk they become capable of giving the maximum symptoms of intoxication with any one of these substances. If, however, they are given each substance in turn it is found that the chain of successive symptoms depends on the order in which the proteids are given.

TABLE 6.

These guinea-pigs were sensitized by subcutaneous injection of a mixture composed of horse serum .01 cubic centimeter, egg white one cubic centimeter, and milk one cubic centimeter. They were subsequently given doses of five cubic centimeters of each substance in turn at intervals of twenty-four hours, and in each case in a different sequence. Nos. 117, 118, 119 were injected beginning on the thirty-fifth day; and Nos. 76, 77, 78 were injected beginning on the fifteenth day; the two series, however, were judged to be comparable inasmuch as the relation of order of substances was considered in relation to the intoxication by the other substances which preceded or followed. The first two intoxications of a series were given subcutaneously, and the last one intraperitoneally.

G.P. No.	Order of Intoxicating Proteid.	Symptoms Corresponding to each Proteid.
76.....	*Egg;* horse serum; milk.	*Marked;* distinct; no symptoms.
118.....	*Egg;* milk; horse serum.	*Severe;* doubtful; slight.
119.....	Milk; *egg;* horse serum.	Severe; *severe;* marked.
78.....	Horse serum; *egg;* ×	Severe; *dead thirty minutes;* ×
77.....	Milk; horse serum; *egg.*	Severe; marked; *marked.*
117.....	Horse serum; milk; *egg.*	Severe; slight; *severe.*

This table is arranged to emphasize the relation of the order in which egg white was placed in serial injection to the resulting symptoms which it caused. A composite table of the different proteids showing the relation of their order of injection to the symptoms which they caused follows:

TABLE 7.

Order of injection on subsequent days.

Proteid.	First Day.	Second Day.	Third Day.
Egg	Marked. Severe.	Severe. Death.	Marked. Severe.
Horse serum	Severe. Severe.	Distinct. Marked.	Slight. Marked.
Milk	Severe. Severe.	Doubtful. Slight.	None. ×

A consideration of these tables shows that after what may be termed a sensitization common to serum, egg, and milk, the initial injection of any one of these substances, after the proper incubation period, will cause maximum symptoms. If injected on the second day egg alone will cause as marked symptoms as if given first; horse serum has already lost part of its potency to intoxicate if given after either of the other proteids; and with milk the loss is greater still. On the third day egg has lost none of its potency, horse serum most of its potency, and milk no longer intoxicates. It is evident that these substances may be arranged in order of their absolute toxicity, egg white, serum, milk, which corresponds to their apparent value after homologous sensitization. And what is more important still is the fact that if, after the mixed sensitization (serum, plus egg, plus milk), the weakest intoxicating substance (milk) is given on the third day it awakens no symptoms. This is clearly evident also in the tables of Rosenau and Anderson. In other words, the reaction which it might have evoked if given early in the order can be in no sense specific since it has evidently been fulfilled by the other two substances.

No particular reference is here made to the pathology and histopathology of the intoxications by egg white, milk, and the serial intoxications produced by injection of the three substances. The lesions caused by these substances differ in details from those which we have described in pure horse serum anaphylaxis and will be considered at another time.

CONCLUSIONS.

The anaphylaxis in guinea-pigs caused by the previous injection of any one of the protein substances, horse serum, egg white, or milk, is only relatively specific. The maximum reaction on second injection is always obtained when the substance which has sensitized is used, but in certain combinations intoxication can be produced by the other two substances. This intoxication, by a heterologous proteid is "partial" and does not occur if the "complete" intoxication produced by the homologous proteid has been effected; when partial intoxication has been produced by one or both of the heterologous substances, complete intoxication may still be effected by the homologous substance. The intensity of an homologous intoxication, after anaphylaxis by a single substance, would seem to depend somewhat on the substance used, the order of toxicity ranging, egg white, serum, and last of all milk. After combined anaphylaxis, produced by initial injection of all three substances, the first intoxication, allowing of course a proper incubation period, may be produced by any one of the substances in question. When intoxications are effected with each substance in turn the serial set of symptoms varies according to the order in which the substances are injected on the subsequent days. When injected as the second or third of the series, egg white alone produces maximal symptoms at all times; horse serum is diminished in toxicity if used after either egg or milk and has lost markedly if used after intoxication with both substances. Milk is very slightly toxic if given second in order and absolutely non-toxic if given third. This would compare with the actual toxic power of each substance as noted after homologous sensitization.

The mixed anaphylaxis, moreover, is only relatively specific since egg and horse serum will completely preëmpt the possibility of intoxication by milk if this substance is given last.

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FURTHER STUDIES IN ANAPHYLAXIS.*

IV. *The Localization of Cell and Tissue Anaphylaxis in the Guinea-pig, with Observations on the Cause of Death in Serum Intoxication.*

F. P. GAY AND E. E. SOUTHARD.

(*From the Pathological Laboratories of the Harvard Medical School and the Danvers Insane Hospital.*)

1. The gross lesions of serum intoxication in the guinea-pig. — In 1907 we reported the occurrence of characteristic hemorrhages in guinea-pigs which had received a second properly timed dose of horse serum.[1] We observed such hemorrhages both in those guinea-pigs which died of the intoxication and in those which we killed at various intervals after intoxication. The hemorrhages were not quite constant in occurrence, but were found in one or more organs in thirty-four out of forty-one guinea-pigs autopsied at intervals within the first twenty-four hours after injection. The hemorrhages occurred in a great variety of organs. The most massive hemorrhages, and those most frequently observed, took place in the stomach wall. Thus we found gastric hemorrhages in thirty-two of the forty-one guinea-pigs examined within twenty-four hours of the toxic injection.

Further observations[2] bring our numbers to a total of eighty-six guinea-pigs with hemorrhages in one or more organs, five with doubtful hemorrhages, eleven without hemorrhages in a series of one hundred and two guinea-pigs examined within twenty-four hours of the toxic injection. Fifty-nine of these guinea-pigs (besides three doubtful) showed gastric hemorrhages. We have three new localizations of hemorrhage to report, the brain (three cases), the peritoneum (two cases), the spinal cord (one case).

Hemorrhages were localized in the complete series (1907 and 1908) as follows: Stomach, 59; lungs, 41; heart, 15;

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cecum, 9; spleen, 9; adrenal, 8; kidney, 4; muscle, 3; lymph node, 3; brain, 3; diaphragm, 2; liver, 2; pericardium, 2; peritoneum, 2; thyroid, 2; spinal cord, 1.

The occurrence of these gross lesions (in eighty-five per cent of our series of guinea-pigs) escaped the attention of the initial writers on anaphylaxis of this type (Otto in Ehrlich's laboratory, and Rosenau and Anderson in the U.S. Hygienic Laboratory). Otto, in fact, in his first paper[3] specifically denied the occurrence of macroscopic lesions in serum intoxication, and Rosenau and Anderson[4, 5, 6, 7] nowhere state the observation of macroscopic or other lesions in the disease. Lewis,[8] however, working under the direction of Theobald Smith is able to confirm our observations fully and states that Smith, one of the earliest observers of anaphylaxis of this type, had seen such lesions in intoxicated guinea-pigs several years before. Since these publications, there has been neither confirmation nor denial of these findings. Besredka[9] remarks that our structural findings may in the future be of service in clearing up the mechanism of the disease.

As we showed in 1907, these lesions have an important bearing on hypotheses concerning serum intoxication. We showed by an extensive histological study that the gross lesions are only indicators of a manifold series of cell-reactions, involving changes in capillary endothelium, voluntary and cardiac muscle fibers, nerve fibers, and gastric epithelium. As with the gross lesions, the microscopic lesions are distinguished by their focal distribution. This focality extends down to the separate cells, in such wise that single nerve fibers and single muscle fibers are found in a state of extreme or maximal fatty change in the midst of wholly normal fibers.

What is the significance of these lesions? The lesions cannot be regarded as anatomically specific for the serum disease. They belong in the same logical group with the multiple focal hemorrhages of the exanthems, of purpura, and of urticaria. They have much in common with hemorrhages due to local endotheliolysis produced by the

application of such substances as venom. Can the reactions be proved by histological methods to show anything histologically or cytologically specific for the serum disease? This question must be answered in the negative. There is nothing in the lesions of this serum disease which approaches a tubercle or a small-pox vesicle in specificity. The importance of our structural observations does not consist in a demonstration of specific lesions, but in the exact demonstration of one method of producing lesions that are common in various types of disease, particularly in the exanthemata.

2. The cause of death in serum intoxication. — A complete delineation of this kind of pathogenesis would comprise: (1) modifications of tissue during the stage of preparation (anaphylactic phase), (2) the lesions of intoxication (critical toxic phase), and (3) the phenomena of repair and secondary anaphylaxis (reparative and secondary anaphylactic phases). In our former paper we confined ourselves largely to the lesions in the critical phase of intoxication, noting that the primary anaphylactic phase yielded few structural data open to observation. In the present communication we offer a continuation of our structural studies, embracing a more particular account of the cause of death in the critical phase, in the light of which some further observations on the anaphylactic and reparative phases are considered. Nothing is more striking than the sudden death following toxic injection by way of the carotid artery or jugular vein. The hemorrhages in brain, lungs, and heart, produced by such toxic injections, do not admit a purely mechanical explanation, since they are absent in control unused pigs injected with the same amounts of horse serum.

The local fatty changes in the capillary endothelium near the hemorrhagic areas in the heart muscle in these experiments must be closely related to the genesis of the hemorrhages.

But, although striking, these lesions are, in the strict sense, hardly lethal. What is the cause of death in the critical phase?

Hypotheses concerning the cause of death in the critical phase are in the field as follows:

1. *The functional hypothesis.* — Lewis[10] thinks the cause of death in the critical phase depends on "the disordered function of a single organ, or broken coördination between several organs." The hypothesis, as stated, gives no clue to the nature of the functional disorder, or to the organ or organs involved. Lewis leaves in doubt whether the intoxication affects the nervous system or the heart or the lungs, or the heart and lungs through the nervous system. The manifold lesions are admitted, but these lesions are not regarded as pertinent to the occurrence of the functional disorders. It does not appear that this hypothesis is more than a restatement of what occurs in the symptomatic course of the disease, or much more than a blank form for whatever might occur in the process.

2. *The latent brain lesion hypothesis.* — This hypothesis was constructed by Besredka and Steinhardt[11] at the outset of their work. They suppose that the sensitized guinea-pig acquires and retains a latent lesion in its brain. The toxic injection serves to start up (réveiller) the latent brain lesion, bringing about severe disturbances or death. If we substitute for the term lesion (with its structural connotations) the term condition (without such specialized connotations), it is evident that the hypothesis of Besredka and Steinhardt involves scarcely more than the assumption that the symptoms of death in the critical phase are effected through the specially sensitized brain. This hypothesis is, therefore, an advance upon Lewis' functional hypothesis in that Besredka and Steinhardt particularize the supposed source of the disordered functions. Although this hypothesis is not sufficient to explain the lesions of remote organs (such as stomach or cecum since described by us), the hypothesis may contain some truth in so far as it applies to some of the prominent and apparently fatal symptoms. The functional hypothesis of Lewis and the latent brain lesion hypothesis of Besredka have, therefore, much in common; but Besredka indicates

the single organ whose disordered function comes prominently into play.

3. *The hypothesis of specific toxic action upon the respiratory centers.* — There is no doubt that the cause of death is often a respiratory one. From such symptomatic observations, Rosenau and Anderson suggest that we here deal with a poison causing death through the nervous control of the respiration. In order to exclude local effects upon the diaphragm, Rosenau and Anderson [12] showed that the phrenic nerve after death is still excitable, and produces diaphragmatic movements on electrical stimulation after death. Vaughan and Wheeler,[13] dealing with similar considerations in poisoning by white of egg, also maintain that the poisonous substance owes its toxicity to a combination with the cells of the respiratory center. Rosenau and Anderson have since [14] generalized their original suggestion, proposing that "profound chemical changes, perhaps in the central nerve cells, are probably produced by the first injection." In a later paper [15] these writers further maintain that the phenomena of the critical phase are also explained by profound chemical changes, probably in the nervous system. It appears from this work, as well as from our own constant observation (see below) of pulmonary emphysema in those pigs which die rapidly, that the respiratory center, or, at any rate, the nervous respiratory apparatus, is prominently affected by the toxic injection. But, as appears from further considerations, this toxic action upon the respiratory center (or nervous apparatus) cannot be regarded as specific.

Briefly, then, the functional hypothesis of Lewis is scarcely more than a hypothesis in name, since it does no more than restate the problem; the latent brain lesion hypothesis of Besredka lacks a convincing demonstration of either the morphological or the chemical nature of the lesions, either before or after they are "réveillé" by the toxic injection; and the respiratory center hypothesis, as first advanced by Rosenau and Anderson, has considerable evidence in its favor, explains the direct cause of death in most or all

instances, and merely errs in suggesting that the toxic substance is a specific respiratory poison.

Our work to the present time has shown that pulmonary emphysema is a constant feature at autopsy in guinea-pigs dying quickly after the second or toxic injection. We are inclined to regard this emphysema as the effective cause of death in the quickly fatal cases. Of course, the pulmonary hemorrhages and cardiac hemorrhages, which we were able to produce by toxic jugular injections with death in four minutes, are important factors as against possible recovery from emphysema in certain highly intoxicated guinea-pigs.

What is the mechanism of this fatal acute emphysema in the intoxicated guinea-pigs? The emphysema is an expression of death in the inspiratory phase with diaphragmatic spasm. All evidence at hand indicates that the diaphragm is under a constant nervous stimulation of excessive degree during the critical phase following the toxic injection. The probable course of the impulses which produce this hyperstimulation of the diaphragm is by way of the respiratory center in the medulla to the phrenic center in the cervical spinal cord and thence through the phrenic nerve.

Our recent experiments with sensitized guinea-pigs bring out the fact that the more readily the horse serum on the second injection can gain access to the nervous respiratory apparatus, the more certainly will excessive and spasmodic diaphragmatic and general respiratory contractions ensue. The fatalities upon injection of serum into the fluid spaces about the brain (we have preferred the post-orbital route for injection) and into the spaces about the spinal cord (notably in the upper cervical region) are very frequent, and with toxic doses of one cubic centimeter, practically constant.

We shall not here report in detail the results of all our localized inoculations with respect to their differential effects upon the nervous apparatus at large, reserving these findings for special neuropathological consideration. The effects of injections in the vicinity of various parts of the central nervous system are to a great degree differential, and may

prove of some importance in determining special reactions of different regions, especially in the spinal cord. The effect upon the respiratory center is marked and early.

The simplest explanation of this effect is that the toxic factor of the horse serum in these local injections comes directly into contact with the properly sensitized nervous apparatus of respiration, and causes it to work excessively. As is well known, expiration is, under ordinary conditions, a purely mechanical matter; but inspiration is under nervous control. Excessive stimulation of the phrenic center leads to death in the inspiratory phase. Even after opening the thorax and excision of the heart and lungs, intense diaphragmatic contractions may be observed in guinea-pigs dying in the critical phase.

It has so far been impossible for us to determine which portion of the respiratory nervous apparatus is most highly sensitized, or whether any portion is more highly sensitized than another.

It is clear, however, that the hyperstimulation may in some instances be effected over two or three separate conducting elements or neurones. A striking experiment, recently accomplished, consists in the production of respiratory symptoms by touching the vagus nerve (lifted from contact with surrounding structures) of a sensitized pig with a pledget of cotton soaked in horse serum. The same procedure in non-sensitized pigs fails to evoke characteristic diaphragmatic contractions, and salt solution fails to produce the effect in sensitized animals.

This experiment would seem to indicate that the vagus nerve is to some extent sensitized in the general anaphylaxis. We have so far been unable to produce death by this procedure. Fatal issue seems to require immediate stimulation of the medullary or phrenic centers, and, so far, we are unable to report finally which of these centers is the more highly sensitized. Experiments to solve these questions are difficult, because it is desirable to avoid mechanical injury to the centers, but at the same time secure quick absorption of toxic serum by a given center, and distinguish the effects of

the local anaphylaxis from those of anaphylaxis in the rest of the body which the serum speedily invades by blood or lymph.

Our vagus experiments may serve for the present to indicate the line of attack on the problems of local anaphylaxis and to bring out the mechanism of one of the most violent and striking of the symptoms of the serum disease. And our local injections with object of differentially intoxicating different parts of sensitized nervous system (to be reported in detail in a later study) serve simply to bring out the strikingly variable accessibility of the respiratory center, when properly prepared, to a poison injected at different points with relation to the nervous system.

Therefore we desire to substitute, for the theories of the cause of death in the serum disease as so far proposed, and above enumerated, a theory of local anaphylaxis of the respiratory nervous centers. By this we mean a condition induced in the respiratory centers, by means of anaphylactin in the fluid media bathing their cells, such that their elements are excessively stimulated when brought in contact with the toxic or assimilable factor of horse serum. The cause of death is, therefore, an indirect one, being founded on hyperstimulation of the phrenic nerve and cessation of respiration in the inspiratory phase. On this hypothesis the means of avoiding death in the critical phase of intoxication following anaphylaxis would be a therapeutic agent depressing the respiratory centers.

This hypothesis differs essentially from those previously proposed. So far from being due to a disorder of function or incoördination between organs, as proposed by Lewis, the cause of death would seem to be due to an excess of functioning of an apparatus, which is working quite properly in response to the stimulation it receives. The new hypothesis is also more specific than the hypotheses of Lewis and of Besredka. Nor is it necessary to suppose with Besredka a latent brain lesion which is started up once more by the second injection. Rather should we say that a condition had

been induced in these cells, as a result of placing an anaphylactic substance in their surrounding media, such that certain results follow upon injection of a toxic substance. There is an essential difference between the idea of slight irritation followed by severe irritation of qualitatively the same character (as indicated by Besredka's metaphor, "réveiller") and the idea which we support that a substance (anaphylactin) bathing, among other structures, the respiratory nerve cells, has the capacity of altering those cells so that a second substance (normally ineffective) can become effective in stimulating them.

It is obvious that our hypothesis has much in common with any which supposes a specific action upon respiratory centers. And, if Rosenau and Anderson were to ground their hypothesis upon the rendering of these centers specifically accessible to certain toxic substances, we should agree with them. But it seems rather that they suppose that a specific toxine has been produced in the process of anaphylaxis or that a toxine is produced by the union of serum constituents and antibodies,[16] such that the wholly unaltered respiratory center can now be affected by this specific toxine. Whereas Rosenau and Anderson suppose a specific respiratory toxine to be somehow newly produced, we prefer to suppose that the anaphylactin of the first injection has altered certain properties of the cells.

Meantime, the insistence of Besredka upon "latent brain lesions" and of Rosenau and Anderson upon "profound chemical changes perhaps in the central nerve cells" is an indication that these workers fundamentally agree that we must look to the cells for the explanation of much of this problem. It does not seem that Rosenau and Anderson would be forced to hint at profound chemical changes in the cells, if the antibody hypothesis were quite convincing.

A consideration of the cause of death, with cessation of respiration in the inspiratory phase under the influence of respiratory central intoxication, which we can hasten by suitable application of serum near those centers, leads us, therefore, to the conception of a local acquired anaphylaxis or

specific lowered resistance of these centers, under the influence of one substance (anaphylactin) so that they become hypersusceptible to another substance (toxic factor).

An extension of these conceptions to the rest of the body requires separate consideration.

3. Tissue anaphylaxis. — We have so far considered two striking features of the critical phase after the second injection, viz.: The multiple focal hemorrhages frequently found at autopsy within twenty-four hours of the toxic injection and the fatal issue which, when it follows, occurs usually within an hour of the toxic injection. We have not found that either of these features is absolutely constant, although our percentage of hemorrhages (eighty-five per cent) is enough to demonstrate their importance, although the fatal issue seems to vary, roughly at least, with the speed and volume with which the horse serum reaches some portion of the respiratory nervous apparatus. We shall later communicate work on the localization and dosage of respiratory central intoxication.

Is there some broader conception under which these two striking features of the critical phase can be united? Further work upon the histological features of the critical phase confirms the opinion expressed in our paper of 1907 [17] that focal fatty changes are found in very numerous tissues of several different sorts. Some of these changes, especially when several altered cells are in close spatial relation, offer loci of lowered resistance permitting hemorrhages. But it is not at all necessary that the altered cells shall be so grouped as to allow hemorrhages. Take the stomach, for example, in which the most frequent and massive hemorrhages and hemorrhagic ulcerations occur: much more frequent than the hemorrhagic areas in these stomachs are areas in which fatty changes of sharply definite character but without hemorrhages are found.

The focal fatty changes of the critical phase are of more fundamental importance than the hemorrhages, which are

possibly but the mechanical expression of *loci minoris resistentiæ*.

Since our former work, we have studied in particular the tissues of guinea-pigs in various stages of recovery from intoxication and during secondary anaphylaxis, as well as the conditions of second and third intoxications.[18] The result of this work has been to confirm the former findings and to show that the disease is essentially a critical one.

The toxic injection can be said to effect no further disturbance than that of the first hour. Though this could have been surmised from the symptoms, still it might be regarded as likely that some further phenomena would characterize the tissues.

We thought, in particular, that an investigation of the blood picture during the course of the disease might exhibit the tissue reactions more exactly.* This work so far indicates that the same type of reaction — hypoleucocytosis followed by hyperleucocytosis — occurs upon injection of horse serum into normal as into sensitized animals. This fact precludes extraordinary stress being laid at this time upon the leucocyte counts in this disease. The difficulty in drawing blood from guinea-pig ears during the critical phase is an interesting point, which may depend upon contraction of peripheral vessels due to an intoxication of the nervous system.

Although it is true that definite blood alterations do follow the toxic injection, it may be suspected that these alterations have to do merely with the elimination of horse serum and with readjustment of the blood cell supply and that they occur in normal animals in similar fashion after serum injections. A study of the spleens of the guinea-pigs, killed at various intervals of hours and days after the toxic injection, demonstrates that in less than six hours the spleen spaces become filled with polynuclear leucocytes. This tendency is so considerable that the spleens may become visibly swollen. This swelling is only in part due to congestion with

* This was undertaken by Dr. M. M. Canavan of the Danvers Insane Hospital, who proposes to contribute the findings in detail shortly.

blood corpuscles. The intrasplenic leucocytosis is prominent still in guinea-pigs killed at intervals up to twenty-four hours after the toxic injection, but in most cases gradually disappears and gives place to mononuclear phagocytosis during the third day. Some guinea-pigs on the fourth day yield little sign in their spleens of the toxic injection; but in some a moderate leucocyte destruction is somewhat persistent. But although a detailed study is contemplated of the conditions of the peripheral and splenic leucocytosis as well as of marrow conditions, it seems at present, from the orienting examinations already performed, that the relation of these pictures to the process of tissue anaphylaxis is quite obscure and that the body exhibits the same signs of eliminative effort in the blood after the first injection as after the toxic injection. In view of our study of recurrent anaphylaxis [19] in the same animal, these findings become interesting, but it is premature to state that the blood reactions have any relation to the process of anaphylaxis.

These studies may serve to show, therefore, that tissue anaphylaxis is very possibly not at all a function of the blood, detectable by cell changes therein. The reactions, both structural and functional, of the critical phase indicate that very numerous tissues of several orders have been altered in anaphylaxis.

What can be found in these tissues during the stage of anaphylaxis? It will be remembered that the only striking changes which we found in the anaphylactic phase in our former work were certain changes in the peripheral nerves and spinal cord, decidedly less often in nerve tissues above the medulla. These changes consisted in blackenings of scattered myelin sheaths demonstrable in Marchi preparations. The alterations were of two sorts, which we termed respectively linear and nodal changes. The linear blackenings, which exhibit the affected fiber as replaced by a row of black globules and signify a severe injury reparable only by regeneration, were decidedly less frequent than the nodal blackenings. The nodal blackenings, found in the peripheral

nerves characteristically, show a limited osmic acid impregnation confined to the regions of Ranvier's nodes in a given fiber. These nodal changes are regarded as quite consistent with rapid recovery of normal conditions in the affected sheaths. It is possible that conduction is not interrupted by such changes but is altered in ways not now definable.

In addition to these myelin sheaths changes there are other and possibly correlative changes in nerve cells demonstrable by the Nissl method after alcohol fixation. We regarded these as within the limits of technical error, but, so far as reliable, consistent with prodromata toward the well known axonal reaction.

We were not eager to regard these changes as indicative of the latent brain lesions of Besredka or of the profound chemical changes of the nervous system proposed on theoretical grounds by Rosenau and Anderson, and, although we have worked much on this problem since and in particular with the sensitizing euglobulins of Gay and Adler, we are still unwilling to say that our findings represent the essential changes of the anaphylactic phase.

In the first place, we have found similar changes in seemingly normal material, and especially in the diphtheria toxone-paralysis material kindly given to one of us by Theobald Smith. It may be that these changes are simply indices of the extreme lability of nerve tissue, meaning by this lability a capacity for relatively rapid physical alterations of nerve cell and nerve fiber, demonstrable by osmic acid and methylene blue methods.

In the second place, our orienting work with the blood pictures in this disease indicates that to some extent the same species of leucocyte variation is present in the anaphylactic phase as in the toxic phase. So many substances are undoubtedly present in horse serum that the chance of changes effected by other constituents than the sensitizing or intoxicating constituents must be strongly considered. It is possible, therefore, that these anaphylactic nerve cell

and fiber changes are purely incidental to the action of unrecognized constituents of horse serum.

Work is in progress with the relatively pure sensitizing substances (euglobulins of Gay and Adler) to discover what differential effects these substances may bring out.

One further word concerning the relation of the fatty changes and hemorrhages of the toxic phase and possible antecedent changes in the anaphylactic phase. Critics have suggested to us that possibly at some time during the anaphylactic phase fatty changes supervene in various foci in various organs, and that the hemorrhages of the toxic phase are simply mechanical expressions of local acquired weaknesses in the vessels. This hypothesis would suggest that horse serum can in the first instance produce qualitatively the same kind of visible changes which characterize the toxic phase. We have made further examinations of the tissues in large numbers of guinea-pigs. The anaphylactic phase is not characterized by such changes. Using the stomach wall as a histologically pure field for this study, we have yet to find, in any pig killed in the anaphylactic phase, convincing evidence of the presence of intraepithelial fat, vascular fat, or early necroses of epithelium under the influence of anaphylactin.

These statements may be generalized as follows: The essential cytological features of the process of anaphylaxis have yet to be discovered. They do not consist in a minor degree of those changes (focal cytolyses) which characterize the tissues in the toxic phase. The process of anaphylaxis is not a cumulative process, and the toxic phase is not the result of a summation of similar stimuli or effects.

Certain changes, expressive of the extreme lability of nervous structures, have been found in the anaphylactic phase in peripheral nerves and spinal cord. These changes may or may not represent essential features of the process of anaphylaxis. The nervous system is not a clear field, histologically speaking, for the study of minor fatty changes, since these may be the expression of numerous uncontrollable

conditions. So far as the changes discovered have any bearing, the alterations produced by anaphylaxis may be regarded as of a physical nature, permitting speedy rearrangement of various contained substances.

Focal histolyses are not the rule in the toxic phase. Separate cells are more likely to be prepared for intoxication and to succumb thereto (focal cytanaphylaxis followed by focal cytolysis). But the diffuse changes in the stomach wall indicate that focal histanaphylaxis followed by focal histolysis may be the rule under some conditions (effect of gastric juice, etc.). Experience with the critical phase indicates that the cells of certain physiological centers may become highly sensitized (cell group anaphylaxis), and that the functional expression of their intoxication may lead to severe symptoms or fatal results.

4. The reparative phase and recurrent anaphylaxis. — We mentioned in our paper of 1907 that the serum disease might be repeated, and expressed the hope that by suitable repetitions chronic conditions would be produced. This hope has not been realized. The lesions do not necessarily repeat themselves in the site of the original lesions or even in neighboring situations. We have not carried our histological work beyond the reparative stages of tertiary intoxication.

Fresh intoxication in new sites is the rule in secondary or tertiary intoxications. But the repetition is virtually a new instance of the old disease and, certainly in the majority of instances, does not effect a cumulative action upon structures formerly attacked.

We hoped to find in the stomach instances of fresh intoxication superimposed on the remains of old and to discover that recurrent anaphylaxis would emphasize that of the sites formerly sensitized. The stomach seemed to be a likely seat for this cumulative anaphylaxis imagined by us, because of the tendency of the stomach to yield focal histolyses instead of the cytolyses of numerous other organs.

We thought that the new areas of local anaphylaxis might

to some extent coincide with or overlap the old areas of histolysis and lead perhaps to chronic ulceration. But, so far, the conditions have not fulfilled such preconceptions.

This branch of the work, therefore, brings out indirectly the features of the anaphylactin hypothesis. It is the habit of the anaphylactin to sensitize focally certain cells or cell groups. The same anaphylactin in recurrent anaphylaxis is disposed to a similar focal sensitization in which, however, fresh elements are affected.

In those cases of cell group anaphylaxis, after which intoxication expresses itself largely in a functional manner (as in local respiratory center anaphylaxis), a repetition of similar toxic effects can be produced. There was no tendency for repeatedly intoxicated pigs to diminish the severity of their respiratory symptoms. It is possible that, on this line of attack, cumulative effects could be eventually demonstrated in certain centers. It is also possible that separate cells of the respiratory centers are always newly involved in recurrent anaphylaxis and repeated intoxication, and that the toxic effects are not due to activities of the same individual members of the cell group involved. These hypotheses, however, evidently transcend the range of immediate proof.

CONCLUSIONS.

The results of this work are in part confirmatory of our previous results and consist in part of novel data.

Eighty-five per cent of guinea-pigs which, after sensitization with horse serum and intoxication by a second dose of horse serum, die in the critical phase or are killed within twenty-four hours of the second injection, exhibit macroscopic hemorrhages in one or more organs. The stomach leads the other organs in frequency of involvement (fifty-eight per cent); the lungs stand next (forty per cent). Three unusual localizations of hemorrhage, not noted in our previous paper, are brain, spinal cord, peritoneum.

The cause of death, when it occurs, is respiratory. Respiration ceases in the inspiratory phase and shows itself anatomically and histologically as emphysema. Death does not

occur, as a result of this disease, except in a critical phase which occupies at most one hour.

The most striking functional feature of the critical phase, after the second or toxic injection of horse serum, is severe diaphragmatic spasm. The spasms are often accompanied by similar shock-like spasms of the accessory inspiratory muscles and of other trunk and limb muscles.

The most rapid deaths are produced by intracarotid, intrajugular, post-orbital, and paraneuraxial injections. The occurrence and rapidity of death in the critical phase, as well as the severity of respiratory symptoms throughout the toxic phase, appear to vary with the nearness of the toxic injections to the respiratory central apparatus.

A new line of research is opened up by the paraneuraxial injections of horse serum in sensitized guinea-pigs. These seem to prove that differential irritative and paralytic reactions can be secured by small localized injections of horse serum adjacent to various parts of the sensitized central nervous axis.

Severe respiratory symptoms can be produced in sensitized (but not in normal) guinea-pigs by local applications of horse serum (not by salt solution) to the exposed vagus. This is interpreted to signify a conveyance of impulses over at least three neurones to the diaphragm, that is, to the medulla, thence to the phrenic center, and thence to the diaphragm. We have not produced death by these vagal applications of horse serum.

To explain these respiratory symptoms, we offer an hypothesis of local tissue anaphylaxis expressed in a relatively specific sensitization of the respiratory centers. We regard as unfounded those hypotheses which consider the respiratory (and other) centers and tissues as unaltered in the anaphylactic or sensitizing phase, and which allege the manufacture of antibodies in the blood serum which later unite with the second dose of horse serum to form new specific respiratory toxines. We regard this change induced in the respiratory centers as of a physical rather than a chemical nature, so far as this distinction is of importance in this connection.

Neither hemorrhage nor respiratory death is an indispensable feature of this disease. Some guinea-pigs show no hemorrhages. Some show slight symptoms. The hemorrhages do not vary in frequency or extent with the severity of the symptoms in all cases.

But all guinea-pigs so far examined in the toxic phase do show focal fatty changes in many tissues of several genetic types. These changes are, in many regions, of an extremely focal character, involving often a single muscle fiber, nerve-fiber, or other cell, as the case may be. The toxic phase is characterized by focal cytolyses of wide distribution. Except in areas of hemorrhage (where local mechanical destruction complicates findings) and in certain diffuse fatty changes in the gastric epithelium (where the local action of the gastric juice may come in play), groups of contiguous cells are not characteristically affected by fatty change: focal histolysis is not the rule.

And, if focal cytolysis (rather than focal histolysis) is the rule in the toxic phase, then it appears that the work of the anaphylactic phase is to sensitize cells in a variable degree (rather than to sensitize several contiguous or regionary cells in a like degree).

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FURTHER STUDIES UPON ANAPHYLAXIS.*

JOHN F. ANDERSON.

(Assistant Director, Hygienic Laboratory, U.S. Public Health and Marine-Hospital Service, Washington, D.C.)

AND

M. J. ROSENAU.

(Director, Hygienic Laboratory, U.S. Public Health and Marine-Hospital Service, Washington, D.C.)

Period of incubation. — In our first publication[1] from a limited series of experiments we found the "period of incubation," or the time necessary to elapse between the first and the second injection, to be about ten days. In order to clear up several interesting details concerning the period of incubation we made three series of experiments: (1) Does the hypersusceptibility come on abruptly or gradually? (2) Do guinea-pigs sensitized in the brain have a shorter period of incubation than those sensitized subcutaneously? (3) Is the period of incubation constant or variable?

We found that the period of incubation appears about the seventh day in guinea-pigs sensitized in the brain and about the ninth day in guinea-pigs sensitized subcutaneously. So far as may be judged, it therefore appears that the period of incubation is somewhat shorter in guinea-pigs sensitized in the brain than in those sensitized subcutaneously. It also seems quite evident that the sensitization comes on somewhat gradually. Judged by our results and the work of others the period of incubation is quite constant.

Lewis[2] states that the period of incubation is not to be considered as abruptly terminating at a given day. He says that he has made an animal quite sick by the intracardiac

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NOTE. — For details of the experiments in this paper see Bulletin No. 45, Hygienic Laboratory, U.S. Public Health and Marine-Hospital Service.

injection of two cubic centimeters of serum on the sixth day after a toxine-antitoxine mixture.

Relation of the amount of the sensitizing dose to the time interval. — It seemed to us at one time in our work that animals injected with a large quantity of horse serum at the first injection had a more prolonged period of incubation or were less sensitive than guinea-pigs given the usual minute amounts. We conjectured that this might have been due to the slow absorption of the serum, thus having somewhat the immunizing effect of repeated injections. We therefore prepared four series of guinea-pigs: One series sensitized with .01 cubic centimeter, a second with .1 cubic centimeter, a third with one cubic centimeter, and a fourth with eight cubic centimeters of normal horse serum. All these animals were subsequently tested by intracerebral injections of the same normal horse serum.

So far as may be judged from this work the period of incubation is not appreciably prolonged by a large sensitizing dose. Further, the animals once sensitized with horse serum alone remained so for a long period of time (at least two hundred and forty-five days).

Time. — The longest time that has elapsed between the first and the second injections in a guinea-pig in our experiments has been two years and two days (seven hundred and thirty-two days). It therefore seems that guinea-pigs once sensitized with mixtures of antitoxic horse serum and diphtheria toxine are sensitive through practically the remainder of their lives.

The effect of heat upon the sensitizing action of horse serum. — In previous publications we reported that the toxic action of horse serum was not destroyed when the serum was heated to 60° C. for six hours, but is entirely destroyed at 100° C. in fifteen minutes. Further work on this subject leads us to conclude that the sensitizing effect of horse serum is gradually influenced by heat and almost entirely disappears when the serum is heated to 100° C. for one hour.

The pigs sensitized with small quantities of horse serum heated to 100° C. for one hour, when subsequently tested, develop very slight symptoms.

Can guinea-pigs be sensitized in the brain? — We were led to make some experiments upon sensitization directly into the brain in view of a statement made by Besredka and Steinhardt[3] that guinea-pigs were not sensitized when very small amounts were injected into the brain. Very minute amounts only, when injected into the brain in our experiments as well as in the few reported by Besredka and Steinhardt, proved negative.

Guinea-pigs may readily be sensitized by intracerebral injections, provided quantities of .0001 cubic centimeter or more are used. We obtained negative results with sensitizing doses of .00001 cubic centimeter.

Attempts to sensitize guinea-pigs with pure proteins. — We have assumed that it is a protein substance in the horse serum, egg-white, milk, vegetable extracts, etc., which sensitizes guinea-pigs and poisons them at the second injection. The substances used by us of course have a very complex composition, so that it became desirable to use solutions of pure proteins for these tests. We are indebted to Dr. Lafayette B. Mendel for a quantity of edestin and excelsin, which are protein substances obtained in a chemically pure crystalline state. Our tests with these two particular proteins resulted negatively, due probably to the difficulty of obtaining a satisfactory solution.

The effect of blood serum and brain substance of sensitized guinea-pigs upon the sensitizing substances. — Normal horse serum, mixed with an equal amount of blood serum of a sensitive guinea-pig, and also normal horse serum mixed with an emulsion of the brain substance of a sensitive guinea-pig and allowed to stand at room temperature twenty-four hours were injected into normal guinea-pigs without causing any untoward symptoms. These pigs were subsequently

tested for their reaction to normal horse serum and were found to respond in the usual way.

These mixtures likewise failed to modify the poisonous action of the horse serum when given to sensitive animals.

Does horse serum decrease in toxicity with age? — Besredka[4] states that freshly drawn horse serum is more toxic than the same serum thirty days old. Our results are not in harmony with his findings, as we have found that a serum ninety-one days old had not decreased appreciably in its toxicity for sensitive guinea-pigs.

The effect of various chemical substances upon the toxicity of horse serum. — Further attempts were made to influence the toxic action of horse serum by treating the guinea-pigs with various chemicals. Animals sensitized by a mixture of toxine and antitoxine were given a subcutaneous injection of various chemicals the day before the second injection of serum. No influence upon the anaphylactic state was obtained by these substances. The following substances were used: Pancreatin, potassium oxalate, sodium sulphate, magnesium sulphate, peptone, calcium chloride, and calcium acetate.

Influence of iodine. — Obermeyer and Pick[5] found that when the aromatic radicals of a protein are combined with various substances the protein loses the power to produce precipitins of closely limited specificity for the original species. Their results suggest that the aromatic groups of the molecule are closely related to the species specificity.[6] This indicates that the striking specificity of proteins of different species depends upon the aromatic groups of the protein molecule and Vaughan has found evidence that the toxicity of the proteins depends upon these same groups.

Fleischmann[7] also found that tryptic digestion destroys this characteristic species specificity.

We made several tests to determine the effect of iodine upon the toxic action of horse serum and it so turned out in

the preliminary experiments that the symptoms appeared to be profoundly modified in the sense that they were either delayed or inhibited. We therefore tested a large number of guinea-pigs to determine this point but found that, so far as the toxicity of horse serum is concerned at the second injection, it was not appreciably modified by the iodine. The iodine was added to horse serum in the proportion of 1.5 grams per twenty-five cubic centimeters of serum and three grams potassium iodide to aid solution.

Nitrites. — A few experiments were made to determine the relation of methemaglobin-producing substances, such as the nitrites, upon the symptoms. Sensitive guinea-pigs were given subcutaneously an injection of sodium nitrite. In thirty minutes the exposed mucous membranes appeared distinctly blue; they were tested for their susceptibility to horse serum and found to react in the usual way. Controls showed that the quantity of nitrite used was not sufficient in itself to kill the guinea-pigs.

Ether. — Besredka[8] reported some interesting observations concerning the prevention of anaphylaxis by ether narcosis. He stated that if sensitive guinea-pigs are etherized to the stage of complete relaxation and while in this state injected intracerebrally with normal horse serum, if the administration of ether be continued a short time, the animal continues to sleep after the injection and at the end of about half an hour awakes without presenting the least symptoms of anaphylaxis. If the guinea-pig is tested on the following day it will be found to be immune.

Of eight guinea-pigs upon which we tried this experiment with ether, seven died from the effects of the second injection of horse serum. It is our belief that the guinea-pig which recovered had masked symptoms while under the influence of the ether and probably would not have died in any case, for we have a certain number of recoveries from the intracerebral injections of .2 cubic centimeter of horse serum. It is true, however, that the narcosis masks the

symptoms. The difference in our results may be accounted for either by the difference in toxicity of the French and the American serums, or by differences in susceptibility of the animals used.

The effect of heat upon the toxicity of horse serum. — Normal horse serum may be heated to 90° C. for one hour and still remain slightly toxic when injected into a sensitized guinea-pig. Its toxicity, however, is evidently markedly affected. Heating to 70° C. for one hour does not seem to diminish appreciably its poisonous properties, but it appears to be affected at 80° C. for one hour. At 100° C. for one hour the toxicity apparently disappears.

Blood serum, of course, cannot be heated to these high temperatures without coagulation, and it is therefore necessary to dilute it in the proportion of one part of blood serum to three parts of distilled water. This dilution may then be heated to a high temperature without producing any visible change other than a slight opalescence.

It appears that there is a slight difference between the sensitizing and the toxic principles in horse serum so far as the resistance to heat is concerned. Serum heated to 100° C. for one hour retains some power of sensitization, but seems to lose its toxicity when given at the second injection. This difference may be more apparent than real, for exceedingly minute amounts are sufficient to sensitize guinea-pigs, while a very large quantity of weakened serum would be necessary to produce symptoms. It must be remembered that in our experiments twenty cubic centimeters of the dilution represents but five cubic centimeters of serum.

These facts must be remembered in drawing conclusions from work upon split proteins, fractional precipitation, or other methods to isolate the sensitizing substance in pure form. A very minute amount of the original protein substance in horse serum clinging to the globulins, or other substances modified by chemical methods, might be sufficient to sensitize guinea-pigs, whereas it would require very large

amounts of such a modified protein to poison a sensitive animal.

The effect of repeated injections of horse serum into guinea-pigs. — We found it desirable to obtain further data upon the effect of repeated injections of horse serum into guinea-pigs. It was found that ten injections of two cubic centimeters each of horse serum, subcutaneously, into normal guinea-pigs did not render them entirely immune. They all developed mild symptoms when tested intraperitoneally fifteen to seventeen days after the last injection.

Five injections of .001 cubic centimeter each, subcutaneously, into normal guinea-pigs, were sufficient to sensitize them, so that when tested twenty-three days after the last injection four out of the six animals tested died.

Two other series, one of which received .001 and the other .01 cubic centimeter subcutaneously daily for twenty days, when tested subsequently for their susceptibility to horse serum, were found to be quite sensitive to injections of small amounts of serum into the brain.

A series of sensitized guinea-pigs was injected with one cubic centimeter normal horse serum diluted with distilled water, one to three, heated to 100° C. for one hour. These animals received from twenty to twenty-five injections subcutaneously. When subsequently tested for their susceptibility to horse serum it was found not to have any appreciable influence.

Specific nature of anaphylactin. — In a previous publication[9] on the specific nature of anaphylaxis we demonstrated that guinea-pigs may be in a condition of anaphylaxis to three protein substances at the same time. Hypersusceptibility to each protein is manifested by a second injection of the corresponding substance. The three reactions may be obtained in the same guinea-pig within a short space of time and are as distinct and specific as three separate infectious diseases.

We now bring forward experimental data proving that the

substance in the blood serum of sensitized guinea-pigs, known as anaphylactin, is also specific in the same sense. In the following table it will be seen that by transferring the blood serum of guinea-pigs sensitized to horse serum, egg-white, and milk, three separate and distinct reactions were obtained in the guinea-pig into which this serum was transferred:

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
9619	.23 cc. toxine No. 7 + 1/600 cc. antitoxic horse serum (Alex. A 249), subcutaneously. 56 days later, 1 cc. cow's milk, subcutaneously. 1 day later, 1 cc. egg-white, subcutaneously. 22 days later, bled; blood defibrinated, centrifuged, and serum used to inject G.P. 9619 A.			
9619 A ..	7 cc. serum of G.P. 9619, subcutaneously.	2	6 cc. milk, intraperitoneally. 2 days later, .2 cc. normal horse (Teddy) serum into brain.	Mild symptoms. Dead in 5 minutes.
9620	Same treatment as G.P. No. 9619.			
9620 A ..	8 cc. serum of G.P. 9620, subcutaneously.	2	6 cc. milk, intraperitoneally. 2 days later, 3 cc. egg-white, intraperitoneally. 2 hours later, .2 cc. normal horse (Teddy) serum into brain.	Slight symptoms. Mild symptoms. Dead in 4 minutes.
9621	.23 cc. toxine No. 7 + 1/580 cc. antitoxic horse serum (Alex. A 249). Subsequent treatment, same as G.P. No. 9619.			
9621 A ..	6.5 cc. serum of G.P. 9621, subcutaneously.	2	6 cc. milk, intraperitoneally. 2 days later, 6 cc. saturated solution egg-white, intraperitoneally. 2 hours later, .2 cc. normal horse (Teddy) serum into brain.	Slight symptoms. Marked symptoms. Very severe symptoms.
9624	.23 cc. toxine No. 7 + 1/560 cc. antitoxic horse serum (Alex. A 249). Subsequent treatment, same as G.P. 9619.			
9624 A ..	7 cc. serum of G.P. 9624, subcutaneously.	2	6 cc. milk, intraperitoneally. 2 days later, .2 cc. normal horse (Teddy) serum into brain.	Slight symptoms. Dead in 5 minutes.

Relation between milk of various animals. — Eight guinea-pigs were sensitized by the subcutaneous injection of one

cubic centimeter of human milk. After an appropriate interval they were tested with cow's milk without response.

A short time later they were again tested with human milk. This time most of them showed severe symptoms.

This indicates very plainly not only the specific nature of the anaphylactic reaction, but shows the differences between the protein matter in human and cow's milk.

Guinea-pigs sensitized with sheep milk react to a subsequent injection of cow's milk. Guinea-pigs sensitized with dog milk do not react to a subsequent injection of cow's milk.

The presence of anaphylactin during the period of incubation. — It is of some interest to determine just when the substance called anaphylactin by Gay and Southard appears in the blood of a sensitized guinea-pig, particularly whether its presence may be demonstrated during the period of incubation.

A series of guinea-pigs was therefore sensitized by the subcutaneous injection of .01 cubic centimeter of normal horse serum. On each succeeding day two guinea-pigs of this series were bled and the serum obtained by whipping and centrifugalization. This serum was then injected into normal pigs. In twenty-four hours they were tested by the injection of six cubic centimeters of horse serum intraperitoneally. In the latter part of the series forty-eight hours were allowed to elapse before the pigs were tested.

We found that no indication of anaphylactin appeared in the blood of sensitized guinea-pigs until the tenth day, that is, just about the time necessary to render guinea-pigs sensitive. It is evident from our experiments that forty-eight hours is a better interval than twenty-four hours for the purpose of demonstrating the presence of anaphylactin in guinea-pig serum.

Our interest in this subject led us to sensitize another series of guinea-pigs with .01 cubic centimeter normal horse serum subcutaneously, but to test for the presence of anaphylactin by injection into the brain. In this series the anaphylactin

was demonstrated in the blood of sensitized guinea-pigs on the ninth day.

Anaphylactin in man and other animals. — The presence of anaphylactin having been demonstrated in the blood serum of sensitized guinea-pigs, it is interesting to know whether man and other animals that have received a previous injection of horse serum also contain a similar substance.

So far as may be judged from our limited experiments we have been unable to demonstrate a similar property in the blood serum of man, the monkey, the rabbit, and the cat when tested upon guinea-pigs. We found that a few of the guinea-pigs showed symptoms, but in every case an interval of at least two weeks had elapsed between the first and the second injections. The slight reactions obtained were probably those which occur when serums of different species are used at the first and the second injections.[10]

Anaphylactin in immune guinea-pigs. — We submit some further work bearing upon the question as to whether guinea-pigs "immunized" against the phenomenon of anaphylaxis are in a refractory state or have returned to the normal or are really immune.

We first made a few experiments to determine whether guinea-pigs immunized by repeated injections of small quantities of horse serum contain anaphylactin in their blood. In this series the guinea-pigs received ten injections of two cubic centimeters normal horse serum subcutaneously, covering a period of seventeen days. Eighteen days following the last injection the pigs were first tested for immunity and then bled.

It developed from the first six guinea-pigs in the following table that ten repeated subcutaneous injections during the course of seventeen days were not sufficient to completely immunize the guinea-pigs, for those tested developed slight symptoms. Indications of anaphylactin were demonstrated in the blood serum of five of six pigs of this series. However, it must be noted that when bled these animals were

certainly immune from the last treatment, as we and others have shown that when a sensitive guinea-pig responds to a second injection immunity is quickly established (see G.P. No. 671 T).

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
1370 A ..	6 cc. serum G.P. 1370, which had received ten subcutaneous injections 2 cc. normal horse serum in a period of 17 days.	2	6 cc. normal horse serum, intraperitoneally.	Mild symptoms.
1373 A ..	6 cc. serum G.P. 1373, which had same treatment as 1370.	2	.2 cc. normal horse serum into brain.	Mild symptoms.
1369 A ..	6 cc. serum G.P. 1369, which had same treatment as 1370.	2	6 cc. normal horse serum, intraperitoneally.	Marked symptoms.
1371 A ..	6 cc. serum G.P. 1371, which had same treatment as 1370.	2	.2 cc. normal horse serum into brain.	Slight symptoms.
1374 A ..	6 cc. serum G.P. 1374, which had same treatment as 1370.	2	6 cc. normal horse serum, intraperitoneally.	Marked symptoms.
1372 A ..	6 cc. serum G.P. 1372, which had same treatment as 1370.	2	.2 cc. normal horse serum into brain.	No symptoms.
671 T....	.0006 cc. toxine A + 1/1538 cc. antitoxic horse serum (PD 09755).	55	2 cc. antitoxic horse serum (Natl. IX.), intraperitoneally. Next day, .2 cc. normal horse (roan) serum into brain. 30 minutes later, bled for serum.	Very severe symptoms. No symptoms.
671 A ...	5 cc. serum of G.P. 671 T, intraperitoneally.	2	.2 cc. normal horse (Frank) serum into brain.	No symptoms.
666 T....	.0006 cc. toxine A + .0002 gm. antitetanic serum (Tizzoni).	55	2 cc. antitoxic horse serum (Natl. IX.), intraperitoneally. Next day, bled for serum.	Very severe symptoms.
666 A ...	6 cc. serum of G.P. 666 T, intraperitoneally.	2	.2 cc. normal horse (Frank) serum into brain.	No symptoms.

The following series of guinea-pigs is a still more conclusive test that anaphylactin may be demonstrated in the blood serum of immunized guinea-pigs.

Guinea-pigs were first immunized by repeated subcutaneous injections of five cubic centimeters normal horse serum. The pigs received from six to twelve such injections, amounting to thirty to sixty cubic centimeters. About eight months

after this treatment the pigs were tested by intracerebral injections, to which some responded with slight symptoms. Two of them showed no symptoms at all. While all the pigs were not completely immunized by the first treatment of subcutaneous injections, they were certainly rendered immune by the second injection given eight months later. Two hours after the second treatment the guinea-pigs were bled, the blood centrifugalized, the clear serum pipetted off and injected subcutaneously into normal guinea-pigs. These normal guinea-pigs so treated were tested two days later by intracerebral injections and all of them showed symptoms.

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
1180.....	Six injections, each 5 cc. normal horse (roan) serum subcutaneously, covering a period of 18 days.	249 after last injection.	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Slight symptoms.
1180 A ..	9 cc. serum G.P. 1180, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Slight symptoms.
1181.....	Six injections, each 5 cc. normal horse (roan) serum in 18 days.	249	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	No symptoms.
1181 A ..	8 cc. serum G.P. 1181, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Marked symptoms.
1182.....	Six injections, each 5 cc. normal horse (roan) serum in 18 days.	249	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Slight symptoms.
1182 A ..	8 cc. serum G.P. 1182, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Marked symptoms.
1183.....	Six injections, each 5 cc. normal horse (roan) serum in 18 days.	249	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Slight symptoms.
1183 A ..	12 cc. serum G.P. 1183, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Slight symptoms.
1186.....	Seven injections, each 5 cc. normal horse (roan) serum in 21 days.	246	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Slight symptoms.
1186 A ..	8 cc. serum G.P. 1186, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Slight symptoms.

Table continued.

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
1187.....	Eight injections, each 5 cc. normal horse (roan) serum in 25 days.	242	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	No symptoms.
1187 A ..	9 cc. serum G.P. 1187, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Slight symptoms.
1188.....	Nine injections, each 5 cc. normal horse (roan) serum in 26 days.	239	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Slight symptoms.
1188 A ..	8 cc. serum G.P. 1188, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Slight symptoms.
1189.....	Ten injections, each 5 cc. normal horse (roan) serum in 32 days.	235	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Slight symptoms.
1189 A ..	5 cc. serum G.P. 1189, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Marked symptoms.
1190.....	Eleven injections, each 5 cc. normal horse (roan) serum in 35 days.	232	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Mild symptoms.
1190 A ...	9 cc. serum G.P. 1150, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Slight symptoms.
1191.....	Twelve injections, each 5 cc. normal horse (roan) serum in 39 days.	228	.2 cc. normal horse (roan) serum into the brain. 2 hours later, bled for serum.	Slight symptoms.
1191 A ..	9 cc. serum G.P. 1191, subcutaneously.	2	.15 cc. normal horse (Teddy) serum into the brain.	Marked symptoms.

It is, therefore, plain that anaphylactin exists in the blood serum of immune guinea-pigs.

The relation of serum anaphylaxis in the guinea-pig to serum therapy. — Besredka and Steinhardt [11] were the first to point out that the second injection may be given into the brain of guinea-pigs. When a small quantity of horse serum is injected into the brain of a sensitized guinea-pig the symptoms appear promptly and often with great violence, and death is a common result.

Besredka [12] believes that intracerebral injections may be

used as a measure for the toxicity of therapeutic serums. He states that, measured in this way, different serums show a wide gamut of toxicity, the fatal dose varying from $\frac{1}{4}$ to $\frac{1}{128}$ cubic centimeter. He believes that this toxicity resides in the serum and not in the cellular elements; further, that the serum of horses living under apparently the same conditions has about the same toxicity, individual variations being rare and of little importance. He concludes that, in a general way, all serums that incite in guinea-pigs grave anaphylactic phenomena in doses of one-sixteenth to one-twentieth cubic centimeter and *a priori* above this amount should be considered toxic.

We doubt whether there is a relation between the toxicity of serums as tested upon guinea-pigs in this way and their power to produce the serum disease or collapse or sudden death in man. It appears to us that in man the symptoms of the serum disease depend partly upon the kind of serum and the amount used. The unfortunate accidents, such as collapse and sudden death, depend more upon the sensitization of the individual than upon the so-called toxicity of the serum used.

Fortunately we are able to obtain two antidiphtheric serums which had been used in two cases of sudden death.

Case No. 1. — Serum No. 2277. Reported by Dr. S. N. Wiley, Norristown, Pa., Journ. Am. Med, Assn., 1, Jan. 11, 1908, 137. Mr. E. W., aged thirty-four years, splendid physique, best of health. Prophylactic injection of one thousand units antidiphtheric serum. Site of inoculation four inches above Poupart's ligament. Within two minutes had violent symptoms — anxious expression, itching, burning, labored breathing; lips, face, and neck swollen and red; paralysis; convulsions. Died within five minutes of injection.

The toxicity of the serum, *i.e.*, another package of the same laboratory number, was tested upon the following series of guinea-pigs:

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
9147	.23 cc. toxine No. 7 + 1/420 cc. antitoxic horse serum (PD 09913).	97	.1 cc. antitoxic horse serum (2277) into brain.	Dead in 7 minutes.
9131	.23 cc. toxine No. 7 + 1/250 cc. antitoxic horse serum (Natl. V. 24).	97	.1 cc. antitoxic horse serum (2277) into brain.	Dead in 8 minutes.
9136	.23 cc. toxine No. 7 + 1/300 cc. antitoxic horse serum (Natl. V. 24).	97	.05 cc. antitoxic horse serum (2277) into brain.	Dead in 35 minutes.
9151	.23 cc. toxine No. 7 + 1/380 cc. antitoxic horse serum (PD 09491).	97	.05 cc. antitoxic horse serum (2277) into brain.	Very severe symptoms.
9161	.23 cc. toxine No. 7 + 1/320 cc. antitoxic horse serum (Mem. C 20).	67	2 cc. antitoxic horse serum (2277), intraperitoneally.	Dead in 3 hours.
9170 ...	.23 cc. toxine No. 7 + 1/330 cc. antitoxic horse serum (Mul. 2438).	67	2 cc. antitoxic horse serum (2277), subcutaneously.	Very severe symptoms.

Case No. 2. — Serum No. 2295. Reported by Dr. H. F. Gillette, Cuba, N.Y., Journ. Am. Med. Assn., I, Jan. 4, 1908, 40. Mr. B., fifty-two years old. Had asthma and bronchial catarrh. Urine and heart normal. Rheumatic attack fifteen years ago. Coughed and raised plenty of sputum. Injection of two thousand units antitoxic serum under left scapula. Prickling sensation in chest and neck, labored breathing, pulse regular and full. Seized with tonic spasm. Died within five minutes after injection.

The serum, that is, another package of the same laboratory number, used in this case was tested for toxicity upon the following series of guinea-pigs:

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
9135	.23 cc. toxine No. 7 + 1/370 cc. antitoxic horse serum (Natl. V. 24).	97	.1 cc. antitoxic horse serum (2295) into brain.	Dead in 20 minutes.
9133	.23 cc. toxine No. 7 + 1/370 cc. antitoxic horse serum (Natl. V. 24).	97	.05 cc. antitoxic horse serum (2295) into brain.	Dead in 22 minutes.
9132	.23 cc. toxine No. 7 + 1/310 cc. antitoxic horse serum (Natl. V. 24).	97	.05 cc. antitoxic horse serum (2295) into brain.	Dead in 11 minutes.
9373	.23 cc. toxine No. 7 + 1/1080 cc. antitoxic horse serum (NY 306).	97	.05 cc. antitoxic horse serum (2295) into brain.	Severe symptoms.
9171	.23 cc. toxine No. 7 + 1/410 cc. antitoxic horse serum (Mul. 2438).	67	1 cc. antitoxic horse serum (2295), subcutaneously.	Dead in 90 minutes.
9164	.23 cc. toxine No. 7 + 1/400 cc. antitoxic horse serum (Mem. C 23).	67	1 cc. antitoxic horse serum (2295), intraperitonally.	Very severe symptoms.

It has interested us very much to find that these two cases, and also others that have come to our notice, were in asthmatics. In our first publication we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man would seem to lend weight to this hypothesis. The knowledge of the fact that the injection of horse serum into some asthmatics is attended with danger must be considered in the use of antitoxine.

In order to determine the comparative toxicity of the above two serums (Nos. 2277 and 2295), we submit the following experiments showing the toxicity of serums which have been largely used in human therapy without untoward effects.

The following serums (Nos. 2364, 2369, and 2442) were kindly sent to us by Dr. A. P. Hitchens, who states that he has had no report whatever concerning any untoward effect resulting from the use of any of these numbers:

No. 2364 is antitoxic serum; No. 2369 is antitoxic globulin; No. 2442 is antitoxic serum.

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
9936	.23 cc. toxine No. 7 + 1/260 cc. antitoxic horse serum (Alex. 192).	47	.05 cc. antitoxic horse serum (Mul. 2442) into brain.	Dead in 4 minutes.
9943	.23 cc. toxine No. 7 + 1/290 cc. antitoxic horse serum (Alex. 192).	47	.05 cc. antitoxic horse serum (Mul. 2442) into brain.	Dead in 5 minutes.
9937	.23 cc. toxine No. 7 + 1/320 cc. antitoxic horse serum (Alex. 192).	47	.1 cc. antitoxic horse serum (Mul. 2442) into brain.	Dead in 3 minutes.
9941	.23 cc. toxine No. 7 + 1/300 cc. antitoxic horse serum (Alex. 192).	47	.05 cc. antitoxic horse serum (Mul. 2369) into brain.	Dead in 8 minutes.
9939	.23 cc. toxine No. 7 + 1/310 cc. antitoxic horse serum (Alex. 192).	47	.05 cc. antitoxic horse serum (Mul. 2369) into brain.	Dead in 5 minutes.
9933	.23 cc. toxine No. 7 + 1/270 cc. antitoxic horse serum (Alex. 192).	47	.1 cc. antitoxic horse serum (Mul. 2369) into brain.	Dead in 3 minutes.
9945 ...	.23 cc. toxine No. 7 + 1/180 cc. antitoxic horse serum (Alex. 192).	47	.05 cc. antitoxic horse serum (Mul. 2364) into brain.	Marked symptoms.
9946	.23 cc. toxine No. 7 + 1/180 cc. antitoxic horse serum (Alex. 192).	47	.05 cc. antitoxic horse serum (Mul. 2364) into brain.	Dead in 12 minutes.
9934	.23 cc. toxine No. 7 + 1/270 cc. antitoxic horse serum (Alex. 192).	47	.1 cc. antitoxic horse serum (Mul. 2364) into brain.	Dead in 3 minutes.
9942	.23 cc. toxine No. 7 + 1/300 cc. antitoxic horse serum (Alex. 192).	47	.1 cc. antitoxic horse serum (Mul. 2364) into brain.	Marked symptoms.

The following diphtheria antitoxic horse serum (123 A) was kindly sent us by Dr. William H. Park, who reports that it is of moderate strength and has given very good results in the hospital:

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
9245.....	.23 cc. toxine No. 7 + 1/480 cc. antitoxic horse serum (Ldrl. 11), subcutaneously.	131	.05 cc. antitoxic horse serum (Park 123 A) into brain.	Very severe symptoms.
9251.....	.23 cc. toxine No. 7 + 1/1000 cc. antitoxic horse serum (Ldrl. 212), subcutaneously.	131	.05 cc. antitoxic horse serum (Park 123 A) into brain.	Very severe symptoms.
9242.....	.23 cc. toxine No. 7 + 1/360 cc. antitoxic horse serum (Mul. 2377), subcutaneously.	131	.05 cc. antitoxic horse serum (Park 123 A) into brain.	Very severe symptoms.
8759.....	.245 cc. toxine No. 42 + 1/320 cc. antitoxic horse serum (Strn. 1429), subcutaneously.	232	.1 cc. antitoxic horse serum (Park 123 A) into brain.	Very severe symptoms.
9253.....	.23 cc. toxine No. 7 + 1/1500 cc antitoxic horse serum (Ldrl. 21 B), subcutaneously.	131	.1 cc. antitoxic horse serum (Park 123 A) into brain.	Dead in 10 minutes.
9239.....	.23 cc. toxine No. 7 + 1/800 cc. antitoxic horse serum (Ldrl. 57), subcutaneously.	131	.2 cc. antitoxic horse serum (Park 123 A) into brain.	Dead in 5 minutes.

The following two serums were kindly furnished us by Dr. E. M. Houghton and were extensively used in human therapy, but no complaints were received concerning them:

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
9635	.23 cc. toxine No. 7 + 1/600 cc. antitoxic horse serum (Alex. A 249), subcutaneously.	87	.05 cc. antitoxic horse serum (PD 08725 C) into the brain.	Very severe symptoms.
9644	.23 cc. toxine No. 7 + 1/680 cc. antitoxic horse serum (Alex. A 249), subcutaneously.	87	.05 cc. antitoxic horse serum (PD 08725 C) into the brain.	Very severe symptoms.
9645	.23 cc. toxine No. 7 + 1/660 cc. antitoxic horse serum (Alex. A 249), subcutaneously.	87	.05 cc. antitoxic horse serum (PD 08725 C) into the brain.	Dead in 4 minutes.
9632	.23 cc. toxine No. 7 + 1/640 cc. antitoxic horse serum (Alex. A 249), subcutaneously.	87	.05 cc. antitoxic horse serum (PD 09043 C) into the brain.	Dead in 8 minutes.
9646	.23 cc. toxine No. 7 + 1/660 cc. antitoxic horse serum (Alex. A 249), subcutaneously.	87	.05 cc. antitoxic horse serum (PD 09043 C) into the brain.	Dead in 4 minutes.
9648	.23 cc. toxine No. 7 + 1/640 cc. antitoxic horse serum (Alex. A 249), subcutaneously.	87	.05 cc. antitoxic horse serum (PD 09043 C) into the brain.	Dead in 15 minutes.

As a control, the following serums — some of them French antidiphtheric and some normal horse serums — are given for comparison:

G.P. No.	First Injection.	Interval in Days.	Second Injection.	Result.
532	.0006 cc tetanus toxine A + .23 cc. antitoxic horse serum (Ehrlich standard).	65	.25 cc. normal horse (roan) serum into the brain.	Dead in 5 minutes.
8497	.24 cc. toxine No. 42 + 1/200 cc. antitoxic horse serum (Cutter 1828).	18	.25 cc. normal horse (roan) serum into the brain.	Very severe symptoms.
8359	.24 cc. toxine No. 9 + 1/1260 cc. antitoxic horse serum (NY 305).	35	.25 cc. normal horse (roan) serum into the brain.	Dead in 5 minutes.
9550	.23 cc. toxine No. 7 + 1/240 cc. antitoxic horse serum (PD 07635).	62	.2 cc. antistreptococcic serum (Past. Inst.) into brain.	Dead in 3 minutes.
9557 ...	.23 cc. toxine No. 7 + 1/280 cc. antitoxic horse serum (Welc. 2 L 839).	62	.2 cc. antistreptococcic serum (Past. Inst.) into brain.	Marked symptoms.
9572	.23 cc. toxine No. 7 + 1/260 cc. antitoxic horse serum (Alex. A 245).	62	.2 cc. antistreptococcic serum (Past. Inst.) into brain.	Dead in 4 minutes.
9580	.23 cc. toxine No. 7 + 1/300 cc. antitoxic horse serum (Alex. A 245).	65	.2 cc. antitoxic horse serum (Lyons) into the brain.	Dead in 3 minutes.
9552	.23 cc. toxine No. 7 + 1/500 cc. antitoxic horse serum (Ldrl. 58 A).	65	.2 cc. antitoxic horse serum (Lyons) into the brain.	Dead in 3 minutes.
9590	.23 cc. toxine No. 7 + 1/320 cc. antitoxic horse serum (Alex. A 245).	65	.2 cc. antitoxic horse serum (Lyons) into the brain.	Dead in 4 minutes.

Lesions. — Gay and Southard,[13] 1907, found, in guinea-pigs dying from a second injection of serum, and in those which had severe symptoms and were later chloroformed, lesions which they interpreted as explaining the mechanism of anaphylaxis. They state that "the study of the histopathology of this serum disease shows us that we have to deal with an intimate cell reaction, demonstrable by definite cell lesions." These investigators state that considerable hemorrhages, rather definitely localized, are the characteristic gross lesions. The hemorrhages may be in one or several organs, gastric hemorrhages being especially frequent. Microscopically there are, in addition to the naked-eye hemorrhages, minute interstitial and oozing hemorrhages. They also claim to have found fatty changes in voluntary muscle fiber, heart muscle fiber, and in nerve fiber.

That the congestion and dilatation of the blood vessels found in the abdominal cavity, and the hemorrhages upon the mucosa of the stomach are not characteristic of death due to anaphylaxis, is evident from the fact that we have found that in violent death produced by large subcutaneous injections of chloral cyanhydrin or hydrocyanic acid, there are somewhat similar congestions and hemorrhages.[14] Further, we have lately had the opportunity to examine a guinea-pig whose death was caused by suffocation in an atmosphere of carbon dioxid. In the stomach and lungs of this guinea-pig lesions were found that, so far as the congestion and hemorrhages are concerned, were similar to those described in guinea-pigs dying from a second injection of horse serum.

We were especially struck by the fact that macroscopic congestions and hemorrhages were frequently absent in guinea-pigs poisoned by a second injection of horse serum given into the brain.

Finally, this congestion and dilatation of the vessels of the abdominal cavity is well known to occur in shock and other states.

We were also unable to confirm Gay and Southard's findings in regard to the fatty changes.

We studied a large number of pigs in which death occurred within thirty minutes of the second injection of serum; also, in a moderate number which were killed by chloroform, or otherwise, from one to four hours after the second injection.

We are indebted to our colleague, Dr. W. W. Miller, U.S. Public Health and Marine-Hospital Service, for the following studies upon the post-mortem appearances and histology of the tissues of guinea-pigs dead of anaphylaxis.

The most noticeable lesion to the naked eye is the marked dilatation of the small veins and capillaries of the body, particularly of the abdominal viscera. Associated with this in about twenty-five per cent. of the cases are hemorrhages in the mucosa of the stomach, and, more rarely, of the intestines — minute hemorrhages one millimeter in size are occasionally observed on the surface of the lungs; no hemorrhages of the heart muscle, spleen, pericardium, or striped muscle as described by Gay and Southard have been seen.

For microscopic study the tissues from sixteen pigs were utilized. These pigs had received the second dose of serum in one of three ways, viz., by subcutaneous, intraperitoneal, and intracranial injection. Material was selected from all the viscera and from the striped muscles and nervous system. Particular attention was given to the study of tissues for the fatty changes described by Gay and Southard; for this purpose sections made with the freezing microtome were used to a great extent, as it is generally recognized that fresh material is superior to that prepared and sectioned in the customary way in celloidin and paraffine, although it must be admitted, as Gay and Southard contend, that such preparations are not as permanent or suitable for photomicrographic purposes. For a general study of microscopic changes tissue was fixed in ten per cent. formalin, in formalin and alcohol (five and eighty-five per cent.), and Zenker.

For fatty degeneration fresh tissue and tissue fixed for twenty-four hours in five to ten per cent. watery solution of formalin was used, and sectioned with a freezing microtome.

For nerve tissue formalin five to ten per cent. Orth's fluid, and Müller's fluid.

As a stain for general purposes, hematoxylin and eosin were used.

For staining fat, Marchi's method was carried out as follows: Fixation in ten per cent. formalin solution twenty-four hours, transferred to Müller's fluid or to formalin and Müller's fluid six to ten days; in Marchi's mixture six to ten days, and kept throughout in the dark; then washed twenty-three hours in running water; hardened in alcohol and ether celloidin as quickly as possible; clove oil celloidin was not used, as it causes general blackening.

For frozen sections of tissue fixed in ten per cent. formalin for twenty-four hours, the admirable method so highly recommended by Schmörl was used, viz., sections placed in Marchi fluid in closed vessels in the paraffin oven for one-half to one hour, washed quickly in water, and mounted in glycerin, or dehydrated over night in alcohol and mounted in balsam.

As controls in this work, tissue was used from normal pigs, from a pig dead of puerperal sepsis with marked fatty changes in liver and kidney, and from pigs killed with diphtheria toxine. Sati's method of osmization was also used with some of the specimens.

Fresh and formalinized tissue was stained by the admirable method of Torrain Smith (Nile blue sulphate); also, Sudan III.

Results. — In sections stained for general study the sole difference from normal tissue consists in the marked dilatation of the veins and capillaries, especially of the stomach and intestines, accompanied by extravasation of blood at points where the vessels are ruptured. The thin-walled veins of the mucosa of the stomach are often greatly distended. In the few instances where a ruptured point in the vessel was seen in section the extravasation of blood was from the portion of the vein nearest the inner surface of the mucosa. The veins of the submucosa participated in the dilatation, but were not so markedly enlarged as the veins and

capillaries of the mucosa. No evidence of general giving way of the capillaries with extravasation of blood into the tissues was noted.

The small veins and capillaries of the intestines, kidney, heart, and muscles were found distended but not nearly to the extent observed in the stomach wall. No hemorrhages were observed in the heart walls or in the liver and striped muscles.

As regards fatty changes in the lining endothelium of the blood vessels, in the gastric mucosa or the striped muscles, none was observed, although carefully sought for. The focal fatty changes described by Gay and Southard were not found. Neither were the "nodal" changes in peripheral nerves made out.

We find, then, that congestion and sometimes hemorrhage takes place in guinea-pigs dead of anaphylaxis, but these lesions are not specific. We were unable to demonstrate the fatty lesions and know further that they occur in other states. We are, therefore, unable to confirm the observations of Gay and Southard along these lines and believe that these changes do not explain the mechanism of anaphylaxis.

The relation of anaphylaxis to the toxemias of pregnancy. — The symptoms which cause puerperal eclampsia and the conditions under which it occurs suggest that anaphylaxis may explain some of the mystery of this state.

It occurred to us that either the blood or protein substances in solution from the fetus or the placenta may first sensitize the mother. A subsequent introduction into the system of the mother of a similar substance may explain the convulsions and the symptoms which occur in a certain class of the toxemias of pregnancy.

"Through the establishment of the pathological anatomy of the condition a general agreement has been reached that puerperal eclampsia must be included among the diseases caused by toxic materials of unknown origin and nature." [15] A certain class of the toxemias of pregnancy are sometimes spoken of as of reflex or neurotic origin.

There seems to be a fair agreement that the placenta must be the source of toxic material, especially as typical cases of eclampsia and pernicious vomiting have been observed in patients with hydatid mole in which cases, of course, toxic matter of fetal origin could be eliminated. Furthermore, eclampsia may appear after the fetus has been removed. Much attention was therefore given to the hypothesis elaborated about four years ago by Veit, Weichardt, and others that, through the entrance of placental cells into the circulation of the mother, an intoxication was caused either by disintegration of the cells and the formation of toxic substances or in the development of anti-substances by the maternal organism.

In spite of much experimentation and discussion, however, no satisfactory conclusions have yet been reached concerning the validity of this hypothesis and Martin has secured some very valuable evidence that, at least in rabbits, entrance of their own placental elements into the circulation in large amounts does not cause any serious disturbance. So far as we are aware we are the first to suggest that certain of the toxemias of pregnancy may be a condition of hypersusceptibility.

Along these lines we first made a number of experiments to determine whether the fetal blood of the guinea-pig could sensitize the mother guinea-pig. We injected a number of female guinea-pigs, both pregnant and not pregnant, with fetal blood and, after an appropriate interval, gave them a second injection of the same material. All these experiments resulted negatively, which was anticipated from our previous studies upon the effect of homologous blood serums. This further confirms the clinical observations that the poisons causing the toxemias of pregnancy do not come from the fetus.

We then made a series of experiments upon female guinea-pigs with placental extracts. The placenta (almost at full term) was ground up and allowed to "autolyze" about an hour at room temperature and some of the resulting extract was injected subcutaneously into female guinea-pigs.

After an interval of twenty-two days the guinea-pigs were again inoculated with a placental extract. This time the placenta was allowed to " autolyze " three hours in the incubator (37° C.). Five pigs were tested with this placental extract; three of them were given six cubic centimeters into the peritoneum, two of these three showing pronounced symptoms of anaphylaxis. The remaining one showed slight symptoms. Six cubic centimeters of the same placenta extract, injected into the peritoneal cavity of two young normal guinea-pigs as a control produced no apparent effect. The remaining pigs were injected with small quantities of the extract intracerebrally, with negative results.

From this limited series it is evident that the mother guinea-pig may be sensitized with the autolytic products of her own placenta. These experiments naturally suggest that there may be a certain relation between some cases of puerperal eclampsia and the phenomenon in the guinea-pig which we are studying. Further studies along this line are now being made.

SUMMARY AND CONCLUSIONS.

The period of incubation of serum anaphylaxis is about seven days in guinea-pigs sensitized in the brain and about nine days in guinea-pigs sensitized subcutaneously. It also appears that the sensitization comes on somewhat gradually.

Judged by our results and the work of others, the period of incubation is quite constant.

It seems that the period of incubation is not appreciably prolonged by a large sensitizing dose.

Animals sensitized with horse serum alone remain so for a long period of time. Guinea-pigs sensitized with the toxine-antitoxine mixture remain sensitive throughout the remainder of their life (at least seven hundred and thirty-two days).

The sensitizing principle is gradually influenced by heat. It disappears almost entirely when horse serum is heated to 100° C. for one hour.

Guinea-pigs may be sensitized by intracerebral injections, provided quantities of .0001 cubic centimeter or more are

used. We obtained negative results with sensitizing doses of .00001 cubic centimeter into the brain.

Guinea-pigs may be sensitized by dropping horse serum upon the eye.

The toxic principle in horse serum is gradually destroyed by heat.

A temperature of 70° C. for one hour does not seem appreciably to diminish the poisonous property of horse serum, but it seems to be affected at 80° C. for one hour. At 90° C. for one hour it still remains slightly toxic, but at 100° C. for one hour the toxicity apparently disappears.

The difference in the effect of heat upon the sensitizing and the toxic principle may be more apparent than real, for exceedingly minute amounts of serum will sensitize guinea-pigs, while it would take a very large quantity of weakened serum to produce symptoms at the second injection.

The toxicity of horse serum does not appear to diminish with the age of the serum.

No influence upon the anaphylactic state was obtained by injecting pancreatin, potassium oxalate, pepsin, sodium sulphate, magnesium sulphate, peptone, calcium chloride, and calcium acetate into guinea-pigs the day before they were tested.

Iodine also apparently had no modifying effect upon serum anaphylaxis, whether dissolved in the serum or injected separately into the guinea-pig.

Methemoglobin-producing substances, such as the nitrites, do not modify anaphylaxis.

Ether narcosis masks the symptoms, but does not prevent the fatal issue of a second injection.

Further attempts to find free antibodies to neutralize the toxic action of horse serum by treating it with the sensitized guinea-pig serum, and also with the brain substance of sensitized guinea-pigs, proved negative.

The specific nature of anaphylaxis is further shown by various experiments. For example, guinea-pigs sensitized with three separate proteins, viz., horse serum, egg-white,

and cow's milk, contain three separate anaphylactins in their blood.

Guinea-pigs sensitized with human milk do not react to a second injection of cow's milk. This indicates, again, not only the specific nature of the anaphylactic reaction, but shows differences between the protein matter of human and cow's milk.

Guinea-pigs sensitized with sheep's milk react to a subsequent injection of cow's milk.

Guinea-pigs sensitized with dog's milk do not react to a subsequent injection of cow's milk.

Guinea-pigs sensitized with hen egg-white react to a subsequent injection of duck egg-white; and guinea-pigs sensitized with duck egg-white react to a subsequent injection of hen egg-white.

The anaphylactic reaction in the guinea-pig, therefore, seems to be specific in the sense that the precipitins are specific. That is, there is a group reaction in the proteins of allied species, but no reaction between the proteins of widely different species, or between proteins of widely different origin.

A substance known as "anaphylactin" is present in the blood serum of sensitized guinea-pigs. This substance is not present during the period of incubation.

We have been unable to demonstrate the presence of anaphylactin in the blood serum of man, the monkey, and the cat.

Anaphylactin is present in the blood serum of immune guinea-pigs.

The mechanism of anaphylaxis. — We find that congestion and sometimes hemorrhages may be present in guinea-pigs dead of anaphylaxis, but these lesions are not always apparent, and, furthermore, are not specific.

We were unable to demonstrate fatty lesions in guinea-pigs dead of anaphylaxis, and know, further, they occur in other states.

We believe that these morphological alterations do not

explain the mechanism of anaphylaxis. It is probable that the mechanism will not be unraveled until the chemistry of the metabolism of proteins is discovered.

Cases of sudden death in man. — Our experiments demonstrate that the horse serum used in cases followed by sudden death is no more toxic for guinea-pigs than antitoxic horse serums used extensively in human therapy without untoward symptoms.

It is our belief that it is not the special toxicity of the horse serum, but the sensitization of the patient which accounts for the collapse or sudden death sometimes following the injection of horse serum.

We are still unable to account for the ways in which man may be sensitized to a foreign protein. It seems perfectly plain, however, that man may be so sensitized.

In previous publications we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man seems to lend some weight to this hypothesis.

The knowledge of the fact that an injection of horse serum into some asthmatics is attended with danger must be considered in the use of antitoxine.

The repeated injections of small amounts sensitizes guinea-pigs.

Repeated injections of large amounts render guinea-pigs partially immune.

Repeated injections of small amounts of serum into sensitized guinea-pigs has no appreciable effect.

Sensitized guinea-pigs cannot be immunized by repeated injections of heated serum (100° C. for one hour).

We suggest a possible relation between the toxemias of pregnancy and anaphylaxis.

Guinea-pigs cannot be sensitized with guinea-pig fetal

blood. This shows that the fetal blood of the guinea-pig does not contain an alien protein for the mother.

Guinea-pigs may be sensitized and subsequently poisoned with guinea-pig placental extracts.

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EPIDERMAL FIBRILS IN THE CLASSIFICATION OF MALIGNANT GROWTHS.*

GUTHRIE MCCONNELL, M.D.
(*From the Laboratory of the St. Louis Skin and Cancer Hospital.*)

The study of the various forms of fibrils found in connection with the different cells of the body has been one to which much time has been devoted. Probably the earliest investigations in this respect were those undertaken by Schroen and Schultze in regard to the epithelial cells of the epidermis and the fibrillar structures associated with them. Later on, Bizzozero called attention to the small nodes present between the epidermal cells. These were also described some years later by Ranvier.

In addition to the intercellular bridges and nodes described by the above and other observers, Herxheimer and Kromayer reported the finding of long fibrils occurring in the basal layer of squamous epithelium and running parallel to the long axis of the cells. Much discussion was provoked as to the origin of these fibrils and as to whether or not they were individual bodies or part of a generalized system.

Numerous articles have been written about the various forms of epithelial fibrils as occurring in the skin of man and the lower animals, but little work seems to have been carried on in regard to their presence in various forms of neoplasms. Wolbach[1] reports a case of squamous epithelioma of the bladder in the cells of which he found well marked fibrils. Thompson[2] gives a picture of a papilloma of the skin in which there has been a very marked increase in the formation of fibrils. He also shows a representation of the fibrils in the skin adjacent to the edge of a rapidly-growing carcinoma of the lip, and in the text states that "This process of fiber production by the cells of the human epidermis . . . may be of importance in identifying or classifying new growths of epithelial origin."

The following paper deals with the results of the examination of one hundred growths from different parts of the

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body. The specimens, with one exception, were obtained from operations, fixed in Zenker's fluid, and stained with Mallory's phosphotungstic acid hematoxylin.

In carrying on this investigation the primary object was to determine whether or not the presence or absence of both "prickles" and fibrils could be used for the purpose of differentiating epithelial from mesoblastic tumors. Also to determine whether either fibrils or "prickles" would be found only in those epithelial growths derived from squamous cells, or whether, like the fibroglia and neuroglia fibrils, they would be present in all epithelial tissues.

In the course of the examinations certain points of interest not bearing directly upon the above two questions but dealing with epidermal fibrils in general were noted and will be mentioned later.

To seek the answer to the first question as to the value of epidermal fibrils in the classification of tumors, sixty-eight growths from the skin and squamous-celled mucous surfaces were taken. Of these forty-six were squamous epitheliomata and twenty-two of the rodent ulcer type. Twenty-six epithelial growths from other parts of the body, breast, axillary nodes, uterus, etc., and six of mesoblastic type were also examined.

The first variety of growth to be described is the typical squamous-celled epithelioma. In the examination of the normal skin surfaces overlying the neoplastic tissue there was noticed a constant feature concerning the development of the intracellular fibrillæ and the prickles. It was seen that these elements were least conspicuous in and between the basal cells, the stratum germinativum. As the succeeding layers of cells were examined, the intracellular fibrils and prickles became more and more prominent till the cells showing advanced cornification were reached. If there were edema of the skin present, the prickles were then most marked.

In those growths in which there were distinct nest formations, the same conditions held good. The epidermal structures were least well marked in the peripheral growing cells, while, on the other hand, they were most prominent toward

the center of the nest, particularly if keratin degeneration with the production of an epithelial pearl had occurred. There was also a difference in the prominence of the structures, according as to whether the growth was of a diffuse character or more restricted in form with the production of distinct cell nests. In the diffuse form the intracellular fibrillæ and the prickles were much less conspicuous, and in a few cases were quite indistinct and difficult to find, but never entirely absent.

When those growths conforming to the type of the rodent ulcer were examined, a very different condition existed. In none of the twenty-two cases were the slightest traces of fibrils or prickles found. From the examinations made of the basal cells of the skin and of the nests in the squamous epitheliomata, this was not expected. As was seen in those instances, there was very little fibril formation amongst these cells. Thompson,[3] in his conclusions, says that "In certain animals, in addition to the protoplasmic fibrils of the stratum filamentosum that are present normally in human epidermis, there are well developed fibrils in the cells of the stratum germinativum. These fibrils are present in the human epidermis only under conditions of increased cell activity. These fibrils, that are prominent in the stratus germinativum of human epidermis under conditions of increased cell activity, seem to increase in direct ratio to rapidity of cell production."

This seems to hold true as long as comparatively normal processes are concerned, but it does not appear to be the case when the increased cell activity is of a malignant type. In none of the specimens of malignant epithelial growths from squamous-celled surfaces was there found any increase in the formation of the fibrils of the stratum germinativum.

The impression obtained from this work was that the protoplasmic fibrils and prickles were found most markedly developed in those cells that had passed middle age and were starting on the downward path of degeneration. Also that they are least conspicuous in the stratum germinativum where the cells are young and vigorous.

As the rodent ulcer is generally believed to be derived

from the basal cells, one would naturally expect from the above findings that the fibrils and prickles would be of a very imperfect type, if present at all.

The absence of these bodies might be considered by some as an indication that the rodent ulcer is not epithelial but endothelial in origin. This conclusion, however, would not be justified, as neither fibrils nor prickles were found in any of the epithelial growths other than those arising from squamous-celled surfaces.

These epidermal bodies, however, do appear to be of some importance in regard to the classification of epithelial growths from the skin, and possibly in the prognosis of a given case. The above discussed structures are found only in the squamous epitheliomata. Although the rodent ulcer or carcinoma baso-cellulare is as a rule not very difficult to recognize, there are cases in which the distinction is hard to make, and in which the ultimate diagnosis would rest upon the presence or absence of the epidermal fibrillæ.

There are also certain skin growths that belong more accurately amongst the true carcinoma. Five specimens have been examined that give the appearance of a diffuse squamous epithelioma in which there has been no keratin degeneration. There is no resemblance to rodent ulcers. The original diagnosis in these cases was that of squamous epithelioma, although the growth was not typical. On account of the absence of epidermal fibrillæ and the lack of resemblance to rodent ulcer, it would seem allowable to consider these five neoplasms as having arisen from the truly glandular epithelium of the skin appendages.

Excluding the five above mentioned skin carcinomata, twenty growths from various parts of the body were examined, but in no instance were any fibrils or prickles found. In addition to the others one fibro-adenoma of the breast and six mesoblastic tumors were investigated with similarly negative results.

It would seem quite evident that these epidermal structures are peculiar to the cells of the squamous surfaces, and that they are not constituents of epithelial cells in general.

In conclusion, it would seem that the presence of epidermal fibrils as a method of diagnosing or classifying epithelial growths is of very limited value. These structures were not found in any growths excepting those derived from surfaces covered by squamous epithelium, such as the skin, lips, etc., so they could not be used to differentiate epithelial tumors in general from those of mesoblastic origin.

The one point of importance is in the classification of tumors arising from the epithelium of the skin and its appendages.

LIST OF SPECIMENS EXAMINED.

Squamous Epitheliomata.		Fibrillæ very distinct.	Rodent Ulcer.		Carcinoma.		Sarcoma.	
Cervix,	5	2	Cheek,	7	Axilla,	6	Face (primary),	1
Cheek,	1	..	Chin,	1	Breast,	7	Face (recurrent),	1
Chin,	1	1	Eyelid,	1	Cheek (muc. mem.),	1	Naso-pharynx (primary),	1
Face,	7	4	Face,	7	Face,	1	Naso-pharynx (recurrent),	2
Finger,	1	1	Forehead,	1	Femur (secondary),	1	Ovary,	1
Forehead,	2	2	Neck,	1	Neck (primary),	1	Total,	6
Groin,	1	1	Nose,	2	Neck (recurrent),	1		
Hand,	4	4	Orbit,	1	Rectum (adeno-car.),	1		
Hard palate,	3	3	Scalp,	1	Stomach (adeno-car.),	1	Breast (adeno-fibroma),	1
Leg,	2	2	Total,	22	Uterus (adeno-car.),	1		
Lip,	7	5			Uterus,	3		
Mouth,	4	4			Temple,	1		
Neck,	4	2			Total,	25		
Node (inguinal),	1	1						
Nose,	1	1						
Skin,	1	..						
Tongue,	1	1						
Totals,	46	34						

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APPARATUS OF SERVICE IN EXPERIMENTAL PATHOLOGY.

FREDERICK P. GAY.
(Assistant in Pathology Harvard University Medical School.)

I. AN AUTOPSY BOARD FOR ANIMALS. — This form of autopsy board, which is depicted in Fig. 1, has been used for the past six years, and is the most time-saving and simple apparatus with which I am acquainted for holding a dead animal in a fixed position for post-mortem examination. The dimensions as given are suitable for holding guinea-pigs of any size and were so arranged that the feet of small guinea-pigs are just caught by the spring clamps, while larger animals are even more firmly held by pushing the legs still farther under the opened clamps. Larger-sized boards would be useful for larger laboratory animals. The mechanism is obvious from the photographs, the two pairs of clamps being forced open by the forefingers, in turn at head and tail end, while with the thumbs the upper and lower extremities are slipped under the opened clamps. A similar operation is sufficient to release the animal, the result being almost instantaneously accomplished.

Dimensions of a board suitable for guinea-pigs: Length of board, fourteen inches; width, 8.25 inches; height outside including supports, four inches; depth of central depression below sides, 1.75 inches; width of clamps, 1.25 inches; length between centers of clamps on one side, eight inches; width between clamps on opposite sides, 5.25 inches; length of clamps between spring base (outside) and flattened top (inside) two inches. The clamps are made of three-sixteenth-inch brass wire, and the sides of the board from seven-eighth-inch material. The board is painted with asphaltum black, which is suitable to withstand the affect of chloroform and alcohol.

II. A SELF-REGULATING WATER BATH MAINTAINING A CONSTANT TEMPERATURE (56° C.) (Fig. 2). — This water bath

was devised, largely on suggestions from Dr. L. J. Henderson, for the purpose of serum work, in many of the procedures of which it is necessary to heat liquids of 56° C. for a longer or shorter period of time (destruction of alexin, or complement). Dealers in this country had nothing in stock fulfilling the requirements, and several attempts by them to produce thermo-regulating apparatus at this temperature were not very satisfactory. The apparatus was modified from the ordinary "Physicians Incubator" (Bausch and Lomb), although a special cylinder could doubtless be built which would be more satisfactory in some respects. This incubator contains a double chamber, the outer one connected with a gauge on the one side; two openings occur on the top which also lead to this chamber. The inner chamber is capped by a removable cover which admits a thermometer; this chamber is filled with water of which it holds three or four gallons. The outer gauge which was originally open at the top was replaced by a tube leading back into the chamber through one of the apertures on the top of the apparatus. Into one of the other apertures was fitted a cock supporting a double ball reflux condenser which offers a surface cooled by running water within and without the central chamber which connects through the stem of the condenser with the outer cylinder chamber. This outer chamber is filled with acetone until a point at least an inch high on the gauge is reached. The boiling point of acetone is from 56.2°–56.3° C. and boiling acetone in the outer chamber will rapidly cause a temperature in the water bath of 56° C. to be reached, beyond which no further rise is possible. The reflux condenser cools and liquefies the vaporized acetone which falls back into the chamber and is used over and over again; in several months of use at intervals it has not been found necessary to replenish the acetone. As an additional precaution against the dilution of the acetone with water, and a subsequent change in the boiling point, the air from the outlet of the condenser is passed through a small calcium chloride bulb.

The entire apparatus is heated by a Simplex electric stove. Within a few hours after turning on the current the water in

the bath has reached and becomes fixed at a constant temperature of 56° C., which is maintained at this point as long as desired. Not only does the temperature remain constant by the necessity of the fixed boiling point of the liquid in the outer chamber, but the rapidity with which such a constant temperature may be attained is worthy of note.

Temperatures other than 56° C. may be obtained by utilizing simple liquids or a mixture of liquids having other boiling points.

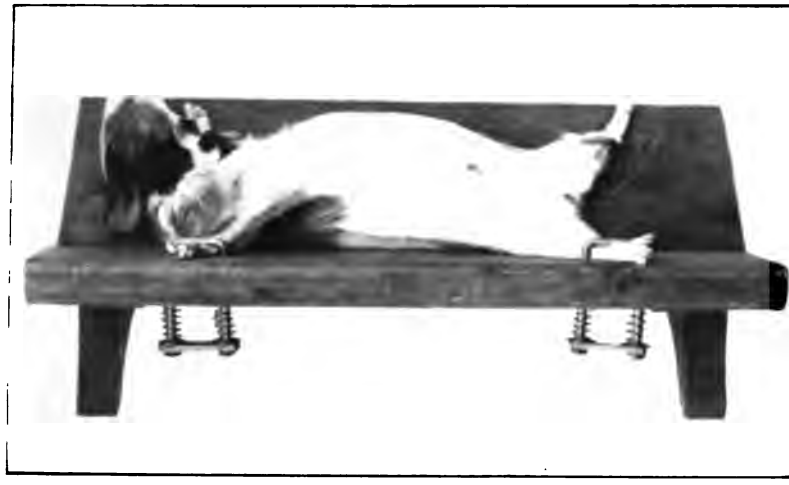

FIG. 1. — Autopsy Board for Guinea-pigs.

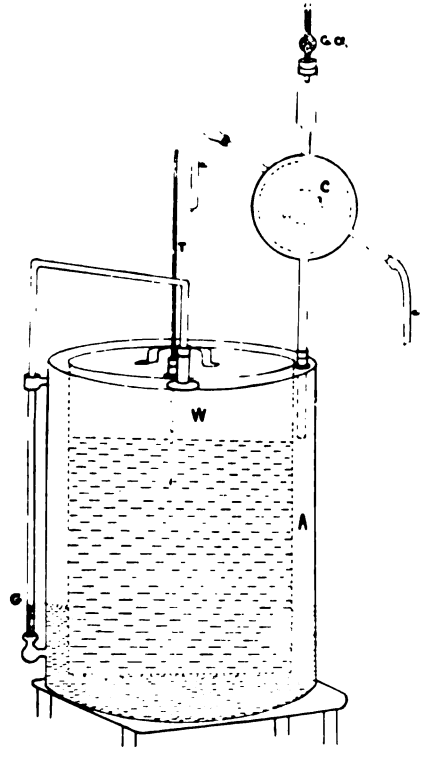

FIG. 2. — W = inner chamber containing water; A = outer chamber containing acetone; G = gauge; T = thermometer; C = central chamber of double ball reflux condenser; a-a = current of water to cool condenser.

STATISTICS OF CONGENITAL CARDIAC DISEASE.

(*400 Cases Analyzed.*)*

MAUDE E. ABBOTT, B.A., M.D.
(*Governors' Fellow in Pathology McGill University.*)

The subject of congenital cardiac disease is one that lends itself well to statistical study, for the conditions, being often complex and of recognized rarity, are usually reported in much detail. Moreover, the cases are so infrequent in any one person's experience that some such method as this, of making use of the available literature, must be adopted in order to arrive at any generalizations.

For another purpose, I have had occasion to make a detailed statistical study of some four hundred and twelve cardiac defects. A few of these are drawn from personal experience, the remainder from the literature. Only well-authenticated cases with post-mortem report attached have been included. The only exception to this statement is formed by three cases included in the series of patent ductus arteriosus diagnosed by characteristic physical signs and by the X-Rays and not confirmed by post-mortem. The results of the analysis of four hundred of these cases are shown in the accompanying chart. This chart is presented here merely as a demonstration of the manner in which these defects were studied, and without any intention of entering at length into the figures. It represents a chart which was originally printed for the analysis of the individual defect, and is here modified in a few particulars to admit of the presentation of the total results obtained.

The chart presents four main divisions. The First Division includes the Classification of the defect, Number of cases analyzed, Age, and Sex. In the classification a simple anatomical order has been followed, based also on the

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principles of the development of the heart, so far as these are known. Thus, defects of the cardiac and aortic septa are followed by transposition of the arterial trunks, due (according to Rokitansky) to a deviation of the aortic septum; and this again by pulmonary and aortic stenosis or atresia (some cases of which are probably likewise due to a deviation of the aortic septum). The cases of pulmonary and aortic stenosis or atresia are sub-classified (following Rauchfuss) according to the presence or absence of defects of the interauricular and interventricular septa, and this affords a clinical grouping of much value. In coarctation of the aorta the distinction drawn by Bonnet* is observed between the infantile form, a simple persistence of the isthmus aortæ, and the typical "adult type" of coarctation in which the aorta is obstructed or even obliterated by a sharp constriction at or above the insertion of the ductus.

Among the defects enumerated in this classification those of clinical importance are:

Defects of the interauricular septum, 28 cases; defects of the interventricular septum, 40 cases; complete defects of the cardiac septa (biloculate heart, etc.), 12 cases; defects of the aortic septum, 14 cases; transposition of the arterial trunks, 44 cases; pulmonary stenosis, 75 cases; pulmonary atresia, 23 cases; tricuspid stenosis, 2 cases; tricuspid atresia, 9 cases; patent ductus arteriosus, 23 cases; coarctation of the aorta, 33 cases; hypoplasia of the aorta, 2 cases. Under "Age" are three columns in which the maximum, minimum, and mean ages of the cases in each group are calculated.

The Second Division of the chart includes those Post-mortem Findings of especial importance in cardiac defects. Here are noted the condition of the fetal passages, whether closed or patent, the presence of hypoplasia or dilatation of the pulmonary artery or the aorta, the existence of a collateral circulation (important in coarctation of the aorta), the incidence of arterial disease, of acute endocarditis, and of

* Bonnet. Revue de Médécine, 1903.

chronic valvular disease, the presence of hypertrophy and dilatation of the different chambers of the heart, and lastly the existence of associated anomalies in the heart, vessels, or elsewhere.

The Third Division notes points of clinical interest, such as the presence of conditions having an etiological bearing on the family history, and in the personal history the incidence of rheumatism, pulmonary tuberculosis, or congenital syphilis, and the proportion of cases recovering from the acute infectious fevers (which cyanotic patients are said to pass through well). Under special symptoms are columns for cyanosis in its different degrees, clubbing of the fingers, dyspnea, dyspneic attacks and delayed development. Physical signs may be vascular or cardiac, and among the latter the occurrence of visible pulsation, precordial bulging, thrill, increased dulness, accentuation of the heart sounds, and the existence of murmurs, presystolic, systolic, diastolic, continuous, double (*i.e.*, systolic and disastolic in rhythm), or indefinitely stated, are noted. Finally, under causes of death we find the defect itself proving fatal suddenly or by failing compensation, or a termination by broncho-pneumonia, cerebral complications or the acute infectious fevers.

The Fourth Division of the chart, that of Relative Frequency, is of the greatest importance. Cardiac anomalies are so often complicated that the number of times a given defect occurs alone or as the primary condition by no means represents its total incidence in the four hundred cases. In this division there are, therefore, three columns. In the first of these stands "the number of cases classified as the primary lesion," the figures of which are identical with those at the beginning of the chart showing "the number of cases analyzed" in each group. The sum of the figures in this column is the four hundred cases analyzed. In the next column stands the number of cases in each group in which the defect occurs complicating other conditions, and this with the number of cases classified as the primary lesion

gives the total incidence of the defect, which is thus sh in the last column of the chart.

The result of this analysis brings out some remark facts, several of which are at variance with accepted id The following points are of especial interest:

1. The frequency of defects of the interventric septum. — While relatively rare alone (thirty-two defect the base among the four hundred), in combination other conditions this is seen to be the most common o cardiac anomalies (one hundred and forty-nine among four hundred cases); next in frequency comes patent f men ovale, under which are included only cases of patency, not simply a valvular or slit-like condition, with hundred and thirty-four cases, and then patent ductus a riosus with one hundred and six. The frequency of tra position of the arterial trunks (forty-six cases) and pulmonary stenosis with defect of the interventricular s tum (seventy-three cases) is noteworthy, while pulmona stenosis with closed interventricular septum is relativ infrequent (seventeen cases).

2. The duration of life is seen to be relatively long uncomplicated defects of the interauricular septum, pat ductus arteriosus, coarctation of the aorta, and pulmona stenosis with closed interventricular septum. In pulmona stenosis with defect of this septum the duration of life seen to be much shorter.

3. Patency of the ductus arteriosus is seen to be rare pulmonary stenosis, though very frequent in pulmonar atresia.

4. The right chambers chiefly are hypertrophied an dilated in defects of the interauricular septum, transpositio of the arterial trunks, pulmonary stenosis and atresia. Bot chambers, but chiefly the right, are enlarged in defects o the interventricular septum and patent ductus arteriosus, th left ventricle chiefly in coarctation of the aorta.

5. Acute endocarditis is seen to be relatively common i

NALYZED)

Murmurs: 2nd +	Presystolic	Systolic	Diastolic	Double	Continuous	Indefinite	Causes of Death: The Defect: Sudden	Failing Compensation	Broncho-pneumon	Cerebral Disease	Infectious Diseases	Relative Frequency: No classified as Primary Lesion	No complicating other defects	Total Incidence
												4	–	4
								4				4	–	4
		1					1	1				5	2	7
		1										3	2	5
		1							1			2	–	2
		1					1	1				3	–	3
	1											5	2	7
												2	4	6
	1											2	3	5
		3		2				1	1			10	124	134
		2									1	5	2	7
2		5						2	1			10	4	14
		1						1				3	15	18
		19		4			3	4	8		5	32	117	149
				1								3	9	12
				2							1	5	2	7
							1	2				3		3
							2					1	4	5
				2			2					4	4	8
							1	1			1	4	2	6
		1	1			1	1	2	2			8	4	12
		2		2				5				6		6
1		8					1 3	1	2	1		27		27
							1 1	1		1	2	13	2	15
2		1		1			1	3	1			4		4
		5	2				2			1	1	7		7
1		6		1				2			2	9	1	10
2		17		4			9	4		7	5	37	5	42
1		12			1		6	3		2	3	22	9	31
1			2				1		1			6	2	8
			3		2			1	1	2		7		7
1			3				3	4				10	4	14
													76	76
		2	1					2	2		1	5	2	7
		2		1				3	2			5	1	6
		1								1		3		3
			2				1	2	1	1	1	10	3	13
		1	3	1			3	3			1	14	45	59
	1	1	1					1			1	2	5	7
		3										7	5	12
	1	1					1					1	6	7
		1						1				2	1	3
												4		4
		2										4	17	21
		6			4	5	1	3		1		19	87	106
		1									1	4	–	4
		1					1				1	5	25	30
3	1	8		2			4	12		1		28	9	37
2								2				2	20	22
												7	26	33
												3	9	12
		1										6	34	40
												3	2	5

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defects of the interventricular septum at the base and in pulmonary stenosis.

6. Cyanosis was absent in most of the defects of the interauricular septum and was not "marked" in any of these cases. A moderate degree of cyanosis was fairly common in defects of the interventricular septum, a marked degree in only three cases. Marked cyanosis was seen chiefly in transposition of the arterial trunks, pulmonary stenosis with defect of the interventricular septum, pulmonary and tricuspid atresia. Cyanosis was usually slight or absent in patent ductus arteriosus and in coarctation of the aorta of the adult type. In six cases of defect of the interauricular and in four of defect of the interventricular septum, the cyanosis was "terminal," appearing only in the last few weeks of life.

7. A thrill was frequent in "pure" defects of the interventricular septum at the base, and in pulmonary stenosis with closed interventricular septum, or with defect of the interventricular septum and patent foramen ovale. A thrill was relatively rare in pulmonary stenosis with defect of the interventricular septum and closed foramen ovale.

8. In the great majority of cardiac defects the murmur, when present, was systolic in rhythm.

9. In some cases of pulmonary stenosis the pulmonary second sound was accentuated.

These are not all the conclusions to be drawn from a study of this analytical table; they are sufficient, however, to show the value of a careful and detailed tabulation of the data afforded by different observers in arriving at general deductions, such as could not legitimately be drawn from the facts in the experience of any single worker.

A STUDY OF THE EOSINOPHILIC CELL AS OCCURRING IN THE HEMATOPOIETIC ORGANS IN DIPHTHERIA AND TUBERCULOSIS.*

GEORGE BURGESS FOSTER, M.D.

(Interne, Philadelphia General Hospital. Formerly Research Worker in the Laboratories of the Municipal Hospital for Contagious Diseases, Philadelphia; and Resident Pathologist, Philadelphia General Hospital.)

(From the Laboratories of the Municipal Hospital for Contagious Diseases, Philadelphia.)

In reviewing the literature on eosinophilia, the earliest reference found is by Wharton Jones, in 1846. He states, in his essay entitled "The Blood Corpuscle considered in its Different Phases of Development," that finely and coarsely granular leucocytes occur in the blood of man and most animals.

Subsequent writers — Rindfleish in 1863, Preyer, Förster, and others — described cells that were probably eosinophiles, but it may be stated with propriety that the existence of the eosinophile was not definitely shown until the publication of the article of Max Schultze in 1865. In 1868 Bizzozero described elements in the red bone marrow which "resemble white blood cells, and which contain accumulations of fine fat granules in a portion of their (otherwise homogeneous) protoplasm." Neumann, Ponfick, Mosler, Henck, and Bieseadecki also published articles dealing with these cells in various conditions. In 1892 Neusser and his followers claimed that eosinophilia is due to stimulation of the sympathetic system, but their findings were rather too radical to be thought of seriously by recent investigators.

Zappert's classical paper "On the Occurrence of Eosinophilic Cells in Human Blood" was published in 1893, and has added much to our knowledge of the subject.

Among the more recent contributors to literature on the subject of eosinophilia, Opie, of Baltimore, whose three

*A portion of the thesis receiving the Pathology Prize, Jefferson Medical College, 1907. Read before the Pathological Society, Philadelphia, Feb. 27, 1908. Received for publication March 27, 1908.

masterly articles have shed so much light on the problem, should be mentioned especially.

Simon, in "A Contribution to the Study of Eosinophilia," has enlightened investigators along this line, especially as to the eosinophilia associated with trichiniasis. The German literature of recent date has presented us with the work of Stschastnye. The vast majority of the investigations on the subject have dealt with the eosinophilic cell as it occurs in the circulating blood. In fact, in gleaning the writings of others for information as to the occurrence of these cells in the hematopoietic organs in fatal cases of diphtheria, the writer was able to find but one reference — the work of Mallory, Councilman and Pearce — on the pathology of diphtheria. In this paper they assert that in their examinations of the thymus gland, "brightly staining eosinophile cells were very numerous in all cases," and that in the lymph nodes "a few eosinophiles are always found, and in some cases considerable numbers of them."

In June, 1906, while examining, histologically, the various tissues obtained post-mortem from cases of contagious disease in the Municipal Hospital, Philadelphia, the writer's interest was aroused by the enormous number of eosinophilic cells seen in the thymus gland of a patient dead of diphtheria. From this time on, the thymus gland, spleen, and various lymph nodes were examined microscopically, to ascertain, if possible, the significance of the eosinophilia — whether it occurred in diseases other than diphtheria, from whence the cells came, at what time they appeared during the process, their role, and their ultimate end.

With this object in view, the investigation, which is recorded in the following pages, was begun. Autopsies were performed on forty-two cases. The variety of conditions met with was as follows:

Diphtheria, uncomplicated, 14; diphtheria and measles, 3; diphtheria and tuberculosis, 2; diphtheria and scarlatina, 2; diphtheria and ileocolitis, 1; diphtheria and enteric fever, 1; diphtheria, scarlatina, and measles, 1; scarlatina,

uncomplicated, 6; scarlatina and rubella, 1; scarlatina and tuberculosis, 1; epidemic cerebrospinal meningitis, 3; tuberculous meningitis, 1; anthrax, 2; typhus fever, 1; dermatitis exfoliativa and tuberculosis, 1; cases of doubtful diagnosis, 2.

The tissues were in an excellent state of preservation. The subjects varied in age from seven weeks to fifty-seven years, as indicated below:

TABLE OF AGES.

Cases.		Per cent.
4	under 1 year	9.5
5	1– 2 years	11.9
4	2– 3 "	9.5
6	3– 4 "	14.2
3	4– 5 "	7.1
1	5– 6 "	2.4
2	6– 7 "	4.8
3	7– 8 "	7.1
2	8– 9 "	4.8
4	9–10 "	9.5
1	10–15 "	2.4
2	15–20 "	4.8
1	20–30 "	2.4
4	30–60 "	9.5

Methods. — Small pieces of spleen, thymus gland, and palpable lymph nodes were taken and placed immediately in Zenker's fluid, in which they were left for twenty-four hours. They were then thoroughly washed in running water for twenty-four hours; after this they were taken through seventy per cent, eighty per cent, ninety-five per cent, and absolute alcohols, remaining in each twenty-four hours. Cedar oil and absolute alcohol, equal parts, followed by pure cedar oil, were used for clearing. The tissues were then infiltrated in three paraffins and blocked. Sections were cut, as a rule, at 3μ, although in a few cases, especially in tuberculous glands, it was necessary to cut them at 5μ. They were then stained in five per cent aqueous solution of eosin for one hour, washed in water, and placed in Unna's polychrome methylene blue (diluted 1-20) over night,

usually about eighteen hours. The sections were differentiated in glycerin-ether mixture (Grübler), (diluted 1–15), until the blue ceased to come away. They were then washed in water, dehydrated in absolute alcohol, cleared in xylol, and mounted in xylol-balsam. In sections where there was the least suspicion of tuberculosis, sections were stained by approved methods to demonstrate the tubercle bacillus. All sections were studied under the one-twelfth-inch oil immersion lens, and the number of eosinophilic cells noted in a hundred consecutive fields recorded. The total number of cells was then divided by the number of fields examined, giving a factor which, for convenience, will be called the eosinophilic index. This index is only approximately accurate because of the distribution of the eosinophiles in the tissues. In some of the specimens as many as twenty cells would be counted in one field, while in a field immediately adjacent not a single cell could be seen. The specimens examined were as follows:

Tissues.	Cases.
Spleen	37
Thymus gland	11
Mesenteric lymph nodes	10
Peribronchial lymph nodes	8
Inguinal lymph nodes	1
Post-cervical lymph nodes	1
Mediastinal lymph nodes	1
Axillary lymph nodes	1
Splenculi	2
Peyer's patches	1

Characteristics and distribution of eosinophiles.— Eosinophilic cells were always found, in varying numbers, in the lymphadenoid structures, in all cases of diphtheria, and in each case where tuberculosis was present as an associated condition; but were absent in all the other diseases encountered. As a rule, the cells were more numerous in the thymus gland than in the other tissues. In individual cases, however, there did not appear to be any constant tissue of election. Eosinophiles were never encountered in the heart, lungs, or kidney; but they were observed in the pancreas

once and in the adrenals in two other cases. In a pylethrombosis of the liver, numbers of these cells were demonstrable in the outer zone of the organizing thrombi and surrounding the thrombosed vessels.

The characteristics of the eosinophilic cells were essentially the same, irrespective of the location in which they were found. They varied considerably in size, some forms being little larger than the erythrocyte, and others showing all gradations of size from these up to those the size of a hyaline cell. Variations in shape were also observed. The majority of the cells were spherical in contour but occasionally bizarre forms were seen. The nuclei were of two forms; one being perfectly spherical and centrally placed, the other being polymorphous. In nearly all instances the latter were bi-lobed. The distribution of the granules in the cells varied greatly. In most cases they were evenly scattered throughout the protoplasm; but many of the cells showed a collection of the granules to one side. Free eosinophilic granules were commonly seen and nearly all sections showed cells that had apparently ruptured, with discharge of their granules.

In the spleen it was not at all uncommon to find free eosinophilic granules which had arranged themselves around certain mononuclear cells, and in many instances the granules were intracellular. The presence of eosinophilic granules in these cells would make eosinophiles tinctorially; but it is doubtful that they were such, functionally. It would seem that the presence of these granules in the mononuclear cells was due to a probable phagocytic property possessed by the cells, and, granting that they were phagocytic, it would be perfectly natural for them to englobulate any free granules with which they came in contact, as they would any other form of detritus. These same cells were also observed in the lymph nodes.

In the thymus gland many of the spindle-shaped connective tissue cells contained scattered eosinophilic granules. Where collections of eosinophiles were found many of them were spindle-shaped, but in all other respects they resembled the

regular type. In regard to a possible relationship between these cells and the connective tissue cells it may be well to speak here of the theory of the origin of the eosinophile, as advanced by S. M. Stschastnye.[4] In a recent article he states that the eosinophile is a product of hemolysis and that these cells may be produced wherever hemolysis occurs. He bases this statement upon the belief that the fragments of the disintegrated erythrocytes are gathered in by the phagocytic action of the mesenchyma cells and worked over so as to form the true eosinophile. Usually this takes place in the bone marrow, lymph nodes, spleen and lung, because the largest accumulations of erythrocytes occur there. He also states in the same communication that "a host of authors claim for all microbes hemolytic properties" and he quotes Besredka, especially, as stating that streptococci, associated with scarlatina, have hemolytic properties. This latter statement, especially, militates against his views, for, in most of the tissues studied personally from fatal cases of scarlet fever, there was a marked accumulation of erythrocytes and numbers of streptococci, and yet not a single eosinophile was demonstrable. In two cases of anthrax hemolysis was evident, the organs were markedly congested and hordes of anthrax bacilli were present; yet no eosinophile cells were found. Repeatedly in sections of spleen, especially, the conditions necessary, according to Stschastnye's hypothesis, have been encountered; but eosinophiles were absent unless the condition was that of diphtheria or tuberculosis.

Sections of all the lymphadenoid tissues, in which eosinophiles were present, showed these cells scattered diffusely through the organs, but there was a distinct tendency to grouping.

In the spleen eosinophiles were seen in greatest numbers beneath the capsule, in the lymph sinuses, and around the vessels. They were rarely seen within the splenic nodules, unless these bodies were the foci of degenerative or necrotic processes, in which event, numbers of the eosinophiles were seen immediately surrounding the necrotic areas.

In the lymph nodes the distribution of the eosinophiles

was much the same as in the spleen; but here the accumulation of these cells around the trabeculæ was notable.

The points of predilection in the thymus seemed to be around the corpuscles of Hassel and in the vicinity of the connective tissue and vessels.

In not a few instances, eosinophiles were seen within the vessels of the part and in the perivascular tissues. One section of the spleen showed, most beautifully, eosinophiles in the vessel walls with one cell in the actual process of migration. It is difficult to say whether these cells were formed in the spleen and were passing into the circulation, or whether they were brought to the spleen in the circulation. Opie[2] thinks that these cells are brought to the spleen from the bone marrow, as in inoculation upon animals he has found them two to four hours after inoculation in the spleen, together with other elements characteristic of the bone marrow. Stschastnye[4] believes that in a slowed circulation the granules, arising from hemolysis, pass through the vessel walls into the perivascular tissues and are converted into eosinophiles and that these eosinophiles may then pass back into the circulation. He stated, however, that he was never able to discern the granules in transition. Opie's view seems most reasonable as in nearly every case some of these cells were undergoing karyokinesis.

Having observed these cells in their different aspects, the question naturally arose as to what was the factor that determined their presence in the hematopoietic organs? The antitoxin used in the treatment of diphtheria was thought of, but this was quickly dismissed, inasmuch as every case, regardless of its nature, admitted to the Municipal Hospital received an immunizing dose of diphtheria antitoxin to guard against the danger of house infection with that disease. Therefore, as an eosinophilia was not observed in cases of scarlatina and meningitis which received antitoxin, this serum was ruled out as a possible factor.

Then in turning to the histories and autopsy notes of the different cases several possible factors arose. Did the age of the individual play a part? Did the disease from which he

suffered have any bearing? Did the clinical course of the disease in any way augment the phenomenon? What were the complications of the disease or the autopsy findings that might account for it? Could the day of the disease on which death occurred explain the condition?

Age was quickly thrown out as a cause, inasmuch as eosinophiles were found in cases ranging from seven weeks to thirty-four years.

It was found that the disease from which the patient suffered had an important bearing as eosinophiles were observed in all cases of diphtheria and tuberculosis, but were absent in scarlatina, epidemic cerebrospinal meningitis, rubella, anthrax, and typhus fever, unless one of them was complicated by diphtheria or tuberculosis.

In considering the clinical course of the disease the pulse and respiration obviously had no bearing, and were given no serious thought.

Several writers on hematology have shown that very high temperatures tend to drive the eosinophiles from the circulation, and it was thought that this might explain their presence in the hematopoietic organs. The temperature ranged as high, however, in scarlatina and other diseases in which eosinophiles were absent, as in diphtheria and tuberculosis, where eosinophiles were present. Another point in reference to the temperature which was thought of was the preagonal rise or fall; but that, likewise, had no bearing.

Bronchopneumonia, myocarditis, and nephritis were the chief complications met with, but they occurred in the absence of eosinophilia as well as when it was present. The day of the disease on which death occurred threw little or no light on the subject.

Inasmuch as it is well known that an increase in the eosinophiles in the blood is augmented by helminthiasis, it was thought that the lumbricoid worms found at autopsy in some of the cases might contribute to the eosinophilia, but the ascaris lumbricoides was found in a case of meningitis which showed no eosinophiles.

Thus eliminating one factor after another, it was obvious

that there was something in the virus of diphtheria and tuberculosis that controlled the phenomenon.

Simon[5] states that tuberculin inoculations produce an eosinophilia in some of the lower animals, and Opie[3] tells us that Nosske produced a local eosinophilia by inoculating animals with the tubercle bacillus, but failed with the pyogenic micrococci. Therefore the writer assumed that the toxins of the tubercle bacillus were responsible for the eosinophilia observed in those cases of the series reported here, in which tuberculosis was an associated condition.

Not being able to find any references, in the literature at hand, as to the occurrence of an eosinophilia following injection of diphtheria toxins, it was decided to try inoculations upon guinea-pigs to ascertain whether diphtheria toxins exercised an effect similar to those of the tubercle bacillus on the eosinophilic cells. Dr. Stewart, of the Bureau of Health Laboratories, kindly furnished me with the toxin used.

Injection of diphtheria toxin. — Weight of guinea-pig, five hundred and twenty-seven grams. Inoculated in abdominal wall with .01 cubic centimeter of virulent diphtheria toxin (Philadelphia Board of Health, No. 240.) Pig began to look ill and refused to eat forty-eight hours after inoculation, grew progressively worse, and died in four days.

Autopsy findings: Localized peritoneal exudate just below focus of inoculation; hypostatic congestion and edema of lungs; cloudy swelling of heart; congestion of spleen; fatty degeneration and punctate hemorrhages in adrenals; acute diffuse nephritis; cloudy swelling of liver.

Microscopic examination: Abdominal wall at site of inoculation showed mostly granulation tissue around which were numerous eosinophiles. On the peritoneum below this focus was a localized exudate composed principally of fibrin, in which were enmeshed large numbers of leucocytes. Numbers of eosinophilic cells (eight to ten in a field of the one-twelfth-inch oil immersion lens) and myriads of small granules, which were so numerous that the sections appeared as if the brightly-staining granules had been dusted over them, were seen in this exudate. These granules were eosinophilic and, as numbers of free nuclei were seen in the exudate, the inference is that they arose from disintegration of the eosinophiles.

It will be seen that the results of this experiment justified the inference that the presence of the eosinophiles might be due to a chemotactic influence exerted by the toxins of the

Klebs-Loeffler organism. Dr. A. C. Abbott tells the writer that while working on experimental diphtheria a number of years ago, he observed collections of eosinophilic cells upon the omentum of guinea-pigs inoculated via the testicle, with diphtheria organisms. This still further tends to show that this organism elaborates a product or products which influence eosinophilia.

Satisfied that the toxins of tuberculosis and diphtheria exercise a specific action on the eosinophile, the next thought was as to why the collection of eosinophiles occurred in the hematopoietic organs? It is thought that these organs act as filters, as it were, through which bacterial toxins and other noxious agents are removed from the economy, and it is here that the battle between the offending material and the body cells is fought. Therefore it seems perfectly natural that these organs should be the site of the eosinophilia a priori, as they evidently play a part in antagonizing the toxins.

This brings us to the consideration of the function of the eosinophile. Of this nothing is definitely known and at best it is but a matter of conjecture. In this study many things have been observed that are at least suggestive and perhaps they are worthy of note.

The arrangement of the eosinophiles around the focus at which the diphtheria toxin was injected in the experiment above recorded, their predilection for the hematopoietic organs, and their constant occurrence in the thymus gland, the internal secretion of which is thought to have an influence in combating infection (according to Osler[28]), would suggest that their function, in diphtheria and tuberculosis, at least, is to antagonize the toxins of the disease.

Since Wright[20] began his classical studies in immunity much has been written on the subject of opsonins and antibodies in the body fluids. Metschnikoff,[7] while admitting that there are sensitizing substances in these body fluids, still clings to his theory of immunity and claims these substances are elaborated by the leucocytes.

"Some time ago," he says, "Pfeiffer and Marx proved that the sensitizing substance originates in three groups of organs — spleen, bone

marrow, and lymphatic glands or the phagocytic organs, for all these organs, without exception, contain and produce phagocytes.

"In corresponding researches Deutsch-Ditre proved the phagocytic organs are the nuclei of the anti-typhoid sensitizing substance.

"On the strength of these researches we may conclude that the phagocytes are able to elaborate and even to excrete into the blood substances which fix themselves onto the microbes and render them more amenable to destruction by the body.

"Acquired immunity is, therefore, superactivity of the phagocytes which manifests itself by the overproduction of sensitizing substances, by their power of reacting strongly toward the introduction of microbes, and their products, and lastly, by their capacity of enveloping pathogenic microbes and destroying them intracellularly."

Simon [5] believes that the eosinophile is a glandular structure in which a secretory product is elaborated, inasmuch as detailed study with adequate stains, such as eosinate of methylene blue, shows that the majority of the eosinophilic granules are in reality little vesicles, composed of a deeper staining outer wall and lighter staining contents, which he believes to be the specific secretory product. Correlating the belief of Metschnikoff that the leucocytes do secrete antibodies, the claim of Simon that the eosinophilic granule is in all probability capable of secretion, and the fact, as shown in this study, that the toxins of diphtheria and tuberculosis evidently exert a chemotactic influence on the eosinophilic cell — is there not a suggestion that the function of the eosinophilic cell is to secrete a substance antagonistic to certain toxins?

To the writer it seems probable that such is the case, although his experience has been too slight and the studies recorded in this paper have been within too narrow limits to make such a statement justifiable. Nevertheless it has long been thought that the eosinophilic cell plays an important part in combating infection. Hardy and Wesbrook [38] demonstrated that, when organisms were introduced into the intestines of animals, eosinophiles migrated into the canal and disappeared from the intestinal wall. Noesske [8] produced an artificial eosinophilia with the tubercle bacillus. Howard and Perkins [39] demonstrated eosinophiles in appendicitis,

salpingitis, and various other processes, inflammatory in character. Eosinophiles have been observed in neoplasma and were found by the writer in the embryonic tissue of organizing thrombi in the hepatic vessels, and, in numerous instances, surrounding areas of necrosis in the spleen. Whether they were here to combat bacterial toxins or endogenous toxins elaborated by the disintegration of body cells cannot be stated. Opie [3] says that:

"Bacteria exert a chemotactic influence upon cells with eosinophilic granulation attracting them from the blood to the site of inoculation and from the bone marrow to the blood. Though rarely phagocytic they have a part in the changes following bacterial invasion."

Still another fact that would go to show that the eosinophile is amenable to the chemotactic influence of certain bacterial toxins is their constant occurrence below mucous surfaces, where organisms are always present, as in the gut, and their infrequency in the deeper tissues unless these tissues are the focus of inflammatory or necrotic processes.

It would seem that the action of the eosinophile is a specific one inasmuch as from personal observations the eosinophile seemed to be amenable to the chemotactic action of only the tubercle bacillus and the Klebs-Loeffler organism. It was shown a number of years ago by Widal and Ravaut, in their article on cyto-diagnosis, that in certain conditions some one variety of cell predominated, as the lymphocyte in tuberculous effusions; and it would seem that we have an analogous condition here, the toxins in diphtheria and tuberculosis attracting the eosinophiles to the hematopoietic organs, while the toxins in the other conditions met with apparently exerted no influence or at most a negative one. From the number of cases studied the observations should be conclusive in diphtheria and rather suggestive in tuberculosis. The number of cases of the other diseases studied is wholly inadequate to say conclusively that eosinophiles do or do not occur in the hematopoietic organs, but they may open up avenues for future investigation.

Animal inoculations. — With the object of finding out the evolutions of the eosinophile in combating infection, it was decided to inoculate several guinea-pigs with diphtheria organisms.

The culture used was isolated from a blood serum growth taken from the throat of a child ill with diphtheria. Tested on a pig, it was found to be very virulent. Transferred to neutral bouillon, there was a luxuriant growth in twenty-four hours, and this growth was used in the experiments.

The pigs chosen were of practically the same weight (three hundred and fifty to three hundred and sixty grams), had been kept under the same hygienic surroundings, and were of identical strains. Each pig, except a control, was inoculated intraperitoneally with .5 cubic centimeter of bouillon culture. The animals were killed fifteen minutes, one hour, two hours, four hours, six hours, and twenty-four hours after inoculation, respectively. Killing instantly was deemed preferable to death by chloroform or other chemical agents, as it was thought that these substances might produce changes in the body fluids which would militate against the accuracy of the results. The peritoneal cavity was immediately opened, and smears were made from the same region in each case — the right iliac fossa. This region was chosen because the fluid was slight in amount and tended to gravitate here.

The spleen was placed in Zenker's fluid and prepared by the same technic as the tissues obtained at autopsy in the human cases, as stated in the first part of this paper. Smears were made from the bone marrow of the femur and, with the smears from the peritoneum, were stained with Wright's blood stain.

The smears from the bone marrow showed such enormous numbers of eosinophiles that it was impossible to make even an approximate estimate of the comparative numbers in the different preparations.

The spleen of the control pig showed an eosinophilic index of 7.2; but Opie[2] states that eosinophiles are normally found in the spleen of the guinea-pig. The spleens in the

fifteen minute, one hour, two hour, four hour, six hour, and twenty-four hour experiments showed eosinophilic indices of 11.4, 7, 9.8, 1.5, 4.75, and 5.25, respectively.

A differential count of the various leucocytes found in the peritoneal exudates of the respective pigs was then made. Atypical cells were found in considerable numbers, and great difficulty was experienced in deciding under which heading to classify them. Several spreads of normal guinea-pig blood and peritoneal fluid were studied, therefore, to enable the writer to become familiar with the cells found normally.

The mononuclear cells, which were larger than the erythrocyte, were classified as large lymphocytes, and those up to the size of an erythrocyte as small lymphocytes. Two distinct types of eosinophiles were observed. Those of the first class were about the size of a large lymphocyte and identical with the eosinophiles found in the blood; were polymorphonuclear and contained large regular granules, which stained brightly with eosin. The eosinophiles of the second class were smaller than those of the first class, their granules were more compact, stained less intensely with eosin, and showed one small, eccentrically placed, round or oval nucleus in most instances, although polymorphonuclear forms were not uncommon. These eosinophiles of the second class were also phagocytic.

In the fifteen-minute exudate the only phagocytes were large endothelial cells. In the one-hour exudate twenty-five per cent of the eosinophiles of the second class, many endothelial cells, and polymorphonuclear neutrophiles acted as phagocytes. The two-hour exudate showed twenty per cent of the eosinophiles containing bacteria and fifteen per cent of the neutrophiles were phagocytic. A few large lymphocytes were also found to be phagocytic, while the endothelial cells were fewer in number and did not show as high a phagocytic index as in the previous specimens. No free bacteria or bacterial inclusions were seen in the four, six or twenty-four hour specimens. In the twenty-four-hour exudate many of the eosinophiles showed a partial polychromatophilia.

It is interesting to note that in the four-hour exudate after phagocytosis had evidently ceased, the type of eosinophiles changed from those of the second class to those of the first class.

TABLE OF DIFFERENTIAL COUNTS OF LEUCOCYTES IN PERITONEAL EXUDATES.

	15 Min.	1 Hr.	2 Hrs.	4 Hrs.	6 Hrs.	24 Hrs.
Small lymphocytes	43.0	58.5	70.0	0	19	0
Large lymphocytes	43.5	40.0	18.5	6	24	0
Polymorphonuclears	1.5	0.0	4.5	21	6	77
Eosinophiles, 1st class	1.0	0.0	0.0	72	51	13
Eosinophiles, 2d class	11.0	1.5	7.0	1	0	10

It will be seen by reference to the foregoing differential counts that the first effect of inoculation was to drive the eosinophiles from the peritoneal fluid, as evidenced in the study of the fifteen-minute exudate. It is also interesting to note that accompanying this temporary disappearance of the eosinophiles from the peritoneum a rise in the eosinophilic index occurred in the spleen. In the four-hour exudate, where the peritoneal eosinophilia reached its fastigium, the eosinophilic index of the spleen reached its lowest point. It would, therefore, seem that the presence or absence of eosinophiles in the peritoneal fluid varied inversely with the findings in the spleen.

Combining these results with those of the previous experiment, and, with the facts elicited by study of the forty-two cases mentioned in the first part of this paper, the writer has formed the following conclusions:

CONCLUSIONS.

1. The occurrence of eosinophilic cells is constant in the hematopoietic organs in diphtheria and tuberculosis.

2. The toxins of the diphtheria and of the tubercle bacillus exert a positive chemotactic action on the eosinophile cell.

3. The chemotactic stimulus which attracts the eosinophile is a selective one, and is not possessed by all bacterial toxins, as eosinophiles were not found in the hematopoietic organs in scarlatina, cerebrospinal meningitis, anthrax, rubella, or typhus fever.

4. The eosinophile elaborates either sensitizing substances or antibodies, which antagonize certain bacterial products, at least the toxins of diphtheria and tuberculosis.

5. Following intraperitoneal inoculations in guinea-pigs with the diphtheria bacillus, the number of eosinophiles in the spleen varies inversely with the number found in the peritoneal fluid.

6. In the guinea-pig the eosinophile cell is phagocytic for the Klebs-Loeffler bacillus.

[I wish to acknowledge my indebtedness to Dr. Randle C. Rosenberger, of the Jefferson Medical College laboratories, who first suggested studying the hematopoietic organs in these infections. I wish to express my thanks to Dr. W. M. L. Coplin, at the time Director of the Department of Public Health and Charities, Philadelphia, and to Dr. A. C. Abbott, Chief of the Bureau of Health, through whom it was my good fortune to receive a special appointment in the Municipal Hospital, where the studies herein recorded were carried out.

I am especially indebted to Dr. B. Franklin Royer, Chief Resident Physician, Municipal Hospital, whose enthusiasm and unceasing interest in the work were a constant source of encouragement. Drs. Aller C. Ellis and John D. Wilson, workers in the laboratories of the Jefferson Medical College Hospital, are also deserving of my thanks for many timely suggestions and valuable references. Dr. E. Burvill Holmes, Resident Physician Municipal Hospital, courteously furnished the anthrax tissues studied in this series.]

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THE VIABILITY OF PROTEOLYTIC ENZYMES IN TISSUES.*

CHANNING FROTHINGHAM, JR.

(*From the Laboratory of the Department of the Theory and Practice of Physic, Harvard University.*)

Besides the proteolytic enzymes present in the alimentary canal during the process of digestion, others have been found in several of the organs and tissues of the body. Thus in the liver, kidney, spleen, and lymph glands proteolytic enzymes have been described which act best in a mildly acid media.[1] In the suprarenal, muscles and thymus proteolytic enzymes have been described without mention of the reaction in which they are more active,[2] while in bone marrow and the globulin of the serum[3,4] proteolytic enzymes are present which are more active in an alkaline media. Also similar enzymes have been described in different animals.[5]

Recently, considerable work has been done on the enzymes contained in the cells of the lymph and circulatory systems. Fr. Müller[6] in 1888 observed that pus from an abscess or a pneumonic sputum would digest proteid, while pus from a tubercular abscess would not. Since then Müller and Kolaczek[7] have confirmed this in regard to pus from an uncomplicated and untreated tubercular abscess.

Opie[8] working with cells from a sterile inflammatory exudate describes two proteolytic enzymes: one, leuco-proteose, contained in the polynuclear neutrophiles, which is active in an alkaline and neutral media, the other, lympho-proteose, contained in the mononuclear phagocytic cells, which is active in an acid media. Both of the enzymes are destroyed at 75° C. The lympho-proteose gradually loses its power from 55°–75° C. and in fifty per cent glycerine solution. It is destroyed by precipitation with absolute alcohol, alcohol and ether, with subsequent drying in the air. Opie raises the question whether the proteolytic

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enzymes of the different organs and tissues may not be due to the presence of these cells within them. Opie's method in brief consists in mixing given quantities of the substance to be investigated with a given amount of albuminous material, and, after incubating for a certain length of time, in determining the amount of uncoagulable proteid present.

The Germans have worked on this question of proteolytic enzymes. They use chiefly a method devised by Ed. Müller.[9, 10] He uses Loeffler's blood serum and incubates at 50°–55° C. The substance to be investigated is placed on the surface of the serum plate. If a proteolytic enzyme is present the substance sinks into the coagulated blood serum; if not, it dries up on the surface of the plate. Müller[10] claims that the polynuclear neutrophiles and mononuclear myelocytes contain a proteolytic ferment, and that it is contained in the cytoplasm of the cells. He also claims that eosinophiles and lymphocytes do not contain a ferment. Erben[11] supports this view, as he found that they stained after incubation at 37° C., while the neutrophiles did not.

In man, Jochmann[12] and Ziegler, with Müller's method, found that the bone marrow, spleen, and pancreas contained a ferment, but that the lymph glands did not. In pathological conditions the following points have been brought out: Müller and Jochmann[9] have shown that blood from myelogenous leukemia has a proteolytic action upon Loeffler's serum at 55° C., while blood from normal individuals and from cases of lymphatic leukemia, pseudoleukemia, cancer, and sarcoma does not show this activity. In a case of myelogenous leukemia the bone marrow, spleen, lymph glands, and tonsils all contained a proteolytic enzyme, but histologically they all contained myelocytes in considerable numbers. Tissues from cases of lymphatic leukemia and pseudoleukemia act practically the same as average autopsy tissue. Müller[13] and Kolaczek showed that while tissues from areas with inflammatory reaction always contained an enzyme sarcoma, carcinoma, gumma, and solitary tubercle did not.

Jochmann[12] and Ziegler took up the question of the

viability of these enzymes in preservatives and found that tissues which had been preserved in some cases for several years in toluol, thymol, sublimate solution, alcohol, Müller's fluid, and formalin still retained their proteolytic enzyme. On the other hand, Kaiserling and Haugsche decalcifying solution destroyed the enzymes.

As a rule, the German writers have not mentioned the reaction of their serum, and so they do not show whether their results are in regard to leuco-proteose or lympho-proteose.

The object of this study has been to work out more definitely the viability of leuco-proteose and lympho-proteose in the presence of a few of the common preservatives. Two other points have come up during the work: Do the enzymes in parenchymatous organs act similarly to the above two enzymes in regard to preservatives? Can this method be used in helping to decide the origin of tumors of the bone marrow by finding out what enzymes their cells possess?

In these observations Müller's technic, with certain modifications, was used. The Loeffler's blood serum was made as nearly neutral as possible with litmus paper, and then enough sodium hydrate or acetic acid was added to make a .2 per cent solution. As coagulating and sterilizing the media changes the reaction somewhat, greater accuracy in the per cent of acidity or alkalinity was not attempted. By this method the media remained at a low enough percentage of acidity or alkalinity not to interfere with the action of the enzymes due to concentration of acid or alkali. Unless otherwise stated it was considered that no enzyme was present if a depression in the media had not appeared within forty-eight hours. As an incubator a paraffin oven kept about 56° C. was used. Bacteria did not usually grow at this temperature. Occasionally a short, thick bacillus appeared on the alkaline media, but it did not digest the serum or interfere with the observation. Throughout no attempt

has been made to make a quantitative study, simply the attempt to decide whether or no an enzyme was present.

For the study of the viability of the enzymes, pus from a breast abscess was used. When fresh this showed, on culture, Staphylococcus pyogenes aureus in practically pure growth. When first obtained it was active on both media. It was kept for about two months at room and cold room temperature before being put in the preservatives. At that time it still digested both media, although the action was more marked on the alkaline media. I assumed, therefore, that both lympho-proteose and leuco-proteose were present. Some of this pus was left in the freezing-room for three days. At the end of that time it still digested both media. Some of that which had been frozen was dried in the air for several days, and then at 56° C. for twenty-four hours. This still digested both media.

Some of the pus that had been preserved in alcohol, ten per cent formalin, and Zenker's fluid was next studied. That fixed in alcohol was still active on both media. That fixed in ten per cent formalin showed no activity on an acid media and only extremely slight activity in forty-eight hours on an alkaline media. The pus hardened in Zenker's fluid showed no activity on either media within forty-eight hours, but in seventy-two hours a very slight activity on the alkaline media was observed. The pus hardened in formalin and Zenker was now ground up and repeatedly washed in water. After this the formalin pus was active on both media, and the Zenker pus on the alkaline one only. That the washing did not introduce an enzyme was shown on the acid media by the fact that the Zenker material did not digest. For a control on the alkaline media a Zenker fixed rabbit's kidney washed in the same manner was tried and found negative.

From the above it may be concluded that drying in the air, freezing, or preserving in alcohol for a short while does not destroy or inhibit either leuco-proteose or lympho-proteose. Formalin, by its presence, for a short while inhibits but does not destroy lympho-proteose and very materially

checks the action of leuco-proteose. Zenker fixation practically inhibits but does not destroy leuco-proteose, but destroys the lympho-proteose.

For the study of the prolonged action of these fixatives, a case of myelogenous leukemia was used which had come to autopsy in 1901. Tissue had been preserved in alcohol, formalin (ten per cent), and Zenker's solution. All the organs and tissues of this case were crowded with myelocytes. All the tissues preserved in alcohol digested the alkaline media. On acid media no digestion manifested itself within forty-eight hours, but after four days very slight pitting of the serum occurred. To make sure that these cases contain lympho-proteose a piece of the spleen from a recent case of myelogenous leukemia, washed free of formalin after a few weeks' preservation, was placed on the sera and showed activity on acid media. That this was not due to the spleen itself is shown by the fact that no other spleen showed activity on acid media. The alcohol preserved material after drying in air still contained an active leuco-proteose but no lympho-proteose. The formalin tissue was inactive on both media. After washing, however, the enzyme was active on alkaline media, but not on acid media. The Zenker fixed material showed a very slight digestive power after forty-eight hours on alkaline media, but none on acid media. After thorough washing, digestion occurred on alkaline media within twenty-four hours, but none on acid media. Thus it appears that leuco-proteose acts practically the same after a number of years in preservatives as after a short time. The inhibitory action of the formalin which was very marked after a short while becomes complete with a longer time, and the Zenker inhibitory action, after years of soaking in alcohol, has slightly worn off. The lympho-proteose by prolonged action of alcohol is practically destroyed, and by the prolonged action of formalin completely destroyed.

Thinking that a case of lymphatic leukemia might contain enough lympho-proteose to be active, even after several

years' preservation in alcohol, a case was studied that had been preserved in alcohol for over nine years. Five pieces of this tissue were placed on both media. No digestion occurred on the acid media, which meant that either no lympho-proteose was present or the prolonged action of the alcohol had destroyed it. On the alkaline media three of the five pieces digested. Histologically, all of the five pieces contained lymphocytes, so that the lymphocytes could not be considered the cause. The ones that digested were, liver and spleen in forty-eight hours, and lung slightly in seventy-two hours. The pancreas and kidney showed no reaction. Bits of tissue were now studied to see if these organs ordinarily contained an enzyme which manifested itself by this method, and if so, if it acted similarly in regard to the preservatives to leuco-proteose or lympho-proteose. For this purpose tissues were used from fresh autopsies, washed with running water or put in alcohol to remove the antiferment of the serum. The results were not absolutely consistent throughout, but in general they showed that the liver, lung, spleen, bone marrow, and pancreas contained an enzyme which acted by this method on alkaline media, and that the pancreas contained a ferment which acted on acid media also. The kidney, adrenals, heart, and brain were not active on either media. This possibly accounts for the action of the liver, spleen, and lung in the case of lymphatic leukemia. Why the pancreas in this case did not digest also is explained by the fact that its enzyme, active in acid, is destroyed after a short time in alcohol, and the enzyme, active in alkaline, is considerably inhibited in a short time and presumably destroyed after a longer while by the presence of alcohol.

Next, pieces of lung, liver, and spleen which had been preserved in alcohol for several years, were studied to see if the enzymes withstood the action of preservatives, but the results were so inconsistent that no definite conclusions could be drawn. As these enzymes may be due to the cells of the circulation rather than the parenchymatous cells, perhaps variations should be expected, depending upon the histological picture of the organs.

The question of using this method as an aid in determining

the origin of tumors was also studied. For this purpose two myelomas hardened in alcohol, two sarcomas hardened in alcohol, and one breast cancer kept over night in a freezing room were used. None of the tissues showed the presence of an enzyme on either media after three days' incubation. It was not expected that the sarcomas and cancer would contain an enzyme, but it was thought that if the myelomas came from myelocytes they would. The fact that they do not adds one more point in favor of their being composed of some other cells.

The following conclusions seem justifiable:

Leuco-proteose is inhibited but not destroyed by the presence of formalin and Zenker's solution, and upon their removal recovers its active power. Neither alcohol, freezing, nor drying in hot air inhibits or destroys it.

Lympho-proteose is destroyed by Zenker's solution, is inhibited, and after a prolonged stay is destroyed by formalin, and is considerably inhibited after a prolonged stay in alcohol. Neither freezing nor drying in hot air inhibits or destroys it.

The presence of enzymes in organs and tissues requires further study in regard to their relation to the two above mentioned enzymes.

This method may be used for helping to determine the origin of bone-marrow tumors even after the tissue has been hardened in the common preservatives.

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PSEUDODIPHTHERIA BACILLUS INFECTIONS AND THEIR RESPONSE TO THERAPEUTIC INOCULATIONS.*

A. P. OHLMACHER, M.D., DETROIT, MICH.

My interest in the question of pseudodiphtheria bacillus infections having been revived through my clinical studies of therapeutic immunization, I propose to recount, as compendiously as possible, some belated observations, and to supplement them by those bearing on the more recent aspects of the subject.

Endemic infection with pseudodiphtheria bacillus. — During my four years' service as pathologist of the Ohio Hospital for Epileptics, it was customary to make thorough bacteriological examinations of autopsy material as a routine practice; the autopsies were usually made promptly, and so far as this factor goes their results are valuable. Many fatalities resulted from the exhaustion of epileptic dementia, generally complicated with a terminal bronchopneumonia. From time to time the pseudodiphtheria bacillus was isolated from these infected lungs. During the spring and summer of 1901 this microbic species was repeatedly recovered from a series of autopsies, until it became clear that the occurrence assumed proportions justifying the pronouncing it an endemic. Several times the bacillus was recovered from the heart's blood as well as from the pulmonary pneumonic lesions, and once it was present in the pus of an abscess of the cheek, as well as in the pneumonic lungs. Those of us who followed these examinations reached the conclusion that this microbe was a not rare agent of the terminal infections to which the chronic insane and epileptic are prone. It was this view of the matter that presented itself to me when I learned of the work of Ford Robertson and his alleged bacillus of paresis, viz., that the organism of Robertson was in reality a member of the pseudodiphtheria or xerosis group

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of bacilli, and entitled to consideration only as an agent of terminal infection.

A case of typhoid and meningitis with B. pseudodiphtheriæ in the brain. — Among the cases of pseudodiphtheria bacillus infection presenting themselves during the summer of 1901 was that of a male epileptic, eighteen years old, who died after ten days of acute illness characterized by fever and stupor. In the evening of the eighth day of illness the patient had three general convulsions, which resembled ordinary grand mal; five similar attacks occurred the following evening, and one on the morning of the tenth or last day. The stupor throughout the illness and the final coma were the particularly striking clinical features. At autopsy typhoidal ulceration of the lower ileum, with swelling of the mesenteric glands and spleen were the principal abdominal findings. There were also cloudy swelling of the liver and kidneys, and bilateral bronchopneumonia. In the central nervous system a sero-cellular leptomeningitis and ependymitis with diffuse cloudiness of the soft meninges, some recent adhesion in the longitudinal fissure, opacity of the ependymal lining, and diffuse redness of the choroid plexus which adhered to the contiguous structures, were presented. The exudate was located in the subarachnoid lymph space, was sparingly cellular, and consisted mostly of large, endothelioid cells (both mono- and poly- nuclear), smaller mononuclear cells (lymphoid), and a few intermediate (plasma?) cells. The cells were most abundant in the exudate over the motor regions. A painstaking bacteriological analysis revealed the typhoid bacillus in the spleen and mesenteric glands; Staphylococcus aureus and Bacillus pseudodiphtheriæ from the heart's blood and the pneumonic areas; and from the subarachnoid fluid these same organisms, viz., Staphylococcus aureus, and Bacillus pseudodiphtheriæ; while from the ventricular fluid the pseudodiphtheria bacillus was alone isolated. Tested by the differential cultural methods then in use, this bacillus answered to the type of the pseudodiphtheria bacillus group. It was non-pathogenic when inoculated

into half-grown guinea-pigs, producing no local or constitutional reaction.

Pseudodiphtheria bacillus as an associate of the gonococcus in acute urethritis. — Three years ago, while engaged in the task of recovering and cultivating the gonococcus, I again encountered the pseudodiphtheria bacillus as an inhabitant of the pus from acute gonorrheal urethritis. The association of this bacillus and the gonococcus was noted on several occasions and from different individuals, and it was one of the features rendering more difficult the recovery of the gonococcus in pure culture. From these observations I concluded that this companionship was not an accident, but that it might be fraught with importance, especially from the standpoint of therapeutic bacterial immunization in gonorrhea.

Bacterial therapy as directed against pseudodiphtheria bacillus when associated in acute gonorrhea. — It has been my experience that therapeutic immunization in acute gonorrhea, with the gonococcus alone, is not as successful as one might be led to expect from the results that follow a similar treatment directed against acute suppurative diseases caused by other pyogenic microbes. But, in several cases of acute blenorrhagia in which I found the pseudodiphtheria bacillus associated with the gonococcus, a vaccine composed of both these bacterial species produced therapeutic responses that had not been previously obtained. Accordingly, it appears to me that we cannot, from the standpoint of bacterial therapy, ignore the pseudodiphtheria bacillus when we find it as an organism of mixed infection in gonorrhea.

Pseudodiphtheria bacillus in chronic unhealed empyema. — In the treatment of a number of cases of chronic unhealed thoracic empyema, I have encountered the pseudodiphtheria bacillus twice, and in each instance inoculations with it were required to promote a satisfactory outcome. One of these cases furnished an interesting illustration of the changing bacterial flora of chronic suppuration, and the influence

exerted on this flora by specific therapeutic bacterial immunization. At the outset of treatment, several careful analyses of the pus from the draining but unhealed pleural abscess yielded only pseudodiphtheria bacillus. Inoculations with this autogenous bacillus were followed by a highly satisfactory clinical response which continued for several weeks, during which time the pus was examined on the occasion of each inoculation, at intervals of five to seven days. At the end of four weeks Staphylococcus aureus began to crop out in the cultures from this pus, and by the end of another month the aureus was alone found. In order to hold the steady improvement in the patient's condition it was necessary to modify the inoculations to correspond with the changes in the bacterial flora, using a mixed pseudodiphtheria bacillus and Staphylococcus vaccine, and later one of the aureus alone.

THE REACTION OF HYPERSUSCEPTIBILITY AS PRODUCED BY BACTERIAL INOCULATIONS.*

A. P. OHLMACHER, M.D., DETROIT, MICH.

With recently acquired information concerning the peculiar reaction to proteid injections, first clearly brought out in connection with the use of horse's serum by V. Pirquet and Schick in their studies of the "serum diseases," it is quite natural to look for some manifestations of a similar nature following the periodic injection of bacterial vaccines required in opsonic practice. In my own experience I have observed, on several occasions in certain patients, immediate symptoms like flushed face, dizziness, and nausea when inoculations were repeated, which could be explained only on the basis of there being a mild manifestation of the phenomenon of proteid hypersusceptibility. No unpleasant consequences followed these temporary symptoms, which, in a very considerable number of cases, were so rare as to be qujte exceptional. Very rarely, also, temporary skin eruptions like the "serum rashes," or attacks of itching, appeared after repeated inoculations.

Besides these evidences of a generalized reaction of hypersusceptibility, I have recently noted a phenomenon which seems allied to the cutaneous tuberculin reaction, being a sharply localized manifestation of hypersusceptibility induced by previous bacterial inoculation, and aroused by subsequent spontaneous infection with the corresponding bacterial species. It happened that a father and his son had been subjected to therapeutic inoculations of Staphylococcus aureus some four to six months before the occasion in question when, apparently by chance, each individual simultaneously developed what looked like a beginning boil. In the case of the father the small, red, indurated lesion was on the arm; while with the son the boil appeared on the shoulder. But instead of progressing like an ordinary

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furuncle, these infected areas showed a small, reddened, slightly tender, nodular center, surrounded by a colorless wheal precisely like the lesion of urticaria. This circular area of edema was at least an inch in diameter, and remained in evidence for two days. In the father's case the incipient boil, from whose serum Staphylococcus aureus was secured in pure culture, aborted; but in the son the edema, which I took to mark the local reaction of hypersusceptibility, subsided, only to be followed by a large staphylococcus boil in the affected area.

Still another manifestation of local hypersusceptibility is that in which the inoculation of a certain bacterial vaccine arouses into activity, as shown by swelling, tenderness, and sometimes redness, the region previously subjected to injection with the same vaccine. I have noted this effect particularly in mixed vaccines composed of the gonococcus and some other organism like staphylococcus obtained from the patient's urethral secretions, and the sites of inoculations several weeks before and those of intervening inoculations all becoming irritated. These effects persist for several days or even longer.

PAPILLIFEROUS CYSTS OF THE KIDNEY.*

JOSEPH MCFARLAND, PHILADELPHIA.

The lesions that form the subject of this paper were discovered in the body of an unknown man secured from the Pennsylvania State Anatomical Board, for the purpose of demonstrating autopsy technic to students of medicine. Of the history of the patient nothing is known. The autopsy protocol is synoptized as follows:

The body was that of a well-developed white man apparently between fifty and sixty years of age. It was not well preserved though decomposition was not advanced. There was a strong odor like urine given off from the cadaver, which became much intensified as the autopsy progressed. There was no rigor mortis. There was marked anasarca, and the skin was very white except the face, which was livid. There were no other signs of disease, and none of injury.

Thorax. — Old dense pleuritic adhesions were present between the left lung and the diaphragm. Hydrothorax was present on both sides, but was much more marked on the right side.

The pericardium was stretched over an immensely enlarged heart, and contained several times the natural quantity of fluid.

The heart was of bovine size, rounded in shape, and showed hypertrophic dilatation of all the chambers. From the appearance of the organ, mitral disease was suspected, but when the viscus was opened all of the valves were found to be competent, their only abnormality consisting in small scattered atheromatous foci upon the mitral and aortic leaflets, and slight inflexibility of the latter. This showed the cardiac enlargement to depend upon extrinsic causes, and it was referred to the kidneys, though the peculiar shape of the organ remained unexplained.

The lungs had undergone considerable post-mortem

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change. Both were the seat of much hypostatic congestion and maceration. There was a doubtful pneumonic consolidation at the right base, but the lung was very pulpy in texture.

Abdomen. — There was a marked ascites.

The spleen was enlarged, dark colored, flabby, and so soft as to be readily torn by the fingers. It was supposed to be the seat of congestion, edema, and post-mortem softening.

The adrenals were soft, the cortex yellow, the medulla grayish and pulpy. No distinct abnormalities were observed.

The liver was slightly enlarged, faintly nutmeg in appearance from a mild degree of congestion.

The gall-bladder and bile-ducts were normal.

The kidneys were much enlarged, dark red in color, congested, juicy, edematous, flabby, with swollen cortex, deep congestion at the bases of the pyramids, and with an easily stripping capsule. The kidney contained a retention cyst the size of a marble, and its cortical substance contained ill-defined yellowish spots beneath the capsule. These were at first regarded as areas of inflammatory infiltration, but upon section one was found to be cystic, one solid, and all rounded in form. Near the center of the kidney, situated chiefly in the medullary substance, a much larger lesion of similar kind was discovered. It measured one and a half by two centimeters in diameter, was bright yellow in color, finely granular in texture, and soft as butter in consistence. The plastic contents could not, however, be expressed or washed out. In the water the cut surface of the lesion presented a frayed or villous appearance at the surface, and after washing showed a peculiar glitter that suggested a crystalline quality. A tentative diagnosis of hypernephroma was made.

Careful examination of the left kidney showed a few superficial lesions of similar appearance.

A tentative diagnosis of subacute nephritis with exacerbation was made.

The stomach was too much softened to make satisfactory examination possible. There were no conspicuous lesions.

The intestines were not opened (the time allotted to the examination did not permit this). There were no external signs of disease.

The bladder and prostate, seminal vesicles and testes were normal.

The pancreas was much softened, probably from edema, maceration, and autolysis. The cut surface was pinkish gray and highly glazed, the lobules indistinctly outlined.

Microscopy. — Subsequent microscopical examination of the kidney showed the lesions to be more diffuse than at first supposed. Morbid changes were present in all the structural elements.

Glomerules. — Numerous glomerules were in a state of arterio-sclerotic atrophy; many showed early chronic thickening of Bowman's capsule; some showed coagulated exudate in the intracapsular space.

Tubules. — The convoluted tubules showed widespread destruction of the epithelial cells many of which were detached. Tube-casts were numerous in the lower tubules. A few retention cysts were observed.

Interstitial tissue. — This showed considerable proliferation, mostly focal in character, and more or less widespread edema.

The neoplasms. — Microscopic examination of the various neoplasms showed them to be of the same nature. The smaller lesions were primarily cysts lined with cuboidal epithelium bearing a general resemblance to that of the collecting tubes. From the cyst wall a varying number of papillary excrescences projected into the cyst cavity, more or less completely filling it according to their numbers.

The excrescences seem to begin as simple papillæ which become more and more elaborated as they grow, until they finally form complicated arborescent or dendritic formations. Each excrescence has a delicate fibrillar tissue framework, containing blood vessels, and over each branching formation the cuboidal epithelium is continued.

In the largest cyst a multilocular appearance is presented, and it may be that this lesion consists of a congeries of small

cysts. In each of the compartments, however, the structure of the primitive cyst is maintained. Portions of the large cyst show retrogressive changes in the form of mucinoid degeneration of the fibrillar tissues and more or less edema.

In the older cysts there is a considerable quantity of cellular and molecular débris. The epithelial cells appear to desquamate but to continue alive for some time subsequently.

During this time abnormal proliferative changes seem to be attempted, as cells with two, three, and four nuclei can be found among them, though none were noticed in their normal environment. Eventually the cells degenerate and are resolved into granular débris. The healthy cells, while resembling those of the collecting tubes, possess relatively less cytoplasm, which seems to be more homogeneous. The nuclei are rich in chromatin. No karyokinetic figures could be found, but this may have been dependent upon the fact that the material was not sufficiently fresh.

That these papillary cysts are genetically different from the ordinary retention cyst is easily established by comparing the two. The latter begins in a simple dilatation of a uriniferous tubule. As this increases, its epithelial lining is apt to undergo signs of deterioration. First one observes loss of continuity of the cells, then attenuation and atrophy of such as remain and, eventually, complete disappearance of most of the cells.

For the sake of comparison, numerous cysts from sixteen cases of cirrhotic kidneys were examined, and in all of these atrophic epithelial changes were observed without a single tendency toward proliferation.

The histogenesis of the cysts is not clear. The epithelial cells, as has been said, bear a resemblance to those of the collecting tubes, but no direct descent from any part of the kidney structure could be made out. The smallest cyst examined lay directly beneath the capsule of the kidney, its wall being so thin as to consist of little more than the epithelium and the capsule of the kidney. This cyst contained numerous simple papillary formations and bore no resemblance to the simple retention cysts.

There is no reason to suppose that these lesions could have been responsible for any functional disturbances. To all appearance they are perfectly benign formations whose existence could not have been suspected during life. How old they may have been, or what their ultimate size might have become, had the patient not died of nephritis is a matter of conjecture only.

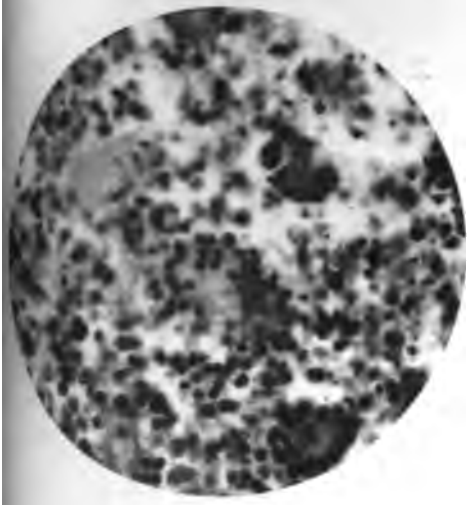
DESCRIPTION OF PLATE II.

FIG. 1. — Section through papilliferous cyst.

FIG. 2. — Desquamated cells from collections in older degenerating parts of the cyst.

FIG. 3. — Further dilatation of uriniferous tubules.

FIG. 4. — Dilated tubules forming a cyst on whose walls most of the epithelium is still present.



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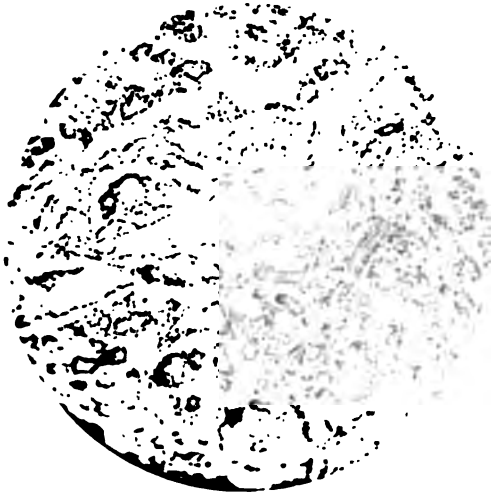

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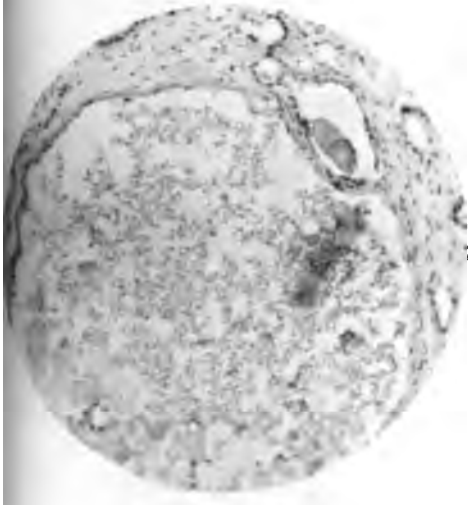

3

4

McFarland.

Papilliferous cysts.

THE RESULTS OF CHRONIC PARATHYROIDITIS AS OBTAINED BY LIGATION OF THE PARATHYROID GLANDULES IN THE DOG.*

R. L. THOMPSON, M.D.

(*Professor of Pathology, St. Louis University School of Medicine.*)

AND

W. E. LEIGHTON, M.D.

(*Instructor in Clinical Surgery, St. Louis University School of Medicine.*)

(*From the Pathological Laboratory, St. Louis University School of Medicine.*)

Following the ligation of the parathyroid glandules in the dog one of two things may happen: (*a*) Functioning islets of gland tissue may persist, keeping the dog alive for a considerable length of time; (*b*) the glandules may eventually be replaced by dense fibrous tissue and the dog die. In either event a train of symptoms of a trophic nature arise, entirely different in character from the severe acute tetanic manifestations that follow parathyroid excision in these animals.

This brings two facts into prominence that we wish to establish in connection with the following experimental work on the parathyroid glandules of dogs. First, which is important for practical surgery, that ligation of the parathyroids, whereby they are left in situ with their usual blood supply destroyed, does not always destroy the glandules and is not the same thing as excision. Second, that by this ligation of the parathyroids, disturbances of nutrition, which may ultimately end in death, can be brought about without producing any tetanic symptoms whatsoever, although the death can be definitely proven to be due to loss of the parathyroid glands.

This work was suggested by the statement of Halsted[1] that "it is in the control of hemorrhage that we sacrifice the parathyroid glandules." We began the work primarily merely to see how much we could interfere with the blood

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supply of these bodies without completely destroying them and what the results would be.

The classic phenomena following removal of the parathyroid glands, which end in convulsive death, need not detain us, since the subject is so well known and has been so frequently reviewed.

Nutritional disturbance following parathyroid operations has been given scant attention, although trophic disturbance following interference with these glandules has not wholly escaped notice. Erdheim[2] has shown that in rats on which a partial parathyroidectomy has been done peculiar trophic disturbances are produced consisting in enamel defects, brittleness, and fracture of teeth and gangrenous stomatitis at times. Cataract formation was also observed by Erdheim in rats. The tetany in these animals was described as of a decidedly chronic character. MacCallum[3] noted that occasionally the violent symptoms in parathyroidectomized dogs gradually gave place to a stuporous condition which lasted several days before terminating in death. That disturbances of a trophic nature may occur in connection with partial loss of parathyroid tissue, then, is a well established fact, and it only remains to emphasize their occurrence apart from tetany, as we have obtained them in the majority of our animals.

We have employed for this work twenty dogs, only fourteen of which, however, are available for report. Three dogs died from the anesthetic, one from hemorrhage, and two from infection.

One might speak, in passing, of some of the difficulties incident to parathyroid ligation in dogs. First of all to be mentioned is the inconstancy of position of the parathyroids in these animals, which makes extended search necessary at times.

Gley[4] noted fourteen variations in the situation of the glandules in thirty-three dogs, and Alquier[5] found the classic situation of the bodies only nine times in fifteen dogs. When we could not find at least four glandules readily at the first

operation the glandules that were found were ligated, and a second and sometimes a third operation done later on.

Although Alquier speaks rather conservatively of parathyroid hypertrophy of remaining glandules after ligation of two or three, nevertheless we have been able to find glandules with comparative ease at the second operation that had escaped our observation at the first.

The frequent occurrence of accessory parathyroids in addition to the four "normal" glandules is at any time liable to complicate the work. It is the occurrence of these accessory glandules that has made it possible for conflicting reports of apparent parathyroid extirpation to appear at times. We have found more than four parathyroids in a number of our dogs. In one case eight glandules were found and ligated at the first operation, four in each thyroid. When these accessory glandules are imbedded deep within the thyroid they are only discovered after the thyroid has been removed. Parathyroids have also been found remote from the thyroid. For this reason a single dog may fail to respond to the ordinary parathyroidectomy, or parathyroid ligation.

We have followed so carefully, by successive operation and microscopic control of ligated glandules, the disappearance of the parathyroids, and observed the results following such loss so frequently that there remains not the slightest doubt in our minds that while the dog can survive a considerable loss (three-fourths if not more) of functioning parathyroid tissue, a complete loss or very near loss is always followed by death. And while this fact is too well established to need confirmation by us, nevertheless the exact control of parathyroid elimination by our method of procedure must help to scatter whatever shadow of doubt may persist in the minds of some who have chanced to read adverse reports on the production of parathyroid death.

Technic. — The dogs were anesthetized by the Grehant method: .01 gram of morphia per kilo, followed by from five to ten cubic centimeters per kilo of a mixture of chloroform five parts, alcohol fifty parts, water fifty parts. Neck opened

and thyroids exposed under careful aseptic routine. Parathyroids identified with as little disturbance as possible. In some instances superficial glandules were separated out and the afferent and efferent vessels tied off. In most instances, however, the glandules, after partial separation from thyroid or capsule and identification of parathyroid artery, were lifted up by wide rat-tooth forceps which were crushed into the underlaying thyroid tissue, and a strong linen ligature passed around the whole mass. This procedure seemed to us to more nearly fulfil the conditions of accidental injury that might occur in connection with thyroid operations whereby granulation tissue might be supposed to form from injured tissue about the parathyroids. The parathyroids themselves were not intentionally injured in this procedure although at times they may have received some injury. Fascia and muscles were closed with mattress suture. Skin closed with subcuticular suture. Collodion dressing.

Preliminary to the more complete operation on the parathyroids the following two dogs were used to see the results of ligation of two or three glandules:

Series I., Dog 4. — Operation: ligation of two (external) parathyroids. No symptoms. Dog killed on the sixteenth day after operation. One ligated glandule wholly sclerotic. The other shows persistence of some degenerated epithelial cells. (See histology 4.)

Dog 13. — Operation: ligation of two (external) and one (internal) parathyroid. No symptoms. Dog killed on twenty-fifth day. Ligated glandules completely sclerotic. (See histology 13.)

These experiments simply confirm the well-known fact that loss of half or even more of the parathyroid tissue gives rise to no notable untoward effects, and shows the slowness with which ligation brings on destruction of the glandules.

Certain dogs have not died despite the apparent destruction of the parathyroid glands. In such dogs it was found that either complete fibrosis of the ligated glandules had not occurred or that an intrathyroideal parathyroid had escaped ligation. These dogs are included in this series.

Series II., Dog 16. — Operation: ligation of two parathyroids on left; thyroid with parathyroids removed on right. Dog appeared sick for twenty-four hours after operation, and refused food, but soon recovered and presented no symptoms save some loss of weight. Killed seventeen days after operation. At autopsy a small intrathyroideal parathyroid was found on the thyroid that was not removed. The ligated parathyroids were not wholly destroyed. (See histology 16.)

Dog 3. — First operation: two (external) parathyroids ligated. No symptoms. Twenty-eight days later second operation; internal parathyroid found on left and ligated. Right internal not found and therefore thyroid removed on right. Following the operation the dog progressively lost in weight, but notwithstanding the fact that one thyroid (with parathyroids) has been removed and the two parathyroids left in ligated. No acute symptoms developed. The dog ran about the yard, took food regularly, and appeared perfectly well despite loss of weight. Twenty days after the second operation dog killed and neck organs removed. Dog extremely emaciated. In the parathyroid that was left behind no unligated parathyroid found, neither were aberrant parathyroids found, although careful search was made for same. Of the ligated parathyroids one (forty-eight days) was completely sclerotic; the other (twenty days) showed persistence of functioning tissue at parathyroid site. (See histology 3A.)

Summary.— Dog not dead in forty-eight days, although two parathyroids were ligated on one side and the thyroid (with parathyroids) removed on the other side. Dog kept alive apparently by functioning islet of parathyroid receiving blood supply from adjacent granulation tissue.

Dog 18. — Operation: ligation of four parathyroid glands. Dog is apparently normal in every way at present, and is being kept for longer observation.

Series III., Death in tetany. — In one dog (for the sake of control) we have produced the usual tetanic symptoms by removing thyroids and parathyroids at a single operation, and in two others have had death in tetany following complete operation, subsequent to partial ligation of the glandules.

Dog 17. — Operation: complete thyro-parathyroidectomy. Two days after operation dog developed tremor and died with the usual severe tetanic symptoms.

Dog. 19. — Operation: ligation of eight parathyroid glands. This is the largest number of parathyroids we have found in any one dog. On the external surface of the right thyroid the upper (external) glandule, quite large in size, was found in normal situation. Separated out with its vessels and ligature passed under it shutting off the parathyroid artery and

leaving the uninjured parathyroid lying on outer surface of right thyroid lobe. Internal parathyroid in normal situation but double, each about one-half the size of the external glandule. One of these internal glandules is in the capsule of the thyroid, the other is within the gland but plainly visible. These ligated "en masse." Near the internal border of the thyroid, half way between upper and lower poles, is a fourth parathyroid, small in size, which is also tied off. On the left side four parathyroids are also found corresponding to those on the right, and are treated in the same way as those previously described.

Following the operation the dog did not recover as is usual, was very quiet, and refused food. Examination three days after operation showed injury of the mouth with some slough (gangrenous stomatitis) (evidently induced by trauma of bar placed in the mouth to hold head in position during operation). The dog developed no acute symptoms, however, and at the end of four days the thyroids were removed together with the ligated parathyroids for histological examination. Three days subsequent to the second operation the dog developed the usual tetany and died. The mouth, although treated, was in bad condition at time of death.

Dog 11. — Operation: ligation of two parathyroids on right. Only one parathyroid found on left and that ligated. An intrathyroideal glandule found on microscopic examination later. No symptoms save loss of weight. Second operation one month later; both thyroids removed. The dog developed acute tetanic symptoms on the fourth day after the complete operation and died in convulsions.

Series IV. — This series includes the chronic death with nutritional disturbance and without tetany that has been obtained in six dogs subsequent to gradual loss of the parathyroid glandules. In addition to the parathyroid ligation one thyroid has been removed (either late or early) in four of these dogs, and both thyroids (late) in one. It seems to make little difference whether or not one thyroid is removed in addition to the parathyroids in regard to these nutritional disturbances. We have our most marked examples of emaciation in dogs when the thyroids were not disturbed (see dog 10), and as far as our present work goes we have not been inclined to consider the loss of one thyroid as influencing the eventual results of the experiments. This subject is undoubtedly, however, worthy of careful consideration.

Dog 7. — Operation: ligation of two parathyroids on right side. Removal of thyroid (with parathyroids) on left side. Dog recovered

from operation and showed no symptoms for several days. On the fifth day slept a good deal and took food sparingly. On the sixth day appeared weak and refused food. Died on the seventh day very quietly, but with the development of a slight tremor just before death. Microscopic examination of the ligated parathyroids showed them to be replaced by fibrous tissue.

Dog 15. — Operation: ligation of four parathyroid glands. The dog did not show the usual temporary recovery that follows either excision or ligation, but appeared sick from time of operation on. Took very little food. Lay curled up in cage in lifeless condition practically all the time. Died very quietly, with no tremor whatever, ten days after the operation. Autopsy showed perfectly healed neck wound, but there was considerable swelling of cervical lymph nodes, and lymph nodes under sternum. The tissue about thymus was edematous. The trachea and bronchi contained pus. Microscopically one of the ligated parathyroids was found not wholly fibrous, although nearly so. The others showed no functioning tissue. Undoubtedly, death in this instance was hastened by infection.

Dog 19. — Operation: ligation of two parathyroids on right. Removal of thyroid (with parathyroids) on left. Dog recovered perfectly from the operation and lived thirty-three days. During this time he progressively lost in weight and strength. About two weeks after the operation cloudiness of the cornea was noted and a keratitis developed, resulting in total blindness. In connection with this was a purulent conjunctivitis. The end of the third week after operation a purulent discharge from the nose appeared which persisted until time of death. At autopsy the ligated glandules were found completely replaced by fibrous tissue, but an intrathyroideal parathyroid was found that had escaped ligation

Dog 10. — Operation: five parathyroids found (the left external double) and all ligated. Despite the apparently complete operation the dog showed no acute symptoms. He lost rapidly in weight, however, and a slight conjunctivitis developed. Forty-four days after first operation neck was again opened and a large (hypertrophic?) parathyroid found on the right (evidently analogous to the double of the left external) that had escaped us at the first operation. This was ligated. Ligatures of our previous operation in place and only connective tissue thickening to be seen macroscopically about them. Neck closed up. Dog showed marked weakness, could scarcely stand on his feet a few days after the operation, and took food sparingly. Despite this great emaciation and weakness (together with some purulent eye discharge) the dog remained in this condition for eighteen days, getting toward the end so weak it was difficult to tell whether he was living or dead. He lay curled up and sleeping for days without change of position and without taking food. Finally found dead in this position. Autopsy: Coat is rough and rubbed off at points of pressure. At edge of first and second molar teeth on either side gums are ulcerated. The deciduous teeth are carious at this point and easily broken off with small forceps. (This is a young dog) The bronchi contains pus and there are areas of broncho-pneumonia in lower

lobe of right lung. Thyroids appear normal. Parathyroids: One gland found that had escaped ligation. Glandules first ligated completely sclerotic. Those ligated at second operation show slight remnants of parathyroid structure.

Dog. 12. — Operation: only two parathyroids found, one on each side, and these ligated. No symptoms save loss of weight. Second operation twenty-seven days later. Still unable to find the other parathyroids, so both thyroids removed. The dog did not recover brightly from the second operation. Was very quiet for two days. Refused food and vomited after drinking water. The third day the dog was very weak and a beginning keratitis was observed. There was occasional slight difficulty with respiration. Dog died quietly on the fourth day. No tremor.

Microscopic examination of the neck organs showed complete destruction of the ligated parathyroids. Although the thyroids were thoroughly searched from pole to pole by frozen sections at close intervals only one other parathyroid found. This was a small intrathyroideal body.

Dog. 5. — Operation: ligation of two (external) parathyroids. No symptoms. Eighteen days later left thyroid removed and internal parathyroid ligated on right. (An intrathyroideal parathyroid found in removed thyroid.) Dog lost weight gradually but progressively. Took food better and showed less loss of strength than animals previously described. Did not develop infection, nor eye lesion. Forty-seven days after the second operation the neck was again exposed. The parathyroids that had been ligated were cut out for histological examination. The thyroid was tied off at upper and lower poles to cut off all blood supply and left in place. The dog made a good recovery from this operation, but died in three days, quietly, with no tremor. Microscopic examination of the ligated parathyroids showed persistence of a few groups of cells corresponding to parathyroid in one ligated glandule, although sixty-five days had elapsed since ligation.

General consideration of the microscopic appearance of the parathyroids after ligation:

A histological study of the ligated parathyroids shows that, although in general we have endeavored to use the same technic in regard to ligation, we have not succeeded in destroying the glandules with any degree of regularity. We have found complete fibrosis to follow our ligation as early as seven days after the operation, and again as late as sixty-five days we have had a persistence of parathyroid tissue despite the ligation. While this uniformity of parathyroid destruction is not of our choosing, still it has led to some interesting results.

Histology. — A complete detailed description of all the ligated glandules would occupy so much space that only the more important findings will be outlined. In general it may be said that following the ligation of a glandule, as we have practiced the same, there is a temporary attempt at hyperfunction as shown by the activity of the eosin staining cells. Proliferative changes ultimately result, however, whereby the epithelial cells of the parathyroid are replaced by dense connective tissue. This result is brought about in a varying length of time. We have found glandules wholly fibrous on the seventh day after ligation, and on the other hand have found parathyroid epithelium persisting sixty-five days after ligation. Accompanying the new connective tissue growth we find in our earlier specimens a varying number of lymphoid and plasma cells. The center of the glandule may become wholly necrotic, simulating somewhat the appearance found in the center of a caseous miliary tubercle. At the periphery of the glandule granulation tissue, rich in new formed blood vessels, which are apparently keeping alive islets of the tissue, has been found as late as twenty-six days after ligation. One of our four-day glandules would indicate that at first there is a marked increase in the number of "functioning" cells, which is, however, only temporary. The appearance of the persisting parathyroid epithelium in more or less fibrous glandules is variable, sometimes the individual cells differ little from normal, but soon various stages and forms of degeneration can be seen consisting of cloudy swelling and vacuolation of the protoplasm, loss of nuclear detail, and finally fragmentation of the nuclei. At times there is observed the fusion of protoplasm of a number of these cells with persistence of nuclei, giving the appearance of giant cell formation. We have never at any time seen an attempt at regeneration of the parathyroid epithelium.

The general appearance of the different cases is briefly as follows:

Dog 19. — Four days after ligation. Young connective tissue, rich in blood vessels, is seen at the periphery of the

glandule. Sharp capsular differentiation is lost. The young connective tissue cells and new formed blood vessels penetrate the glandule, so that it is difficult to distinguish these from the parathyroid cells and original vessel bearing stroma of the gland. Deeper in the gland large islets of partially degenerated "functioning" cells are seen. The protoplasm of these cells has in places flowed together and is homogeneous, rather than coarse granular. The capillary spaces are distended and in places there is hemorrhage. The central part of this glandule shows beginning necrosis. Other of the glandules, ligated at the same time, show much more necrosis, with considerable hemorrhage and a fair sprinkling of leucocytes.

In this destruction the periphery appears, as a usual thing, to persist most strongly. The center is soon rendered necrotic from loss of blood. The periphery is supplied by the new formed granulation tissue for a time at least.

Dog 7 (seven days after ligation). — Section of the ligated glandules shows a mass of connective tissue. At the periphery of this mass the fibers present a circular arrangement. The nuclei are spindle-shaped. Towards the center the tissue is richer in nuclei, some of which are round or oval, closely crowded together. A considerable number of lymphoid and plasma cells are included in this mass. No parathyroid epithelium can be made out.

Dog 15 (ten days after ligation). — Ligated glandules are fibrous save one. This presents a thickened fibrous capsule in which only a few small blood vessels are seen. The central part of the gland is necrotic. In one segment next the capsule is seen an area somewhat fibrous, but still evidently parathyroid in structure (Fig. 1).

Dogs 4 and 16 (sixteen and seventeen days after ligation). — One glandule is similar to the case last described save that the capsule is somewhat more vascular. At the edge of the glandule is a completely closed blood vessel with thickened fibrous walls. High power examination of the cellular structure within the gland shows young connective tissue, polymorphonuclear leucocytes, and lymphoid and plasma

cells. Near the periphery an occasional group of cells persist, corresponding to cells of parathyroid tissue. While individually it is difficult at times to distinguish these cells from polyblasts, still their arrangement in threes or fours at times is suggestive, and degenerative changes in them may be distinct. At times the protoplasm of a group of these cells fuses together leaving the nuclei clumped in a single protoplasmic mass suggesting a typical giant cell (Figs. 2 and 3). One of the seventeen day glandules presents pretty examples of degeneration of the "principal" cells. A small hyalin islet suggests the degeneration of a mass of "functioning" cells. The other ligated glandules in these dogs are fibrous or necrotic.

Dog 13 (twenty-four days after ligation). — Practically complete sclerosis (Fig. 4).

Dog 3A (twenty-eight days after ligation). — Incomplete sclerosis in one glandule. Either our ligature was not successfully placed or there was a small accessory parathyroid at the edge of the ligated parathyroid, for a tiny glandule is seen in which there is little change. Outside of this is considerable loose connective tissue rich in blood vessels (granulation tissue) in which compact irregular islets of parathyroid tissue persist (Fig. 5) as remnants of the ligated glandule.

Dogs 11 and 12 (thirty days after litigation). — Complete sclerosis in general. One glandule shows suggestions of some of the original cells which appear as closely set nuclear masses (Fig. 6). In one ligated area a tiny mass of practically normal parathyroid tissue is found which we interpret as a minute accessory glandule that was not included in the ligature (Fig. 7).

Dog 3B (forty-eight days after ligation). — Completely sclerotic glandules. One with necrotic center (Fig. 8).

Dog 10 (sixty-two days after ligation). — Glandules completely sclerotic.

Dog 5 (sixty-five days after ligation). — The connective tissue at the point of ligation is dense and runs irregularly in broad bands practically devoid of nuclei. There are no

blood vessels to be made out. Between the broad connective tissue septa several fusiform or narrow slit-like openings appear in which masses of cells corresponding to those of the parathyroid are seen. These cell masses have no resemblance to original arrangement, but are closely crowded together. The cell nuclei are sharply defined, but the protoplasm is scant. Near the center of the section the tissue is necrotic.

SUMMARY AND CONCLUSIONS.

Following the gradual destruction of the parathyroid glandules in the dog a train of symptoms arises different from those obtained by parathyroid excision. After ligation of all parathyroid tissue the dog passes the time limits of tetanic death that occurs after excision of the glandules, practically without symptoms. Gradually, however, chronic symptoms, trophic in nature, arise. These consist in gradual but progressive loss of weight and strength, greatly diminished resistance to infection, and a final stuporous condition ending in death without tetany.

These nutritional disturbances are as marked when the thyroid is not injured as they are when the thyroid is removed on one side.

These observations should lead to a modified consideration of diseases that are supposed to be hypo-parathyroid in origin, and suggest a revision of the epitomized statement of Jeandelize, "that insufficiency of the thyroids causes nutritional disturbances, while insufficiency of the parathyroids causes acute convulsive troubles." The preferable statement regarding the parathyroids as the result of our work is, that while sudden loss of the parathyroids results in acute convulsive troubles, slow destruction of the same gives rise to chronic nutritional disturbances, which eventually end in death without tetanic manifestation.

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DESCRIPTION OF PLATES III. AND IV.

FIG. 1. — Parathyroid glandule ten days after ligation, showing persistence of parathyroid structure at periphery of gland just under thickened capsule.

FIGS. 2 and 3. — Parathyroid glandules sixteen days after ligation, showing fusion of masses of degenerated parathyroid epithelium.

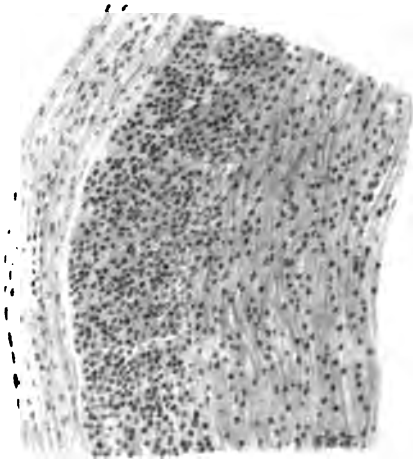
FIG. 4. — Site of parathyroid twenty-four days after ligation. Glandule replaced by connective tissue.

FIG. 5. — Granulation tissue at edge of persisting islet of parathyroid tissue twenty-eight days after ligation.

FIG. 6. — Site of parathyroid thirty days after ligation. Only a slight suggestion of original structure remains.

FIG. 7. — Small nodule of practically normal parathyroid tissue apparently separate from ligated glandule. Found at site of ligation thirty days after operation.

FIG. 8. — Replacement of glandule by dense connective tissue, the center of which is necrotic, forty-eight days after ligation.



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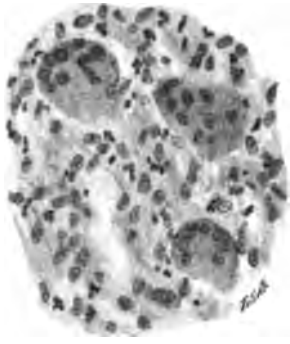

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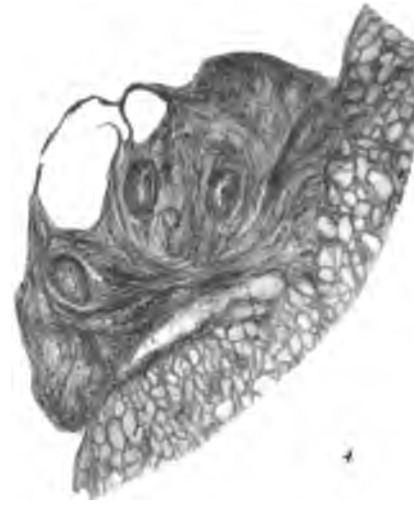

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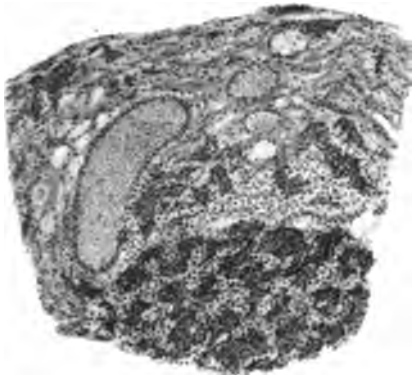

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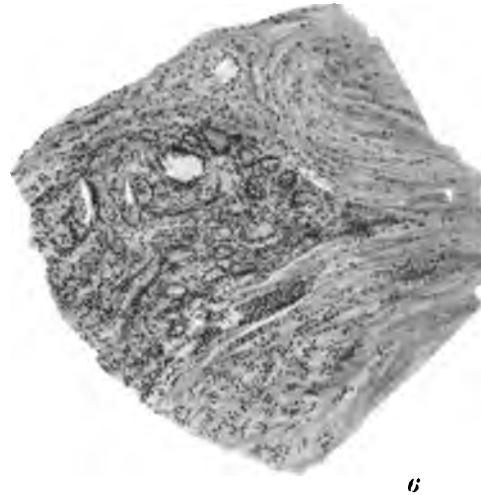

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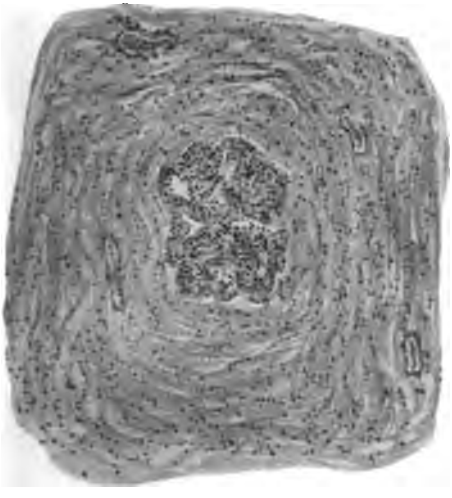

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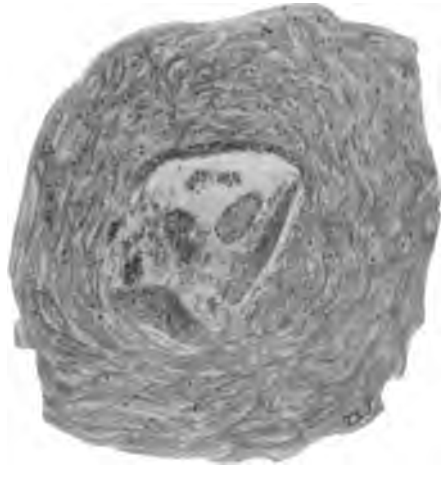

8

A CONSIDERATION OF THE PATHOLOGICAL HISTOLOGY OF THE PARATHYROID GLANDULES, AND A REPORT OF A PARATHYROID-LIKE TUMOR.*

R. L. THOMPSON, M.D.

(*Professor of Pathology, St. Louis University School of Medicine.*)

AND

D. L. HARRIS, M.D.

(*City Pathologist and Bacteriologist, St. Louis.*)

(*From the Laboratory of the Board of Health of St. Louis, and the Laboratory of Pathology, St. Louis University.*)

Since we know by classic animal experiments and by accidents that have happened to man following struma operations that the loss of the parathyroid glandules is followed by loss of life with acute and severe tetanic manifestation, and even partial loss of glandules by very definite symptoms, it would seem necessary that the examination of such important organs should be a part of every properly conducted autopsy routine. The fact that such routine examination may add but little to our knowledge of these structures is only an instance of the well-known limitations of pathological anatomy and, while less interesting and prolific in results than experimental study, is offered here that a few morphological notes may be added to the observations that have already been made along these lines.

This report includes the study of the parathyroid glandules from two hundred and fifty routine autopsies at the St. Louis City Hospital. The glandules were fixed in Zenker's fluid or in formalin and imbedded in paraffin. A wide variety of stains were employed, of which Mallory's phosphotungstic acid-hematoxylin was considered the most useful for general purposes. For fat determination frozen sections after formalin fixation were stained with Scharlach R.

At first we had in mind a comparative study of the functioning cells of the glandules, and for this numerical

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determination many cases were cut in serial section. There seemed to be no rule to cover variations in the number of these cells, however, and so this part of the work was not followed up. In all, we have examined upward of seven thousand paraffin slides in addition to many frozen sections that were necessary for the fat determination and for purposes of orientation.

We will pass over the gross appearance, size, situation, and number of these bodies as our observations in these respects differ in no essential from the oft-repeated description of previous investigators who have done any considerable work on these glands.

(There are four parathyroid glands. Any more or any less in a given case is due to errors of development. One lacks sympathy with authors who state that there are three parathyroid glands, based on an examination of a dozen neck organs. We have found at least four glandules in more than ninety per cent of all cases in which we have made careful search for them. In fifty neck organs given to second-year medical students who had never searched for parathyroids before, the average number found was in excess of three per case, microscopically controlled.)

Since these glands were first described by Sandstroem in 1880 they have attracted more or less sporadic attention, but the most of the work, thanks to the discovery by Gley of their great importance, has been along physiological lines. Restricting our references to purely morphological observations relating to deviations from the described normal histology of the parathyroids, the following authors may be cited who have contributed to their pathological histology, or who have suggested diseases that might be preferred to such pathological alteration.

Sandstroem mentioned that cystic degeneration, and amyloid infiltration of the vessel walls and capsule occurs in certain cases. Müller called especial attention to fatty change. Königstein studied especially the secretion of the glandules from a histological standpoint, but stated he could not bring anatomical changes into correlation with clinical

conditions. Verebely in one hundred and thirty-eight cases described various lesions of the glandules, including two instances of tuberculosis, three cases of cyst, three of hemorrhage, and one tumor. Getzowa called especial attention to the colloid content, which was found present in nearly all cases over ten years of age. Pepere noted a number of progressive and retrogressive changes, including suppuration. Guizzetti described dense mononuclear cell infiltration of the parathyroid in two cases of tetanus. Yanase found hemorrhage thirty-three times in eighty-nine children showing tetanoid conditions. Kohn described hemorrhagic cysts. Peterson, who examined one hundred cases, noted the frequency with which degenerative changes are found in the glandules from cases over twenty years old. Among these changes he found atrophy of the parenchymatous cells brought about by fatty changes, cloudy swelling, and cystic degeneration very frequently. In twenty-five of his cases he found cloudy swelling; in fifteen, colloid; in six, cyst formation; in twenty-one, fatty infiltration. This author was unable to correlate changes in the gland with clinical conditions.

Benjamins, in twenty cases of goiter, found no progressive changes in the glandules but a variety of retrogressive changes. In a general study of the parathyroids he found hydropic degeneration twenty-five times, pigment atrophy, connective tissue increase, and frequent colloid. He described a tumor of the parathyroid the size of a child's head.

Erdheim found glycogen and colloid frequently and observed mast cells in the connective tissue of certain of the glandules. He also noted the frequency of cysts. He found hemorrhage in eight cases.

One of us has called attention to the condensation of the cytoplasm at the edge of the cell in these glands, due to various degeneration products such as fat, glycogen, and colloid. This produces the optical appearance of an intercellular framework which characterizes many of these glands. In a later paper by the same author, degenerative and especially progressive changes were described in these glandules in cases of primary infantile atrophy.

Forsyth, who regards the cells of the parathyroids as all of a single type representing different stages of activity and rest, has described excess of colloid, connective tissue proliferation, both general and perivascular, and also speaks of instances (in animals) where there is a similarity between thyroid and parathyroid structures.

MacCallum, who has worked more extensively on these glandules than any one else in this country, found in certain of the glandules examined following thyroid removal for exophthalmic goiter some increase in fibrous stroma and moderate atrophy of the cells. In general, however, the parathyroid tissue was abundant and normal in these cases. A tumor of a parathyroid and hyperplasia of the glandules in gastric tetany has also been noted by this author.

A summary of these various pathological conditions, together with certain other cases, may be given as follows:

Degenerative changes. — Peterson, Benjamins, Erdheim, Thompson.
Amyloid infiltration. — Getzowa, Escheich, Sandstroem, Pepere.
Progressive changes. — Benjamins, MacCallum, Thompson, Forsyth, Verebely, Erdheim, Pepere, Guizzetti.
Hemorrhage. — Kohn, Benjamins, Peterson, Erdheim, Verebely, Yanase, Getzowa.
Cysts. — Sandstroem, Kohn, Benjamins, Peterson, Schaper, Nicholas, Kursteiner, Maresch, Peucher, Aschoff, Verdum, Erdheim, Verebely.
Tumors of the parathyroids have been reported by De Santi, Askanazy, Hulst, Benjamins, Weichselbaum, MacCallum, Erdheim, and Verebely.

The attempt to establish a symptom complex for diseases of these glands has been due to the results of physiological experimentation rather than to histological findings, and a great range of diseases, in which tetanic symptoms are present, have been advanced as due primarily to deficiency in parathyroid secretion.

In many of these diseases examination of the parathyroids has failed to reveal constant morphological change when symptoms were such as to suggest severe or complete loss of their functioning power.

Exophthalmic goiter. — One of the first conditions for which a parathyroid etiology was claimed (by Mossu, Gley, and Edmunds) was exophthalmic goiter. This theory was not supported owing to the work of Benjamins and of MacCallum, who found (l.c.) no constant lesions in the parathyroid glandules in this disease.

Post-operative tetany and death following thyroid removal in man will not be discussed here except so far as to note that in these cases histological search reveals the loss of the parathyroid glands. Reverdin, Kocher, Erdheim, and others have contributed adequately to the surgical phases of this question.

Adult tetany. — A loss of parathyroid function was first suggested by Jeandelize as the cause of tetany in adults and later emphasized by Pineles, who grouped together thyroid tetany, occupation tetany, tetany of childbirth, children's tetany, and gastric tetany. This identity of different forms of tetany was accepted by Chvostek, who stated that functional diseases of the parathyroids is the most plausible explanation of tetany. Microscopic lesions have been described in the parathyroids in various forms of tetany. MacCallum found hyperfunction of the glandules in gastric tetany, and Königstein also reported a case in which similar changes were found. In tetanus Guizzetti found infiltration of mononuclear cells in two cases in which the disease had lasted for four and seven days respectively. Two other cases were negative.

Tetany of children. —Erdheim (three cases) and Königstein have found hemorrhage in the parathyroids in children exhibiting tetanic symptoms. Verebely, and also Thiemich, have found hemorrhages in cases where there was no tetany. The most convincing work in this line has perhaps been done by Yanase who examined the parathyroids in eighty-nine children showing tetanoid conditions and found hemorrhage in thirty-five cases. Degeneration of the parathyroids in a case of tetany in course of a case of tuberculous meningitis has been described by Escherich.

Eclampsia. — The greater amount of work on this condition has been experimental, but several authors have examined the parathyroids in this condition. Pepere found changes in the glandules in four cases. Zangfrognini found only two glandules in a case, but these were both normal. Erdheim in four cases found hyperemia, circumscribed injury once, hemorrhage once.

Epilepsy. — Erdheim found sclerosis of all four parathyroids in a case of epilepsy. In another case he found the glandules normal.

Paralysis agitans. — Insufficiency of the parathyroids was suggested as the cause of this disease by Lundburg, by Berkeley, and by Alquier, but no changes in the glandules were described by these authors. In autopsies on nine cases of paralysis agitans, one of us found the glandules negative. Erdheim also failed to find a parathyroid hypoplasia in three cases of this disease.

Myxedema. — In a case of myxedema with atrophic thyroid, Forsyth found six parathyroids showing marked sclerotic change and much colloid.

Other authors (Erdheim, Maresch, Peucker) have noted their presence in congenital absence of the thyroid without describing the lesion of the same. Vincent and Jolly state that in their belief myxedema must be more complex than simple thyroid insufficiency.

Rachitis. — Escherich, under whom the work of Yanase previously cited was done, has suggested congenital parathyroid hypoplasia as an etiological factor in rachitis. This assumption is based on the frequent coincidence of tetany and beginning rachitis as well as Erdheim's findings in the teeth of parathyroidectomized rats. At present this hypothesis lacks morphological confirmation. Schmorl found no changes in the parathyroids in four cases of rachitis.

Osteomalacia. — In two autopsies in this disease Erdheim found hyperplasia of the parathyroids in one case and normal glandules in the other case. Schmorl in four cases found the glandules normal three times and hyperplasia in one case of one of the upper glandules.

Primary infantile atrophy. — In this disease one of us found constant changes in the parathyroids, practically all of a progressive nature, which the author considered the result of this condition rather than an etiological factor in the same. As tetany was never observed in these cases the findings are interesting as tending to show that extensive changes may take place in the parathyroids without exhibition of any tetanic symptoms, a finding that might serve as a check, perhaps, upon a too liberal interpretation of morphological change in cases that do exhibit tetany.

In the present paper we have made no effort to go into the study of the parathyroid glandules in individual diseases, but rather have attempted to pave the way for such study by a careful histological examination of these bodies in routine autopsies, being willing to accept a parathyroid pathology as a factor in any condition in which sufficient proof of the involvement of the glands should offer itself. While as we expected, and as has been the experience of other authors, we have been unable to correlate to any extent clinical symptoms and morphological parathyroid alteration, we nevertheless add the following few morphological notes of certain histological findings in these glandules that have seemed to us to be of interest:

Fat. — The fat content of these glandules is so constant in the adult that it gives a distinct yellow color to the gland and serves as a macroscopic aid in differentiating parathyroids from lymph nodes, accessory thyroids, or thymus, sympathetic nerve ganglia, or other bits of tissue which make

the search for these organs more or less difficult, especially to one who has not had considerable experience in their isolation. Microscopically one should differentiate perhaps between the fatty content of the connective tissue of the gland and the fatty content of the cells of the parenchyma, although as a matter of fact it is doubtful if one occurs to any marked extent without the other being present. We have seen a great number of the glandules in which there was a replacement of considerably more than half the parathyroids with fatty tissue and in which, in addition, the principal cells of the gland contained fat; but such cases showed nothing clinically that would serve to call attention to a lack of parathyroid function.

While we believe that in general more fat is to be found in elderly individuals than in those of middle age, still we would hesitate to accept the view that a regular and constant increase of fat is an accompaniment of increasing age. We found a number of glandules in individuals over sixty years of age that are only moderately fatty, and on the other hand we found glandules in patients from twenty to thirty years of age in which there was marked fatty change both in parenchyma and stroma. We would not, therefore, limit the diagnosis of fatty degeneration to the earlier years of life, although we admit the increased difficulty of making such a diagnosis in the later years.

The fat content is, as previously stated, so physiologically variable that one hesitates to attempt any classification for fatty degeneration of the glandules. We can only say that the most marked changes in our cases, the factor of age being kept in mind, have been found in the following conditions: cirrhosis of the liver; chronic nephritis, especially chronic parenchymatous nephritis; chronic heart affections with the usual associated lesions; chronic tuberculosis; diabetes. Especially are the glandules apt to be fatty when an acute infection is superimposed on a chronic condition. The most constant and marked fatty change in any one series of cases was in five instances of ascending infection of the genito-urinary tract with pyelonephrosis. In all these cases, which

were of various ages, we found marked fatty change in the parathyroids. These cases are representative only of a type of rather long continued acute infection where considerable chronic disease of the lungs, heart, and liver was present.

The association of marked fatty change in the parathyroids with cases of infection of the gall-bladder and ducts, with extreme jaundice in four cases that we had of this condition, might be noted in passing. In malignant diseases, carcinoma especially, either of comparatively long or comparatively short duration, we found no fixed condition of fatty content in the parathyroids. At times these organs showed marked fatty change; at times there was no apparent increase of fat. The same was true in regard to the parathyroids in cases dying from uncomplicated acute infectious diseases of short duration such as lobar pneumonia. A case of tertiary syphilis (the only one in our series) showed marked fatty degeneration of the glandules.

Colloid. — It would be unfair to exclude the presence of colloid unless serial sections are made of all the glandules, although if colloid is present at all it is usually more or less widely distributed in a given gland. We found colloid in about fourteen per cent of all our cases, and agree that the presence of a certain amount of colloid in individuals over twenty years of age is not to be considered abnormal. The interesting point in regard to colloid is the fact that its secretion not infrequently leads to appearances in the parathyroid that makes circumscribed areas within them exceedingly suggestive of thyroid structure. These areas begin by a dozen cells, more or less, assuming an alveolar arrangement. In the center so formed a droplet of colloid appears. Continued secretion of colloid pushes back and flattens the cells so that finally a follicle, similar to those seen in the thyroid gland, appears. If enough of these are formed in juxtaposition, thyroid-like structure results. Usually, however, these colloid follicles are discrete, or the amount of colloid is not sufficient to alter the general typography of the glandule.

Even though a picture somewhat like thyroid structure may be produced, one should remember that on embryological, anatomical, and physiological grounds there is no relationship between human thyroid and parathyroid, save that of propinquity. We consider them wholly independent from each other, and see no reason for assuming that one acts for the other, although it is probable that there is some interaction between the two. We do not believe, as stated by Forsyth, that histologically intermediate stages between thyroid and parathyroid are common, in the human being at least, nor that the difference in the glands is merely a difference in the amount of secretion, neither have we reason to suppose that the parathyroids exhibit a partial change to thyroid structure with advancing age as claimed by Rogowitsch.

Vincent and Jolly find that parathyroid tissue left behind after thyroid extirpation "approximates in appearance to ordinary thyroid tissue" and believe that the parathyroid functionally replaces thyroid. Their view is directly opposed by Hagenbach, however, who obtained a typical cachexia thyroprivia when two parathyroids are left behind.

We do, however, find in this region appearances which we choose to consider accidents of propinquity and in which there is an apparent transformation of one organ into the other, but which we think should be interpreted on more rational grounds than transformation of parathyroid into thyroid.

In this case (which showed at autopsy caseous tuberculous pneumonia, chronic pleuritis, localized peritonitis, and peri-hepatitis) there were fairly firm adhesions in places between the capsule of the thyroid and surrounding tissue. The upper parathyroids were normal; left lower not found; left right lower pole showed a circumscribed thickening of the surrounding structures and was excised. Microscopically, section of this showed, on the outer edge, fairly typical parathyroid structure penetrated by a dense connective tissue stroma. The inner part of the section showed typical thyroid structure with a similar increase of stroma. There

was no line of demarcation between the two, but one seemed to run into the other so as to suggest the transformation of parathyroid into thyroid tissue. It seems more rational to assume, however, that a peri-thyroiditis leading to proliferative changes in both glands joined the two organs together in this peculiar manner, the connective tissue ingrowth being so distributed that both appear to be one and the same organ.

Degenerations. — Acute degenerative changes occur in the parenchymatous cells of the parathyroid glandules, but a diagnosis of "cloudy swelling" or "acute degeneration" is to be made only when one can exclude post-mortem changes and other adventitious factors that might arise wholly apart from intrinsic parathyroid changes. In many cases the glandules are macroscopically enlarged, are soft and pale, or firm and tense. These changes are usually due to increased fluid content (edema) and are practically always a part of a general edema of the neck organs. Microscopically the cells in such glandules are larger than normal, the cytoplasmic granules are more distinct than usual and the cell nuclei large and pale. Frequently the usual structure of the gland is lost and no good cell pictures obtained. We have been unable to fix such appearances as being of significance.

Hemorrhage. — We found hemorrhage in our cases only three times. The rarity of hemorrhage in adults has been noted by Erdheim, who found it six times in children but only in one instance in an adult. Getzowa found it only once in the adult. Yanase also speaks of the infrequency of hemorrhage in these glandules in adults although he has been able to demonstrate it frequently in the first year of life, as previously noted. Verebely found hemorrhage only once in the adult (twice in children) in his one hundred and twenty-five cases, Benjamins and Peterson only report single cases, the latter in one hundred autopsies. Our cases of hemorrhage were found in connection with toxic glomerulonephritis, marked general anemia secondary to syphilis, and acute parenchymatous nephritis, respectively. In none of these cases was there any clinical manifestation of tetany.

Fibrosis. — The amount of connective tissue found in the

parathyroids is, in general, subject to wide variation. The gland may consist of a continuous mass of epithelial cells penetrated by a considerable capillary network, unaccompanied by connective tissue, or there may be a continuous reticulum running throughout the gland. When the gland is broken up into distinct islets by a decided connective tissue stroma there is in the gland more connective tissue than should be considered normal for the structure. Some authors, however, choose to classify this as a particular "type" of gland. In any event the widening of such a stroma and the decreased size of the islets leads to the different degrees of what may be termed "chronic interstitial parathyroiditis." Or, as Verebely, who found the condition well marked in two cases, terms it, "parathyroiditis chronica fibrosa." The best examples of this condition, and the only condition in which fibrosis is constant, has already been described by one of us in connection with primary infantile atrophy.

In the present series of cases we have met with every possible variation in connective tissue content of these glandules. We will leave out of consideration all save the more advanced cases, although in a number of glandules that are excluded fibroglia fibrils can be demonstrated in many instances, especially in thickened connective tissue in the neighborhood of blood vessels; an index to the beginning of this process.

The cases exhibiting connective tissue increase in the parathyroids are, in our series, almost without exception poorly nourished individuals showing at autopsy chronic heart lesions with general chronic passive congestion, cirrhosis of the liver, and chronic tuberculosis. However, the greater number of cases exhibiting the above lesions show no changes in the parathyroids, so that chronic fibrous parathyroiditis is not necessarily an accompaniment of these conditions, although it may be most frequently found in connection with such.

That specific infectious agents may bring about this condition is suggested by the extreme sclerosis found in a lower parathyroid in a case of acute miliary tuberculosis. The reaction in this case can be compared to the appearance

sometimes seen in very chronic, tubercle bacilli poor, tuberculosis of lymph nodes, where there is little or no caseation or tubercle formation but marked connective tissue hyperplasia.

In our opinion the fact that even in extreme age and in a great variety of severe disease the parathyroids are so comparatively free from lesion is more noteworthy, and a better proof of their importance than would be the frequent finding of lesions that would seriously impair their function. In none of our cases that show fibrosis was there any clinical manifestation of tetany. The progressive destruction of the parathyroids has been brought about experimentally without tetany and is discussed in another paper. (This number, p. 121.)

Cysts. — Cysts occurring in connection with the parathyroid glands may be broadly classified as (1) retention cysts; (2) polycystic degeneration; (3) cysts arising either without, or in the neighborhood of, the parathyroids (branchial cysts). The production of cysts of the former class has already been discussed under colloid. These small cysts are quite common. We have found them in about five per cent of our cases They may be single, or three or four may be found in a single glandule; rarely do these cysts exceed the diameter of a low power field. We have not found a single example of polycystic degeneration, although such a condition has been found by other authors — Schaper in the parathyroid of a sheep; Erdheim in an eighty-three-year old woman. Verebely also describes a similar picture in the parathyroid, but on account of the variation in the lining epithelium of the cysts prefers to class his case as a branchial polycystoma.

It would seem that colloid filled spaces are in general so frequent that there is no necessity for considering these "retention cysts" in detail unless they assume a number or size that brings them into relation with the condition described elsewhere, namely: true cysts and thyroid-like structure.

The cysts included in class (3) are to be considered as

developmental anomalies. Verdum has contributed extensively to their embryological development, and more recently Erdheim has sought a classification for these branchial cysts. The latter author finds two different types of cysts in relation with the upper parathyroids which arise from the fourth gill pouch. The more common and better known cysts are, however, in relation with the lower parathyroids which arise from the third gill pouch. Verebely describes in detail two cysts in connection with the upper glandules, one of which he terms a post branchial cyst, the other a branchial cyst.

We have found only two cysts of the parathyroid of any considerable size. The first in a woman seventy-two years old with atrophic thyroid (lateral lobes measured only 3 x 2 x .5 centimeters), upper parathyroids in normal position but quite small (5 x 1.5 x .5 millimeters). Left lower parathyroid not found. At the base of the right lower lateral pole of the thyroid is a parathyroid gland forming a flattened cap to a cyst which measures two by two by two and one-half centimeters in diameter. The cyst wall is lined with a single layer of flattened epithelium.

The second cyst was also in connection with a lower glandule and practically its equivalent in size (7 x 4 x 3 millimeters). This was a simple cyst.

Tumors. — Tumors of the parathyroid have been reported by a number of writers as previously mentioned; the total number being twelve. Most of these tumors represent only reproduction of parathyroid tissue, and while some authors designate the growths as adenoma, Hulst raises the question whether some, at least, should not rather be designated as hypertrophy and hyperplasia. Weichselbaum leans towards the designation of adenoma for his tumor but calls attention to the fact that the boundary line between adenoma and hyperplasia cannot be sharply drawn. Erdheim describes three cases as adenoma and refers to two more as hyperplasia of the parathyroid. Most of these growths have been comparatively small. The cases of Hulst, Verebely, MacCallum, and Erdheim average about two and one-half by one and one-half by one and one-half centimeters. The largest

described tumor is that of Benjamins, which developed within three years to the size of a child's head. This tumor, as well as the one described by Hulst, was intrathyroideal. The others were outside the thyroid, either in or replacing parathyroid glandules.

Verebely very logically brings up the point that the origin of these intrathyroidal tumors (Benjamins and Hulst) may be called into question. In Benjamin's case the tumor was removed at operation and therefore its relation to the parathyroids geographically could not be determined. Hulst also failed to describe the parathyroids in his case. As Verebely says, there is a great similarity between the cells of the parathyroid and rapidly-growing parenchymatous thyroid nodules, so that one must keep in mind the question of congenital fetal anomalies in the origin of these growths; such, for instance, as the failure of closure of the central canal of Prenant. The work of Getzowa and of Langhans has thrown much new light upon these epithelial forms of malignant struma and brought up the question of the origin of certain types, at least from the post branchial bodies.

It is with these difficulties of classification in mind that we present the following tumor, which was removed at operation so that no careful dissection of the neck could be made. The extreme similarity of the greater part of the structure to parathyroid tissue justifies its discussion in this place, although certain parts of it suggest the possibility of its origin from the post branchial body. We have chosen to call the growth simply a parathyroid-like tumor and regret that circumstances were such that neither a careful topographical study of the neck region could be made in the case, nor even, owing to the way the tumor was received, could the differential histological study, especially in regard to glycogen content, be done that was desired.

We wish to express our indebtedness to Dr. O. H. Elbrecht, Superintendent and Surgeon in charge of the St. Louis Female Hospital, who removed this tumor, both for permission to report the case and for the very complete records which we have abstracted briefly.

Clinical history. — Patient, white female, age twenty-three years; occupation, housework; born in Missouri. Family history negative. Previous history negative. Present illness: "Began when an infant and has grown gradually up to present time. Thyroid gland is enlarged to about the size of patient's head. Lobes can be palpated and also isthmus connecting them. Not painful, only symptoms those due to pressure." Diagnosis, goiter.

Abstract from operation report. — "The growth was extremely large and involved both lobes of the thyroid, but more on the right than on the left. The growth occupied the whole anterior portion of the neck between the sterno-cleido-mastoid muscles and hung downward to a point two or three inches below the upper end of the sternum. It was lobulated on each side and in several areas on the anterior surface. The tumor mass was rather firm in consistency and caused a serious obstruction to respiration, so much that the patient could not be anesthetized in the dorsal position on account of pressure on the trachea. The tumor was carefully freed from its attachments on the lower and outer sides, held outward and divided at its base just to the right of the isthmus." Patient suffered considerably from primary surgical shock; pulse rapid and weak; died six hours after operation.

Gross appearance of tumor. — The specimen is a nodular encapsulated mass (15 x 10 x 6 centimeters), weighing two hundred and fifty grams. Of the nodules, some push sharply above the level; others are low, broad, and seemed fused together. The larger reach a diameter of four centimeters; the smaller measure one-half to one centimeter. The tumor is firm throughout. The capsule is thick, fibrous, and tense, and entirely covers the mass with the exception of an area, four by five centimeters, which represents the severed point of attachment. This capsule dips between the nodules and marks their outlines. The color is brownish yellow, mottled by scattered hemorrhagic areas in and upon the nodules. There are no large or congested vessels to be seen.

On section (Fig. 1) the mass is found to consist of many discrete and confluent light brownish yellow areas separated more or less by fibrous trabeculæ. These areas correspond to the superficial nodules. They are firm, homogeneous, and friable; their cut surface is a milky exudate, rich in cells. Variations in the appearance of these areas depend, in the main, upon the blood content. Some are pale and bloodless, some show congested capillaries; others contain bright

red or dark brown areas of hemorrhage. In one nodule the cut surface is marked by translucent, silver-gray anastomosing bands of connective tissue which arise from the capsule. There are a few small cysts filled with a gelatinous semi-solid material.

The capsule, which on the surface is distinct both in color and structure, loses its fibrous character as it passes into the mass and fades into a broad infiltrated brownish yellow band. Numerous small blood vessels course along the connective tissue trabeculæ.

For microscopical study a segment about five millimeters in thickness, cut from the median zone, was carefully plotted so that in the end we were able to reconstruct the entire cut surface of the gross specimen in the photograph. Histologically, the specimen is an epithelial tumor in which the cells are arranged as tubules or in nests and chords resembling the structure of parathyroid glandule (Fig. 2). The connective tissue in parts is prominent and forms anastomosing septa between the epithelial islands. In other parts it is present only as a delicate basement membrane or very fine interlacing reticulum between the chords and clusters of loosely lying epithelial cells. Different lobules show wide variations in this proportionate distribution of epithelial cells and struma. In an individual lobule this relationship is constant. The epithelial cells vary in diameter from twenty to twenty-five microns. The nuclei measure from four to eight microns and stain deeply. The protoplasm is vacuolated and stains lightly. As a rule, the cell boundary is not sharply defined. For the most part the cells are cuboidal or columnar and rest upon a distinct but delicate basement membrane.

The structural formation follows the type of a simple gland with a small lumen, or the cells are grouped into small solid nests of six or more cells. From this original gland-like type, two variations arise. In the one the lumen becomes dilated, with the formation of numerous small cysts, the epithelium becomes flattened, and papilliferous outgrowths arise from the walls (Fig. 3). In the other variation the cells are freed from their attachment to the basal

membrane and appear as compact or loose clusters, chords, and nests; the lumen disappears and all structural regularity is lost (Fig. 4).

For the most part the tumor is rich in capillaries upon whose delicate walls the cells are attached directly. In a few nodules the blood supply is surprisingly scant, entire fields being apparently free from determinable vessels (Fig. 5). The hemorrhagic areas are numerous in certain lobules, the blood lying in large lakes and tubules and nests. The absence of large blood vessels with well developed walls is striking.

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DESCRIPTION OF PLATE V.

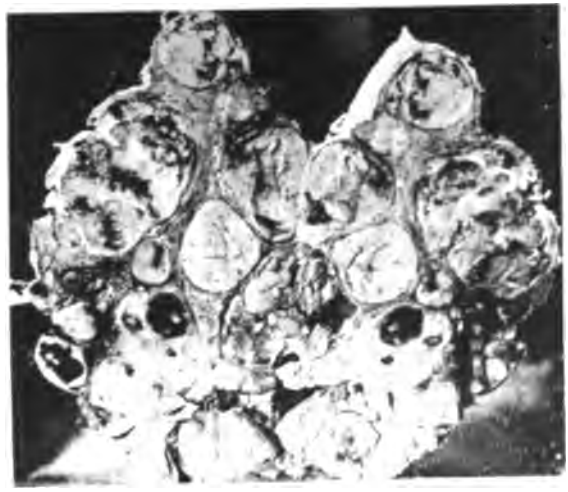
FIG. 1. — Photograph of tumor. Section through middle showing both halves.

FIG. 2. — Photomicrograph of tumor nodule showing resemblance to parathyroid glandule structure. x 150.

FIG. 3. — Section of tumor showing cystic spaces with ingrowth of epithelium. x 100.

FIG. 4. — Section of tumor showing loss of structural regularity. x 150.

FIG. 5. — Section of tumor area much magnified showing relationship of cells to basal membrane. x 400.



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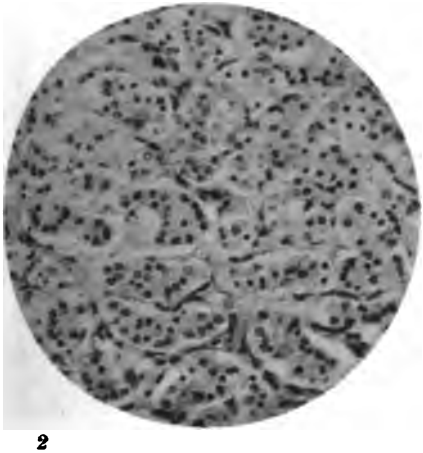

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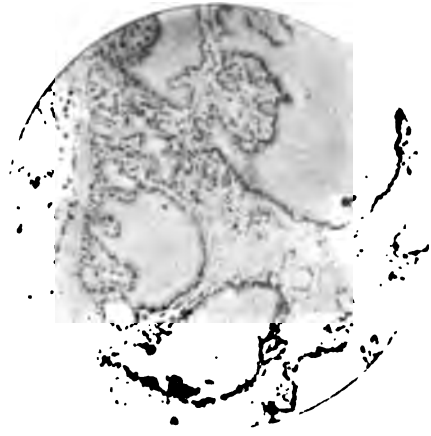

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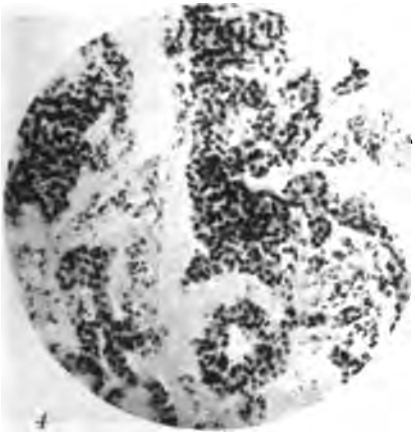

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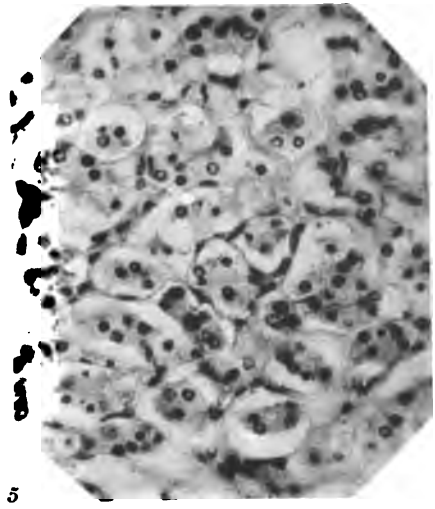

5

Thompson and Harris. Parathyroid glandules

MULTIPLE HERNIAS OF THE CEREBRUM AND CEREBELLUM, DUE TO INTRACRANIAL PRESSURE.*

S. B. WOLBACH, M.D.
(*Instructor in Pathology, Harvard University Medical School.*)

Multiple hernias of the brain following increased intracranial pressure are small masses of brain tissue which are forced into and through the dura. They may enter the large sinuses of the cranium or they may cause the formation of pits in the bones of the skull. In extreme cases these hernias have perforated the bones of the vault.

Multiple hernias of the cerebrum have been described by von Recklinghausen and by Beneke. The former demonstrated a single case in 1870, the latter reported two cases in 1898, and these three cases are the only ones recorded. The increased pressure in these cases was due to intracranial tumors. Similarly produced hernias of the cerebellum have not been described.

This paper is based upon the study of nine cases of increased intracranial pressure: six were due to tumors, two to acquired internal hydrocephalus, and one to massive cerebral hemorrhage. These cases, with the exception of one (number nine, for which I am indebted to Professor E. E. Southard), represent autopsies by myself upon consecutive cases of increased intracranial pressure. It is evident, then, that multiple hernias of the brain in cases of increased pressure are common and, in my experience, constant. The absence of mention of these hernias in autopsy protocols and in reports of brain tumor cases is certainly due to the failure of pathologists to examine carefully the sinuses and both surfaces of the dura. It is also probable that these hernias have been mistaken for arachnoid villi of unusual size. "In cases of brain tumor — glioma, sarcoma, gummata of dura (case of Lancereaux), carcinoma, etc., — one often finds an excessive development of the Pacchionian granulations

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Entrance may also take place into the large granulations in the neighborhood of the superior longitudinal sinus. After entrance, the hernias extend along the lymph spaces and Beneke found a similarity between the spaces thus filled with brain tissue and the pictures obtained by Key and Retzius by injection. The correspondence of the hernias with the locations of the arachnoid villi, namely, along the superior longitudinal sinus, and in the anterior portions of the middle fossæ, is emphasized. The pits in the bone, Beneke believes, are largely performed by the arachnoid villi and the hernias enlarge them through pressure atrophy. He does not, however, exclude the possibility of a new formation of pits. No microscopic examinations of the pitted bone were made. As a factor in the causation of the hernias, Beneke suggests the possibility of an increased lymph flow through the arachnoid villi, which softens the underlying tissue. A momentary further increase in pressure is supposed to cause rupture of the pia and the escape of brain tissue into the granulations from whence it spreads. Beneke finally states that these hernias are excessively rare.

In the presentation of the cases which have furnished the material for this paper, only those facts are included from the clinical histories and autopsy records that are of direct interest in the consideration of multiple hernias of the cerebrum and cerebellum. Three cases of tumor are complicated because of the formation of cysts after decompression operations. In two cases, numbers I. and VI., these cysts were of very large size and unquestionably produced some of the hernias found at autopsy. And in these two cases, the localization of the hernias is dependent upon two independent sources of pressure.

Case I. (L. I. H. 06: 22) is that of a man twenty-nine years old, who had symptoms of intracranial pressure for more than one year. Fourteen weeks before death a decompression operation was done, in which a large portion of the skull, involving the left parietal and temporal bones, was removed. A large hernia of the brain took place through this opening and the pressure symptoms became worse. Death followed immediately after a second decompression operation upon the right side. At

autopsy a glioma in the right half of the cerebellum was found. The dimensions of the tumor were 3.5 x 2 x 2 centimeters. Beneath the soft tissues covering the field of operation on the left side there were many cysts, one to two centimeters in diameter, containing clear liquid. Fifty cubic centimeters of liquid were obtained from these cysts. Hernias of the cerebrum were found in the left middle fossa, distributed along the middle meningeal vessels. The largest hernias were one to five millimeters in diameter and one to four millimeters deep, and occupied pits in the bone. Many small elevations of the dura, which were just visible, proved on microscopic examination to be hernias. There was general thinning of the bones of the skull. An occluding thrombus was found in the right lateral sinus just external to the torcular. There was acute inflammation of the right middle ear.

Case II. (L. I. H. 06:23) is one of hemiplegia in a woman thirty-nine years old. Death occurred thirteen days after the stroke. A massive hemorrhage into the right basal ganglia was found at autopsy. There were many firm white villus-like bodies in the right lateral sinus, midway between the torcular and jugular foramen. These bodies projected from the inferior surface of the sinus; the largest were two millimeters in diameter. Microscopic examination showed large arachnoid villi containing, at their bases, small masses of distorted cerebellar tissue.

Case III. (H. M. S. 2618. Reported by E. W. Taylor, Boston Med. and Surg. Journal, clvi, No. 6) began six months before death to have symptoms of pressure upon the spinal cord. Three months before death there were symptoms of increased intracranial pressure. The autopsy revealed a diffuse sub-pial, small round cell sarcoma of the spinal cord and marked internal hydrocephalus. The amount of liquid in the lateral ventricles was estimated at eighty cubic centimeters. There was marked flattening of the convolutions. Small hernias with corresponding pits in the bone were found in the anterior portion of the left middle fossa.

Case IV. (H. M. S. 2666) is that of a middle-aged woman who had symptoms indicating increased intracranial pressure for about one year. A decompression operation was done several months before death, in which large portions of the occipital bone were removed on each side of the median line, below the level of the lateral sinuses. The autopsy revealed chronic basal leptomeningitis and marked internal hydrocephalus. The flattening of the cerebral convolutions was extreme. Small hernias of the cerebrum, one-half to three millimeters in diameter, were found in both middle fossæ. The left Gasserian ganglion was cystic, and in the microscopic examination of the overlying dura a small hernia was found. The arachnoid villi along the superior longitudinal sinus and the sinuses in general contained no hernias.

Case V. (H. M. S. 3416) is that of a powerfully built man of thirty-nine years, who had pressure symptoms for several years, and bitemporal hemianopsia for two years before death. At autopsy a large tumor was found in the median line in the position of the pituitary body. The optic

tracts were flattened and separated by the tumor which projected upwards into the third ventricle, forming there a smooth rounded eminence. The tumor was connected below with a large extradural tumor mass which had destroyed portions of the basilar processes of the occipital and sphenoid bones and adjacent portions of the middle fossæ. The sphenoidal sinuses and the superior nares were invaded. There was no acromegaly. Microscopically, the tumor is an adeno-carcinoma. Hernias of the cerebellum were found into the left lateral sinus. These hernias consisted of two hemispherical masses of soft white tissue, each about four millimeters in diameter and situated midway between the torcular and the jugular foramen.

Case VI. (H. M. S. 3851) is that of a man thirty-seven years old, who had symptoms of increased intracranial pressure for five years. Seven months before death a decompression operation was done in which a large opening six by nine centimeters was made on the left side, involving the parietal and frontal bones. At the time of the autopsy there were a large hernia of the brain through the opening in the skull and many small cysts in the meninges beneath the scalp at the edges of the opening. With the scalp removed, the hernia appeared as a translucent walled cyst overlapping the opening in the skull for a distance of one to two centimeters. This cyst contained remains of brain tissue consisting of delicate strands and a thin lining layer of soft brownish yellow material. The floor of the cyst was crater-like and composed of necrotic brain tissue; it communicated with the left ventricle through two small openings. There was moderate internal hydrocephalus. The opening in the skull was surrounded by a sharp ridge of newly-formed bone. At the base of the brain to the left of the median line and anterior to the pons, was a large tumor, adherent to the brain, and connected with a large subdural tumor mass which extended through the floor of the left middle fossa into the neighboring sinuses and the nose. Microscopically, the tumor is composed of small round cells supported by a reticulum. The cells resemble those of the lymphocyte series and the tumor is one commonly called small round cell sarcoma. Multiple hernias of the cerebrum were found into the arachnoid villi on both sides of the superior longitudinal sinus, and into the floor of the right middle fossa. There were corresponding pits in the bone. There was a single hernia into the right lateral sinus, from the superior surface, in about the middle portion. The tip of the occipital lobe was adherent at this point. There were no hernias of the cerebellum.

Case VII. (H. M. S. 4495) is that of a man fifty years old, who had symptoms of increased intracranial pressure for about six months. At the autopsy a tumor 2.5 x 2 centimeters was found in the cerebellum, involving the left half and the superior worm. There were several small tumor masses in the posterior end of the left superior frontal gyrus. Microscopically, the tumor proves to be carcinoma, in type suggesting an origin from the alimentary tract. Multiple hernias of the cerebrum were found into the arachnoid villi upon the left superior frontal gyrus and in

both middle fossæ. These hernias were of minute size and there were no demonstrable pits in the bone. Multiple hernias of the cerebellum with corresponding pits in the bone were found in the left posterior fossa, along the external border of the occipital sinus. The largest of these hernias were two to four millimeters in diameter. The left lateral sinus was nearly completely filled with hernias of the cerebellum for a distance of three to four centimeters from the torcular. The largest of these hernias were five millimeters in diameter. The dura on the external surface presented a series of parallel white striæ between which were tags of cerebellar tissue.

Case VIII. (H. M. S. 4564) is that of a man fifty-two years old, with a history definitely indicative of increased intracranial pressure for six months preceding death. A decompression operation on the right side was done one month before death. A similar operation on the left side was done eleven days before death. The two openings in the skull were of large size and symmetrically placed, and each included the temporal and parietal bones. At autopsy there was a slight hernia of the brain through the operation opening on the right side. There were many small cysts between the brain and the tissues which covered the opening in the bone. The largest cysts were about one centimeter in diameter. On the left side there was no hernia and the brain was not adherent to the overlying tissues. The cause of the cerebral pressure was a large infiltrating glioma of the right temporal lobe, which projected into the posterior horn of the lateral ventricle, forming there a smooth rounded mass. The cornu ammonis was pushed upwards and inwards; the optic thalamus was pushed forwards and upwards. The tumor extended forward in the substance of the temporal lobe to within a few centimeters of its tip. The diameter of the infiltrated region in the middle portion of the temporal lobe was five centimeters.

Multiple hernias of the cerebrum were found in the arachnoid villi on the right side of the superior longitudinal sinus, in the floor of the right middle fossa, and in the right lateral sinus. There were deep pits in the middle fossa on both sides of ridges of bone which lay beneath the branches of the middle meningeal vessels. The pits for the arachnoid villi at the vertex were of unusual size and there were slight corresponding elevations of the external table. The hernias through the superior wall of the lateral sinus were small. Multiple hernias of the cerebellum were numerous into the right lateral sinus, and there were a few minute hernias into the left sinus. The right lateral sinus also contained an occluding thrombus filling the sinus for a distance of two centimeters external to the torcular. The middle ears were normal.

Case IX. (Danvers 1072) is that of a woman, age thirty-nine years, who had symptoms of cerebral pressure for several years. There was a history of apoplectiform seizures for eight years before death. The autopsy showed a large infiltrating glioma involving the median portions of both frontal lobes, but lying chiefly within the left frontal lobe. The dimensions

of the tumor roughly were, 6.5 centimeters in the vertical line, four centimeters horizontally, and 3.5 centimeters in the antero-posterior line. The flattening of the convulsions was extreme. Many multiple hernias of the cerebrum of large size were found. There were scores in both temporal and frontal regions with corresponding pits in the bone. The arachnoid villi along the superior longitudinal sinus were of unusual size and contained hernias. The hernias of the temporal lobes into the middle fossæ showed the usual distribution about the branches of the middle meningeal vessels.

The following table is inserted in order to give in compact form the data of greatest importance concerning duration and cause of pressure, together with the localization of the hernias. It is obvious that in the early cases many minute hernias must have been overlooked, and especially those into the sinuses and arachnoid villi along the sides of the superior longitudinal sinus. This is indicated by the greater range of distribution of hernias found in the later cases. The data, however, point clearly to a relationship between the location of the source of pressure and the distribution of the hernias.

Case.	Duration.	Location and Cause of Pressure.	Location of Hernias.
I.	1 year: decompression operation 2½ months.	Glioma of cerebellum; right side. Cysts from decompression operation; left side.	Left middle fossa.
II.	13 days.	Right basal hemorrhage.	Left lateral sinus (cerebellar).
III.	6 months.	Acquired internal hydrocephalus.	Left middle fossa.
IV.	1 year.	Acquired internal hydrocephalus.	Both middle fossæ; left Gasserian ganglion.
V.	2 years.	Base of brain. Adenocarcinoma of pituitary body.	Left lateral sinus (cerebellar).
VI.	Years. Decompression operation several months.	Base of brain, left side, small round cell sarcoma. Cysts after decompression operation in right temporal region.	Both sides of superior longitudinal sinus; right middle fossa; (left perforated by the tumor); right lateral sinus (cerebral).
VII.	6 months.	Metastatic carcinoma; left side of cerebellum.	Left superior frontal gyrus; left lateral sinus (cerebellar); left posterior fossa; both middle fossæ.
VIII.	6 months. Decompression operation 1 month.	Glioma of right temporal lobe. Cysts from decompression operation in right temporal region.	Right side of superior longitudinal sinus; right lateral sinus (cerebellar and cerebral); right middle fossa.
IX.	Years.	Glioma, infiltrating and involving the median portions of both temporal lobes.	Both sides of superior longitudinal sinus; both temporal (middle fossa) and frontal lobes.

The constancy of cyst formation, after decompression operations over the parietal and temporal lobes, is worthy of

consideration by surgeons. Case VIII. was the only case in which cysts of small size were found occurring after a recent operation.

Sections through these cysts and the underlying brain show that the cysts have formed beneath the outer endothelial layer of the arachnoid, which is adherent to the overlying tissues covering the field of operation. The presence of nests of endothelial cells surrounding the cysts suggests the possibility of the cysts being formed in or beneath the arachnoid villi. Much more work would be necessary to determine satisfactorily this point. It seems plausible, however, that the disturbances of the normal relations of the arachnoid villi, and the formation of adhesions to the overlying soft tissues, after the removal of the dura, may account for the accumulation of liquid in the arachnoid.

If this theory is correct, the formation of these cysts probably would be prevented by the removal or destruction, by cauterization, of the arachnoid and pia. The possibility of injuring the brain substance does not need to be considered in cases where the total destruction of the same area is expected to follow as a result of a post-operative hernia.

The conclusions to be drawn from these autopsies, in regard to the transmission by the brain, of pressure caused by tumors, are confirmatory of the results of the experiments of Leonard Hill (The Physiology and Pathology of the Cerebral Circulation, 1896) and Harvey Cushing (Physiologische und anatomische Beobactungen über den Einfluss von Hirnkompression auf den intracraniellen Kreislauf und über einige hiermit verwandte Erscheinungen. Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, ix, Heft 4 and 5, 1902). Both of these experimenters recorded the transmission of pressure in dogs by means of manometers and thus determined that the falx cerebri, the tentorium cerebelli, and the falx cerebelli act as barriers and serve to localize the effects of pressure exerted upon any given area of the cortex. This is especially true of the tentorium as the falx cerebri may be readily dislocated laterally (Cushing). That downward

dislocation of the pons and medulla in cases of cerebellar tumors occurs to a much more marked degree than with tumors of the cerebrum is well known. The effect of the tentorium is well shown in Case VII., where most of the hernias were found in the lateral sinus and floor of the posterior fossa on the side where the tumor was situated. The hernias of the cerebrum were very small and may have been caused by the cerebral metastases. Tumors at the base of the brain also are liable to produce cerebellar hernias (Cases V., VI., and VIII.). The best example of widespread transmission of pressure is that of Case IX., where the tumor involved both frontal lobes. In Case I. it is probable that hernias into the sinuses were overlooked. The hernias into the left middle fossa were unquestionably the effect of the cysts which formed after the decompression operation. Case VIII. illustrates the limitation of hernias to one-half of the cerebrum in a case of a tumor of the right temporal lobe. Internal hydrocephalus, which is very common with tumors of the cerebellum, is probably the cause of hernias of the cerebrum, when the pressure is below the tentorium.

From the above facts and because of the reasons advanced by Beneke, it is certain that the one cause of multiple hernias of the cerebrum and cerebellum is increased intracranial pressure. In two cases of internal hydrocephalus and one case of a huge extradural tumor in infants, autopsied since I began this study, no hernias were found. The rapid growth of the bones of the cranial cavity increase its capacity and so prevent the creation of a marked increase in pressure and hernia formation.

That multiple hernias of the brain may be caused suddenly is proved by Case II. (cerebral hemorrhage), where fragments of cerebellar tissue were forced into the lateral sinus, behind arachnoid villi. Microscopically, one can distinguish between the rapidly and slowly formed hernias by the nature of their contents.

Aside from the localization of the hernias in or near the large sinuses of the dura, the most striking anatomical

relationship is that to the vessels of the dura. When hernias of large size were found, they were always clustered about the vessels. This was always particularly marked about the middle meningeal vessels and their branches in the middle fossæ (Fig. 1.)

Von Recklinghausen did not attempt to determine the relation of the hernias to anatomical structures in his case. Beneke determined the occurrence of hernias into the arachnoid villi. In the description of his second case he mentioned the existence of endothelial lined fissures of the dura which he states run from the inner to the periosteal surfaces. He believed the fissures contained small arachnoid villi.

In the present study, many hernias were studied by means of serial sections and in every instance remains of arachnoid villi could be found. In most hernias in the neighborhood of the superior longitudinal sinus, the brain tissue literally injects the larger villi and there is no difficulty in determining the exact location of the extruded brain tissue. Similarly the hernias of the cerebellum often partially fill easily recognizable villi. The hernias at the base of the brain, however, often show no gross evidence of connection with arachnoid villi, and are never branched in the manner of the hernias in the large villi at the vertex. This difference in the shapes assumed by the hernias is explained by the differences in size and shapes of the arachnoid villi found in the neighborhood of the great sinuses and of those found at the basis of the brain, in relation to the meningeal veins.

Key and Retzius have determined, by means of injection methods, that arachnoid villi occur in the following positions: the superior longitudinal sinus and especially the lacunæ laterales or accessory sinuses, the transverse sinus, the superior petrosal sinus, the cavernous sinus, the middle meningeal veins in the middle fossa, the meningeal veins close to the superior longitudinal sinus, a small sinus occasionally found on the outer side of the first division of the Gasserian ganglion and the straight sinus (from the cerebellum).

No mention is made of arachnoid villi in the lateral sinuses. The frequency of hernias into the lateral sinuses and, upon microscopic examinations, the finding of structures identical with the arachnoid villi led me to search for them in a normal young male adult. Arachnoid villi of small size were found on both sides coming from the arachnoid of the cerebellum, and entering small accessory sinuses which are similar to the lacunæ laterales of the superior longitudinal sinus. These villi are easily visible in sections without magnifying. The largest ones are branched and are identical in structure with those found in the neighborhood of the superior longitudinal sinus. The regions examined were those in the vicinity of the entrance of the inferior cerebellar veins. This was done by removing the cerebellum with the intact dural covering and then sectioning through both structures. Other villi were found external to the entrance of the inferior cerebellar veins, and these villi were found by noting delicate adhesions between the dura and arachnoid (Fig. 6). The existence of arachnoid villi in the floor of the posterior fossa near the median line, along the occipital sinus, has been determined by finding their remains in sections of hernias from this location.

Since the number of hernias may far exceed the number of visible arachnoid villi in any given location, search was made in normal and pathological cases for microscopic villi. Serial sections were made of the middle meningeal veins and surrounding dura from the middle fossa and at the level of the inferior border of the parietal bone, and the presence of minute villi was established. They were also found accidently in a set of serial sections made from the superior frontal gyrus. The smaller of the microscopic villi consist simply of tufts of endothelial cells projecting into endothelial lined fissures of the dura in the neighborhood of small veins (Fig. 9). The larger are branched and run obliquely for long distances into the dura and these are the only ones that remain attached to the dura when the latter is removed from the brain. They consist of connective tissue covered by endothelium continuous with that of the arachnoid. The

connective tissue may be compact or loose in texture, except at the extreme tip, where it is always loose meshed (Figs. 8 and 10). It is probable that the distribution of minute arachnoid villi is far more widespread than has been believed. The few observations I have made were done in order to verify the finding of remains of arachnoid villi, which are always demonstrable at the periphery of hernias of the cerebrum and cerebellum. In view of the above facts it is safe to conclude that multiple hernias of the cerebrum and cerebellum always enter the dura through fissures occupied by arachnoid villi.

The gross appearances of the hernias have been accurately described by Beneke. The shape naturally varies according to the location. Those in the superior longitudinal and the accessory sinuses are often branched, as may be those from the cerebellum in the lateral sinuses. The majority are spherical or pear-shaped, having taken place into simple forms of arachnoid villi. Hernias which do not penetrate above the level of the dura may be extremely variable in shape or not noticeable except by microscopic examination. These latter hernias, as will be explained later, are rapidly formed and consist of softened brain tissue, forced like an injection mass into preëxisting spaces. The large hernias occupy pits in the bone; a covering of dura is always found, and sometimes when the hernias can be pulled out from the dura a smooth covering derived from the arachnoid is demonstrable. The fissuring of the dura noted by von Recklinghausen is, I believe, simply an exaggeration of normal structure in the neighborhood of arachnoid villi due to the pressure atrophy. This is especially well seen on the inferior surface of the lateral sinuses and along the superior longitudinal sinus. The tufts of brain tissue which represent the pedicles of the hernias are always found between the strands of dura, but the clefts are simply the enlarged fissures occupied by arachnoid villi (Figs. 1, 2, 3, and 4).

In two cases, microscopic examinations were made of the bone containing pits occupied by hernias, and proof was obtained of the active absorption of the bone. The absorption

of the bone is shown by the presence of osteoclasts and of lamellæ cut at all angles where the trabeculæ abut on the walls of the pits. Groups of giant cells about small fragments of bone are occasionally found on the outer edge of the dura. Besides these two cases, in every case of hernias of large size, giant cells were found attached to the dural covering in sections of the hernias where there had been contact with bone.

Beneke's opinion that the pits in the skull were always partly preformed by arachnoidal villi cannot be opposed, though in some locations it is extremely rare to find pits for arachnoid villi in normal cases. Luschka (Arch. f. Path. Anat. u. Physiol. u. f. Klin. Med., xviii, 1860, quoted by Key and Retzius) gives the following locations where pits due to arachnoid villi may be found, aside from the neighborhood of the superior longitudinal sinus: the anterior half of the parietal bones and especially close to the spheno-temporal sutures; the great wing of the sphenoid; the tegmentum tympani and occasionally in the vicinity of the petro-squamous suture. These locations agree closely with those of the pits in cases of hernias of the brain, however, since intracranial pressure may cause thinning of all the bones of the cranial cavity it is possible that hernias into microscopic villi may alone be responsible for pit formation.

Before beginning the microscopic description of the hernias, and the relations of the contents to the arachnoid villi and dura, a short description of the structure of normal arachnoid villi is necessary.

According to Key and Retzius the smaller villi are pear or balloon shaped and consist of a loose mesh-work of connective tissue. The base of a villus is continuous with the arachnoid of the brain; the surface is covered with endothelium continuous with that of the arachnoid. The smallest villi may not reach the veins or sinuses, but all project into the dura. Some may lie in contact with veins without entering. When projecting into a vessel, they always receive from the dura or vessel wall a thin coat of connective tissue

and endothelium. The connective tissue may be lacking so that only two layers of endothelium cover the surface of the villus. The space between the villus and the investment from the vessel is lined by endothelium continuous with that of the inner surface of the dura and is traversed by delicate strands of connective tissue, covered by endothelium. The larger villi are branched and may anastomose with neighboring villi. Liquids injected into the subarachnoid space fill first the villi, then the space enclosed by the dural covering, and finally pass into the sinuses. Suspensions of cinnabar in water pass through the villi as well as solutions of dyes. Liquids injected into the subdural space over the villi will not pass into the villi, though it does pass easily into the sinuses. These results were verified upon living dogs.

The application of modern histological methods can add nothing of great importance to the above description of the structure of the arachnoid villi. It is certain, however, that the arachnoid villi vary in structure, but chiefly in the compactness of their connective tissue. Those in the neighborhood of the superior longitudinal sinus may be composed of very dense connective tissue and this is usually the case when there is thickening of the pia, which is usually most marked in their region. Such dense or sclerosed villi, even in cases of long continued increased intracranial pressure, do not contain hernias. The normal arachnoid villus contains spaces between the bundles of fibrous connective tissue which at the base of the villus seem to be continuous with those of the subarachnoid mesh-work. At the periphery of the villus there are definite spaces lined with endothelium which in "wet brains" are usually injected with finely granular material. These endothelial lined spaces, as near as I can determine, are most easily demonstrable beneath the nests of endothelial cells, which are always found on the surfaces of arachnoid villi. In sections of villi projecting into the sinuses the double investment of endothelium from the inner surface of the dura and from the vessel wall can be made out. Often these two layers enclose a small amount

of connective tissue. Between the endothelial covering of the villus and the investment derived from the dura and vessel wall is the subdural space filled with finely granular material, probably precipitated from liquid present before fixation. There are no blood vessels in arachnoid villi. Between the brain and the base of an arachnoid villus there is only a delicate mesh-work of connective tissue, containing blood vessels, though the number and arrangement of vessels is not peculiar to this location. The more compact layers of the pia and arachnoid extend upwards into the villus, around the periphery of its base. This arrangement practically makes a break in the pia arachnoid covering of the brain and probably is of mechanical importance in the formation of hernias.

In the discussion of the mechanics of multiple hernia formation, the following facts must be taken into consideration: (1) That the brain is enclosed in an unyielding case of bone. (2) That the veins and sinuses of the dura are compressible (the lateral sinuses probably yielding only where there are arachnoid villi). (3) That local compression is possible (because of the existence of compartments in the cranial cavity and the compressibility of veins). (4) That the weakest points in the dura are those where the arachnoid villi enter.

General readjustment of brain tissue takes place in every case of increased intracranial pressure as is shown by the flattening of the convolutions, the obliteration of the sulci, and in the downward dislocation of the brain as a whole.

The movements of the brain tissue, when under pressure, must be towards yielding points: the veins and sinuses. The greatest movement of any given point must be at the place of yielding, which necessarily determines the situation of the resultant of pressure lines. The movement of the brain tissue is probably responsible for the softening at points below the arachnoid villi.

Microscopically, in the flattened sulci, indentations of the vessels of the pia are plainly visible and injury to the elevated brain tissue is proved by very marked neuroglia increase.

The veins in cross section show no distortions or flattening, and it is probable that the diminution in volume is compensated by contraction of the vessel walls. Rapidly-formed hernias are those which lie wholly within the dura and which may run for considerable distances along the sides of veins (Figs. 2 and 3). It is difficult to decide upon the exact location of the brain tissue, though in some instances, at least, it is certain that it gets into lymphatics. In these rapidly-formed hernias there is no increase of neuroglia and of course no pitting of the bone. The brain tissue shows all degrees of necrosis and the only evidence of reaction is the presence of leucocytes, compound granular cells, and other phagocytic cells containing cell detritus. Small blood vessels are carried along with the brain tissue and may be found in the hernias far removed from their original locations. The brain tissue at the base of the hernias shows varying degrees of distortion due to the rearrangement of the ganglion cells and also the granular remains of nerve fiber sheaths and degenerated ganglion cells. The spaces of the arachnoid villi may be distended with granular material and even fragments of nerve fibers and cell remains. The subdural space surrounding the villus may be markedly distended with granular material.

In hernias which have occupied pits in bone there is always a large amount of neuroglia, and in many instances the hernia contents consist almost wholly of dense neuroglia tissue. The dural covering may be very thin and on the outer surface giant cells and occasionally spicules of bone surrounded by osteoblasts may be found. The only reaction in the dura is a slight infiltration with lymphoid and plasma cells. There may be a few polynuclear leucocytes grouped about necrotic nerve elements. In general, the microscopic findings are simply those dependent upon destruction of brain tissue and the removal of the same through the agency of polynuclear leucocytes and mononuclear phagocytic cells. The neuroglia reaction is secondary and could be dismissed at once were it not for certain peculiar relations found

between the neuroglia cells and the connective tissue of the arachnoid villi and the dura.

The proliferation of subpial neuroglia cells with the formation of circumscribed elevations between the vessels of the pia has been mentioned above. Similar circumscribed neuroglia masses are also found beneath arachnoid villi and many small hernias are capped by such neuroglia masses. After very careful study of many sets of serial sections of small hernias I am forced to the conclusion that the neuroglia in contact with connective tissue, and under pressure to a degree that is destructive to the connective tissue, is capable of active proliferation and, to a slight extent, of invasion of the connective tissue of the arachnoid villi and dura. It is possible that many slowly-formed hernias may enter villi behind advancing zones of neuroglia cells. This behavior of the neuroglia is strikingly illustrated in some instances, in the form of outgrowths into the connective tissue surrounding small vessels which enter the cortex from the pia (Fig. 12). Such outgrowths of neuroglia are found only in cases of long-continued pressure and the most plausible interpretation is that connective tissue, in contact with the brain, when injured by pressure yields and disappears before the growth of neuroglia tissue. Beneath arachnoid villi of microscopic size I have found slight elevations of the cortex with a mass of neuroglia cells apparently invading the pia and here also the process is probably secondary to degeneration of the connective tissue (Fig. 11). The peripheries of hernias show the best illustrations of neuroglia activity. Here large masses of neuroglia cells seem to push into the connective tissue, and extend in a direction parallel to the connective tissue fibers. In large hernias, similar nests and advancing cells of neuroglia are found in the dura, and in these instances the connective tissue and endothelium of the villus has disappeared (Figs. 11, 12, and 13).

SUMMARY.

The series of cases furnishing the material for this report practically prove the constancy of multiple hernias of the

cerebrum and cerebellum in cases of increased intracranial pressure.

The distribution of the hernias in relation to the position of the source of pressure adds to the proof for the local compressibility of the brain, and the directions of transmission of pressure, as influenced by the falx cerebri, the tentorium cerebelli, and the falx cerebelli.

In the general rearrangement of brain tissue following increased pressure, the greatest movement is at yielding points. The arachnoid villi, because of their structure and relationship to compressible veins and sinus, furnish yielding points in the encasement of the brain. The softening of the brain tissue, which permits the free extrusion into the arachnoid villi, is most probably due to the mechanical injury following the movement of the tissue towards the yielding points. The widespread distribution of the hernias and the identification in all of them of the remains of arachnoid villi illustrates the large number and general distribution of arachnoid villi, both of which are greater than even the observations of Key and Retzius would indicate. Microscopic arachnoid villi are often the seat of hernias.

Two types of hernias may be distinguished; those rapidly produced by sudden increase of pressure, such as voluminous cerebral hemorrhage and those produced slowly by gradual increase in pressure, such as accompanies intracranial tumors. The hernias produced slowly are attended by marked and peculiar reactions of the neuroglia, which indicate the survival and proliferation of neuroglia cells under conditions destructive to connective tissue.

DESCRIPTION OF PLATES.

PLATE VI.

FIG. 1. — Sketch of outer surface of dura, showing hernias of cerebrum and relation to vessels (middle meningeal). Natural size. Case IX.

FIG. 2. — Photomicrograph of a section through base of a rapidly-formed small hernia of the cerebrum into the dura. x 45. Case I. From the middle fossa.

FIG. 3. — Photomicrograph of a section through a rapidly-formed hernia at one edge of the base, showing a vein surrounded by brain tissue. x 45. From the middle fossa. Case I.

FIG. 4. — Photomicrograph of a section through the base of a large hernia of long duration which occupied a pit in the floor of the middle fossa. The dura is very much thinned, and upon the outer surface many giant cells were found. x 14. Case I.

PLATE VII.

FIG. 5. — Drawing of a small hernia into an arachnoid villus from the superior frontal gyrus, Case VII. The small mass of neuroglia above the vein on the left is connected with the larger hernia mass at another level. The neuroglia proliferation is characteristic of slowly-formed hernias.

FIG. 6. — Low power drawing of arachnoid villi from the cerebellum. The lateral sinus is collapsed. The villi project, in some instances, into sinuses similar to the accessory sinuses of the superior longitudinal sinus.

FIG. 7. — Photomicrograph of a section through a large hernia of the cerebellum into the lateral sinus. x 14. The coverings from the sinus and the pia-arachnoid are well shown on the left edge, Case V.

PLATE VIII.

FIG. 8. — Drawing of a microscopic arachnoid villus, from the superior parietal region.

FIG. 9. — Photomicrograph of the smallest type of arachnoid villus which consists of endothelial cells chiefly. x 64.

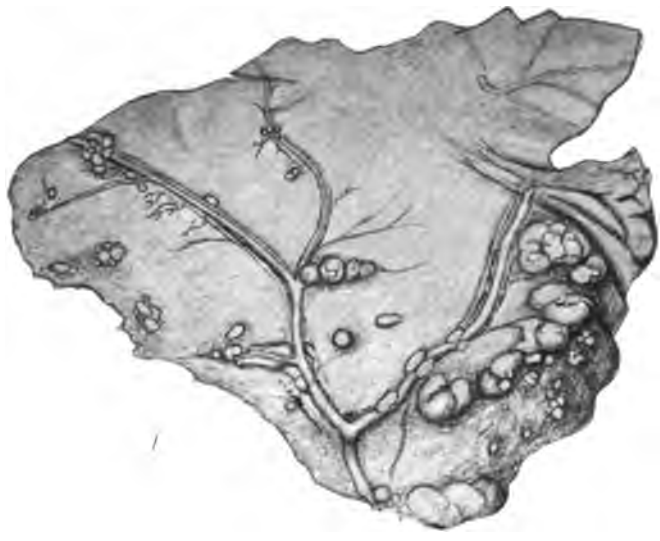
FIG. 10. — Low power drawing of a microscopic arachnoid villus from the neighborhood of the anterior branch of the middle meningeal vein.

PLATE IX.

FIG. 11. — High power drawing of a slowly-forming hernia into an arachnoid villus from the superior frontal gyrus, Case VII. Note the proliferation of the neuroglia and the invasion of connective tissue by neuroglia.

FIG. 12. — High power drawing, Case VII., showing the extension of neuroglia along a small vessel.

FIG. 13. — High power drawing of the edge of a large hernia into an arachnoid villus projecting into an accessory sinus of the superior longitudinal sinus, Case VIII. To show the invasion of connective tissue by neuroglia.



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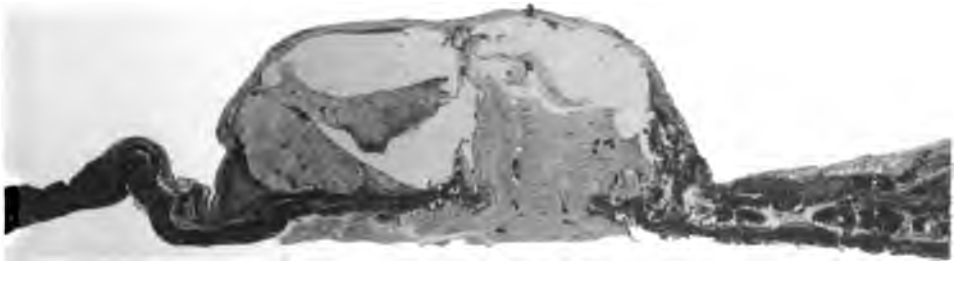

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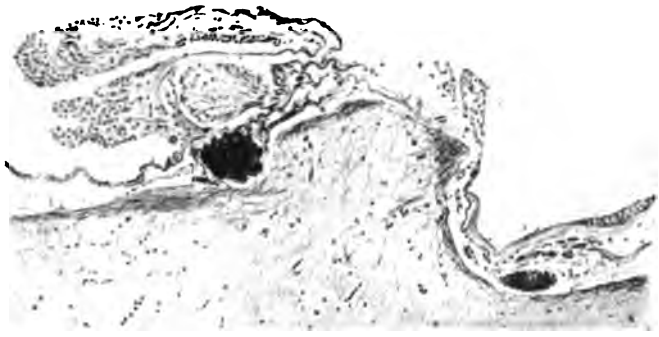
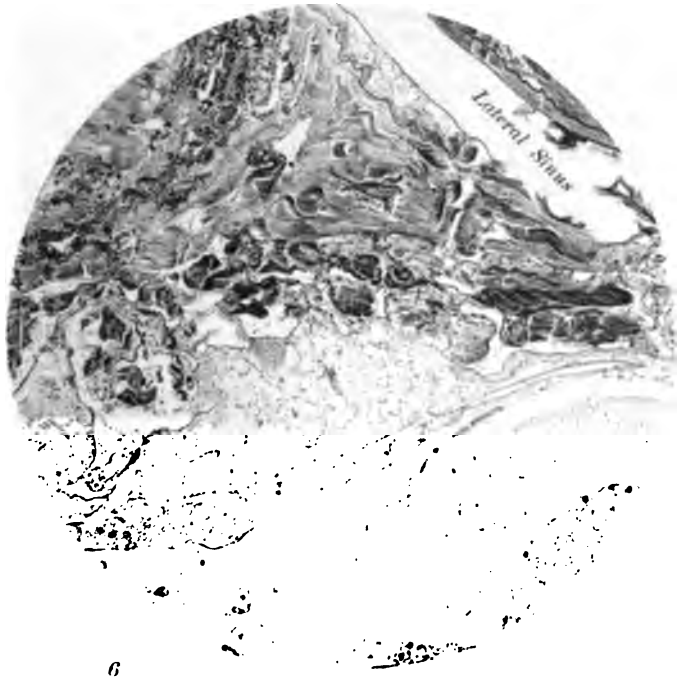
Wolbach.

Multiple hernias.

5





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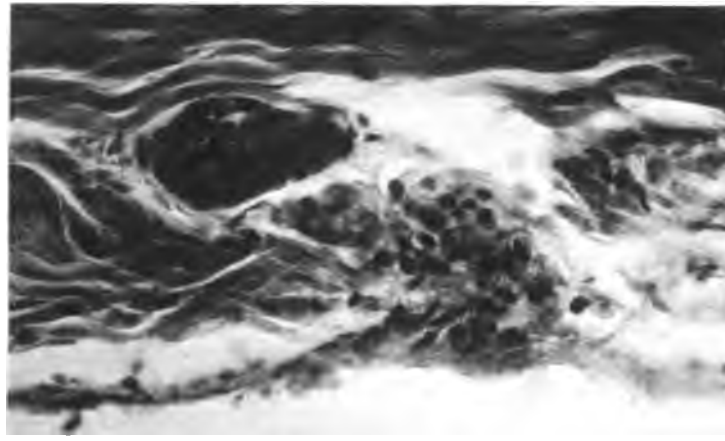

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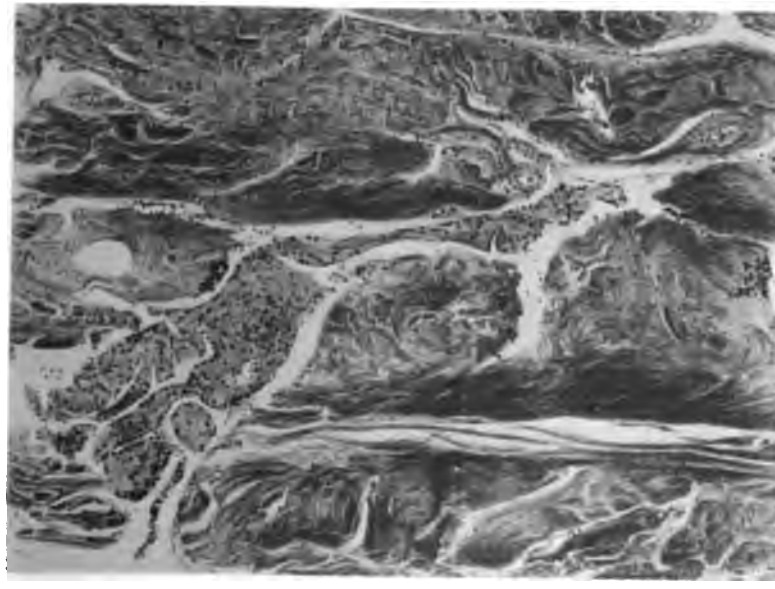

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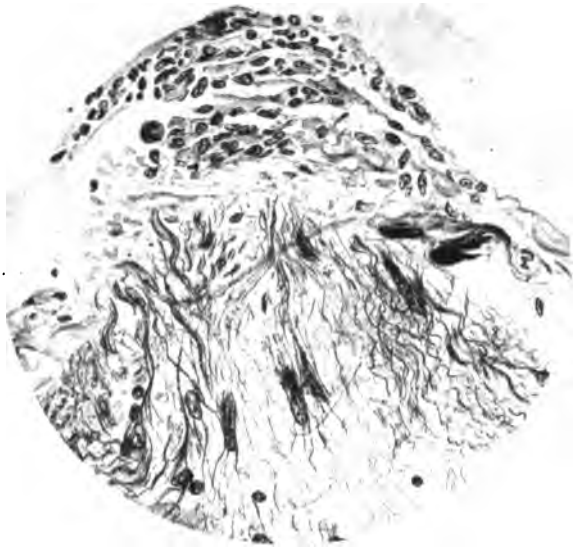

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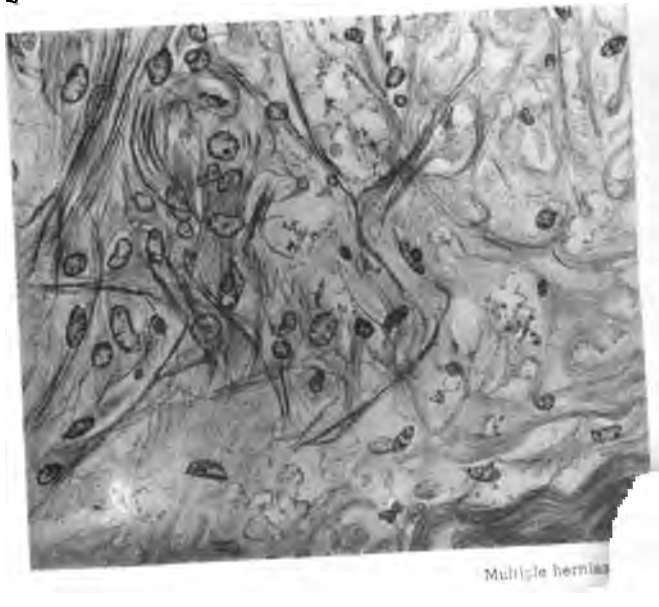

13

Multiple hernias

THE COLON–AEROGENES GROUP OF BACTERIA.*

D. H. BERGEY, M.D.
(*Assistant Professor of Bacteriology.*)
AND
SYLVESTER J. DEEHAN, M.D.
(*Second Assistant in Bacteriology, University of Pennsylvania.*)

There still exists amongst bacteriologists considerable confusion and uncertainty with regard to the identity of the bacteria capable of fermenting carbohydrates which are encountered in feces, in water, in milk, or elsewhere.

During the past two or three years this group of bacteria has been studied in detail by a number of competent observers in England, Germany, and in America, and we are to-day in a better position to identify these carbohydrate fermenting bacteria than we have ever been before. The investigations that have been of value in differentiating this group of bacteria are those of MacConkey,[1] Twort,[2] Vourloud,[3] and Winslow[4] and Walker.

The studies of these investigators have shown that the bacteria belonging to the colon-aerogenes group may be differentiated from each other by their fermenting powers when tested upon various carbohydrates. Besides the power to ferment carbohydrates, MacConkey has shown that several other factors are of great service in differentiating the members of this group of bacteria, namely, the presence or absence of liquefaction of gelatin, the presence or absence of indol production, the presence or absence of motility, and the presence or absence of the Voges and Proskauer reaction.[5]

After testing the fermenting powers of the bacteria of this group upon quite a large number of different carbohydrates MacConkey found that the carbohydrates which were of greatest importance in the differentiation of the various members of the group were saccharose, dulcit, adonit, inulin. MacConkey tested the fermenting powers of these bacteria in

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the ordinary nutrient media containing the different carbohydrates, as well as in bile-salt media colored with neutral red to which the carbohydrates had been added. Twort used the usual peptone solution to which the carbohydrates had been added in the proportion of two per cent. Vourloud employed the ordinary two per cent agar to which the carbohydrates had been added, using litmus as his indicator. In our own work we have employed the Hiss serum-water media colored with litmus, to which the different carbohydrates were added in the proportion of one per cent.

There has been so much uncertainty with regard to the biological characters of various members of this group of bacteria that some of them have been described under several different names. The better known organisms of this group are, Bacillus coli communior and Bacillus coli verus, two types of colon bacillus, both of which ferment dulcit, the former also fermenting saccharose, Bacterium aerogenes (Escherich), Bacterium acidi lactici (Heüppe), Bacterium pneumonicum (Friedlander), Bacillus cloacæ (Jordan), Bacillus enteritidis (Gaertner), Bacillus vulgaris (Hauser), Bacillus icteroides (Sanarelli), and Bacterium Neapolitanus. To these organisms MacConkey has added Bacillus Grünthal, an organism found by Fischer in cases of meat poisoning;[6] Bacillus levans, an organism which Wolffin[7] found to be the cause of fermentation of dough; Bacterium coscorobæ[8] which was found to be the cause of an epidemic in swans; and Bacterium oxytocum (Wyssokowitsch) an organism which has been isolated from stale milk.

MacConkey has found that Bacillus cavicida (Brieger) and Bacillus mustilæ septicus are identical with Bacillus coli verus. He found also that Bacterium rhinoscleromatis and Bacterium ozenæ are likewise identical with Bacterium pneumonicum (Friedlander), while Bacterium aerogenes and Bacterium capsulatus (Pfeiffer) are also identical.

The value of the Voges and Proskauer reaction has been called in question by Harris,[9] but MacConkey contends that the reaction is reliable, and our experiments confirm this contention of MacConkey's. In making the test it is important

that the culture medium employed be not highly colored with beef extract, otherwise there may be some uncertainty as to the presence or absence of this color reaction. The reaction in question is a change of color which takes place in the fluid of the fermentation tube after the absorption of the carbon dioxid by means of sodium hydroxid. The presence of the reaction is indicated by the change of color of the fluid to one closely resembling eosin. The substance produced is acetylmethylcarbinol.[10] This substance seems to be produced by some bacteria that decompose dextrose. The characteristic color when this substance is present begins to show itself about twenty-four hours after the sodium hydroxid is added, and is usually most intense after forty-eight hours.

The value of the different tests employed in the differentiation of the bacteria of this group is dependent directly upon the stability of the different cultural characteristics of bacteria. If it is possible by any means whatever to make bacteria acquire properties which they do not possess ordinarily, then the value of these tests becomes practically nothing. MacConkey is emphatic in his statements that it is impossible to make bacteria take on new characteristics, even by long continued cultivation. For instance, if an organism is capable of fermenting only one of the four carbohydrates which he has found serviceable for purposes of differentiation, such organism will always ferment this carbohydrate and none of the others. Twort[11] claims to have been able to bring about the fermentation of carbohydrates by bacteria that ordinarily do not possess this power. In our own experiments we have failed entirely to arouse fermentative powers in an organism that did not originally possess these powers, and in the same way all our attempts to induce bacteria to alter their biological character with regard to the liquefaction of gelatin, and the production of the Voges and Proskauer reaction, have failed completely. In this respect we are fully in accord with MacConkey's contention, and are unable in any particular to uphold the statements of Twort. Our position is in accordance with the accepted views on heredity, since all the more important scientists of the present

day claim that acquired characteristics are not transmissible.

It is well known to what a marked degree cultures of known organisms may become altered as to their morphologic, biologic, and pathogenic properties, but careful investigations into the nature of these alterations show that they are degenerative in character and not evolutionary. When, by any means, we increase the virulence of an organism, we merely restore to it in full degree its normal properties — properties that have been lowered through the unfavorable influences of environmental conditions.

The studies embodied in this report were made upon the bacteria isolated from fifty samples of milk, from one sample of kefir, and from eight samples of sewage. The sewage organisms were isolated and studied by Dr. Deehan, who is entitled to equal credit for the results embodied in this study. The organisms obtained from milk were isolated and studied by Dr. Bergey. Several of the samples of milk examined were samples of "certified" milk, but none of the organisms included in this study were found in this milk. All the other samples of milk were ordinary market milk, derived from milk shops in different parts of the city. One of the organisms was isolated from a sample of kefir. The samples of sewage were collected from several sewer outfalls along the Schuylkill River.

Only such organisms have been included in the present study as are closely allied to either Bacillus coli or Bacterium aerogenes in general cultural characters and in the possession of the power of fermenting dextrose.

Tests applied to the organisms isolated. — All the gas-forming organisms isolated from the various samples of sewage and milk were studied with regard to the following characters: (1) Morphology in twenty-four-hour agar cultures; (2) motility in twenty-four-hour broth cultures. These were examined microscopically for the presence or absence of motility by the hanging drop method. Any organism was

considered to be motile when there was distinct translation from place to place; (3) staining by Gram's method; (4) production of indol. Broth cultures about eight to ten days old were used. About ten drops of sulphuric acid were added to each tube and about one cubic centimeter of a .02 per cent solution of sodium nitrite; (5) the Voges and Proskauer reaction; (6) percentage of gas produced in dextrose broth; (7) ratio of H to CO_2 (fermentation tubes containing one per cent peptone (Witte), one-half per cent sodium chlorid, and one per cent dextrose in water were inoculated with the various organisms and incubated at 37° C. After forty-eight hours' growth they were taken out of the incubator, allowed to cool, and the amount of gas produced noted. The bulb was then filled with ten per cent NaHO solution, the opening closed with the thumb and the tube manipulated so as to bring the gas in contact with the NaHO. After all the gas had again been collected in the closed arm the thumb was removed and air allowed to enter the bulb to replace any CO_2 absorbed. The ratio of H to CO_2 was then ascertained and the tubes allowed to stand at room temperature for forty-eight hours when the presence or absence of the Voges and Proskauer reaction was noted); (8) growth on nutrient agar; (9) growth on nutrient gelatin, noting especially the presence or absence of liquefaction of the medium. This test was carried out at 37° C. during a week or ten days; (10) growth in nutrient broth; (11) growth in litmus milk; (12) fermentation of various carbohydrates, alcohols, etc., in the Hiss serum media.

We have grouped the bacteria of the colon-aerogenes group according to their powers of fermenting saccharose, dulcit, adonit, and inulin, and with regard to the presence or absence of motility, indol production, the Voges and Proskauer reaction, and liquefaction of gelatin. Taking the latter four characters, we may have sixteen different possibilities as to the presence or absence and combinations of these reactions. In the same way taking the power of fermenting the four different carbohydrates, we may also have

sixteen different possibilities with regard to these powers. We have conceived, therefore, that it might be possible to have two hundred and fifty-six different combinations of these eight reactions. We have arranged the bacteria of this group, therefore, into sixteen sub-groups, which we have numbered from I. to XVI.

In a general way the system of classification followed here is that proposed by MacConkey. We have rearranged the location of the different factors in the charts and have extended the number of possible combinations that may occur.

MacConkey has included in his chart the percentage of gas formed in one per cent dextrose bouillon and the relation of the proportion of hydrogen gas formed to the amount of carbon dioxid formed. While we have found these factors of some value they are, however, of less importance than the others on which we have laid greater stress.

While it is evident that this method of classification is of great value, it fails to allow us to differentiate some organisms that have been regarded as distinct species. Extended study along these and other lines must eventually prove or disprove the value of the method followed.

GROUP I.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
1...	.....	....	....	....	....	60	3/2	....	....	....	....	....	....	1
2...	.....	....	....	....	....	....	....	....	....	....	+			
3...	.....	....	....	....	....	45	3/1	....	....	+	....	1	....	3
4...	Bacillus Grünthal	....	....	....	....	50	2/1	....	....	+	+	3	....	1
5...	.....	....	....	....	....	....	....	....	+					
6...	.....	....	....	....	....	50	1/2	....	+	....	+	5		
7...	.....	....	....	....	....	....	....	....	+	+				
8...	.....	....	....	....	....	50	2/3	....	+	+	+	5		
9...	.....	....	...	....	...	....	....	+						
10..	.....	....	....	....	....	....	....	+	....	....	+			
11..	.....	...	....	....	....	...	....	+	....	+				
12..	.....	....	....	....	....	....	....	+	....	+	+			
13..	.....	....	....	....	....	....	....	+	+					
14..	.....	....	....	....	....	....	....	+	+	....	+			
15..	.....	....	...	....	....	....	....	+	+	+				
16..	.....	....	....	....	....	....	....	+	+	+	+			

The organisms in Group I., comprising the first sixteen of the two hundred and fifty-six possibilities, are those which ferment none of the carbohydrates. In this group the only known organism is Bacillus Grünthal, an organ isolated by Bernard Fischer[12] from prepared meat which had caused gastro-enteric fever in ten persons at Grünthal, Germany. This organism is colon-like in its general appearance and is highly infectious for mice and guinea-pigs.

Three of the bacteria found in milk were identified as Bacillus Grünthal, though one of these was not agglutinated with the serum of a rabbit immunized against another organism believed to be Bacillus Grünthal. Aside from these there were found in milk three other members of this group corresponding to the following numbers in the detailed classification: One corresponding to No. 3, five to No. 6, and five to No. 8. Those corresponding to No. 6 are all alkali

producers and represent the only typical alkali producing bacteria which we found in milk and sewage. No bacteria corresponding to Group I. were isolated from sewage. MacConkey isolated from sixteen samples of milk one organism corresponding to Bacillus Grünthal, and three corresponding to No. 3, and one organism corresponding to No. 1 which he obtained from water.

GROUP II.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
17..	.....	+												
18..	.....	+	....	....	....	30	2/1	....	....	....	+	2		
19..	Bacterium coscorobæ	+	....	....	....	30	2/1	....	....	+				
20..	.....	+	....	....	....	40	3/1	....	....	+	+	1		
21..	.....	+	....	....	....	....	....	....	+					
22..	.....	+	....	....	....	60	2/1	....	+	....	+	2		
23..	....	+	....	....	...	....	....	....	+	+				
24..	....	+	....	....	....	....	....	....	+	+	+			
25..	.....	+	....	....	....	....	....	+						
26..	....	+	....	....	....	20	3/1	+	....	....	+	1		
27..	.....	+	....	....	....	....	....	+	....	+				
28..	Bacillus vulgaris	+	....	....	....	20	2/1	+	....	+	+			
29..	.....	+	....	....	....	....	....	+	+					
30..	Bacillus cloacæ.....	+	....	....	....	75	1/1	+	+	....	+	....	8	2
31..	....	+	....	...	....	....	....	+	+	+				
32..	.....	+	....	...	....	....	....	+	+	+	+			

The organisms in Group II., comprising the second sixteen possibilities of our classification, are those which ferment saccharose but none of the other three carbohydrates. The known bacteria of this group are Bacterium coscorobæ, Bacillus vulgaris (Hauser), and Bacillus cloacæ (Jordan).

Bacterium coscorobæ[18] was found to be the cause of an epidemic in a particular race of swans, "Cygne de Coscoroba."

None of these known organisms was isolated from milk, but on the contrary four other representatives of the group were found, *viz.*, two corresponding to No. 18, one corresponding to No. 20, two corresponding to No. 22, and one corresponding to No. 26. Bacillus cloacæ was the only representative of this group isolated from sewage, and this organism was found in each of the eight samples of sewage analyzed. The only organism of this group which MacConkey found was Bacillus cloacæ.

GROUP III.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
33..	.....	....	+											
34..	Bacillus enteritidis	....	+	....	....	50	2/1	....	....	....	+	....	....	1
35..	.....	....	+	....	....	30	3/1	....	....	+	....	1	....	5
36..	Bacillus coli verus.....	....	+	....	....	25	2/1	....	....	+	+	....	4	2
37..	.....	....	+	....	....	....	....	....	+					
38..	.....	....	+	....	....	35	2/1	....	+	....	+	2		
39..	.....	....	+	....	....	....	....	....	+	+				
40..	.....	....	+	....	....	....	....	....	+	+	+			
41..	.....	....	+	....	....	....	..	+						
42..	.....	....	+	....	....	....	....	+	....	....	+			
43..	.....	....	+	....	....	....	....	+	....	+				
44..	.....	....	+	....	....	25	3/1	+	....	+	+	....	....	3
45..	.....	....	+	....	....	....	....	+	+					
46..	.....	....	+	....	....	....	....	+	+	....	+			
47..	.....	....	+	....	....	....	....	+	+	+				
48..	.....	....	+	....	....	....	....	+	+	+	+			

Group III. comprises those organisms which ferment dulcit but none of the other carbohydrates. The known representatives of this group are Bacillus enteritidis (Gaertner) and Bacillus coli verus (Escherich). Neither of these bacteria was found in milk, but two other representatives were found corresponding to No. 35 and No. 38. Bacillus

coli verus was isolated from four of the eight samples of sewage, and this was the only representative of the group found in sewage. MacConkey found both Bacillus enteritidis and Bacillus coli verus, and he also found five organisms corresponding to No. 35, and three corresponding to No. 44.

GROUP IV.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
49..	.....	..	....	+										
50..	Bacillus icteroides.....	....	....	+	....	20	3/1	....	....	....	+	1		
51..	Bacterium acidi lactici ..	....	....	+	....	30	2/1	....	....	+	....	1	5	1
52..	.....	....	....	+	....	40	3/1	....	....	+	+	....	....	1
53..	.....	....	....	+	....	....	....	....	+					
54..	.....	....	....	+	....	....	....	....	+	....	+			
55..	.....	....	....	+	....	....	....	....	+	+				
56..	.....	...	....	+	....	....	....	....	+	+	+			
57..	.....	....	....	+	....	....	....	+						
58..	.....	....	....	+	....	....	....	+	....	....	+			
59..	.....	....	....	+	..	....	....	+	....	+				
60..	.....	....	....	+	....	....	....	+	...	+	+			
61..	.....	....	....	+	....	...	....	+	+					
62..	.....	....	....	+	....	....	....	+	+	....	+			
63..	.....	....	....	+	....	....	....	+	+	+				
64..	.....	....	....	+	....	....	....	+	+	+	+			

Group IV. comprises those organisms which ferment adonit but none of the other three carbohydrates. The known organisms of this group are Bacillus icteroides (Sanarelli) and Bacterium acidi lactici (Heüppe). One representative of each of these bacteria was isolated from milk. Bacterium acidi lactici was isolated from five of the samples of sewage analyzed. MacConkey found Bacterium acidi lactici once, and he also found another organism belonging in this group corresponding to No. 52.

GROUP V.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
65..	.....	....	....	....	+									
66..	.....	....	....	....	+	....	...	....	....	....	+			
67..	.....	....	....	....	+	....	....	....	....	+				
68..	.....	....	....	....	+	....	....	....	....	+	+			
69..	.....	....	....	....	+	....	...	....	+					
70..	.....	....	....	....	+	....	....	...	+	....	+			
71..	.....	....	....	....	+	....	....	....	+	+				
72..	.....	....	....	....	+	....	....	....	+	+	+			
73..	.....	....	....	....	+	....	....	+						
74..	.....	....	....	....	+	....	....	+	....	....	+			
75..	.....	...	....	..	+	....	....	+	....	+				
76..	.....	....	....	....	+	....	....	+	....	+	+			
77..	.....	....	....	....	+	....	....	+	+					
78..	Bacillus levans	....	....	....	+	60	1/2	+	+	....	+	....	1	
79..	.....	....	....	....	+	...	....	+	+	+				
80..	.....	....	....	....	+	....	....	+	+	+	+			

Group V. comprises those bacteria which ferment inulin but none of the other three carbohydrates. The only known representative of this group is Bacillus levans. This organism was isolated by Alexander Wolffin[14] from sour dough. He regarded this organism as the cause of fermentation in dough. No organisms of this group were found in milk but Bacillus levans was found in one of the samples of sewage. MacConkey found no organisms belonging in this group.

GROUP VI.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
81..	.....	+	+											
82..	.....	+	+	....	...	....	....	....	....	....	+			
83..	Bacterium Neapolitanus.	+	+	....	....	30	2/1	....	....	+	....	1	....	4
84..	Bacillus coli communior.	+	+	....	....	30	2/1	....	....	+	+	2	8	1
85..	.....	+	+	....	....	....	....	....	+					
86..	.....	+	+	....	....	45	1/2	....	+	....	+	1		
87..	.....	+	+	....	....	....	....	....	+	+				
88..	.....	+	+	....	....	50	2/3	....	+	+	+	1		
89.	.....	+	+	....	....	....	....	+	....					
90..	.....	+	+	....	....	....	....	+	....	....	+			
91..	.....	+	+	....	....	....	....	+	....	+				
92..	.....	+	+	....	....	....	....	+	...	+	+			
93..	.....	+	+	....	....	....	....	+	+					
94..	.....	+	+	....	....	....	....	+	+	....	+			
95..	.....	+	+	....	....	....	....	+	+	+				
96..	.....	+	+	....	....	....	....	+	+	+	+			

Group VI. comprises those bacteria which ferment saccharose and dulcit but neither of the other two carbohydrates. The known organisms of this group are Bacterium Neapolitanus (Escherich) and Bacillus coli communior (Dunbar). Both of these organisms were found in milk, the former once, and the latter twice. Aside from these, two other representatives of this group were found in milk, one corresponding to No. 86 and one to No. 88. Bacillus coli communior was found in each of the eight samples of sewage analyzed. MacConkey found Bacterium Neapolitanus in four samples and Bacillus coli communior once.

GROUP VII.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
97..	....	+	....	+	....	50	2/1	....	....	....	....	....	....	2
98..	.....	+	....	+	....	....	....	....	....	....	+			
99..	.....	+	....	+	....	30	3/1	....	....	+	....	4	....	1
100.	.....	+	...	+	....	....	....	....	....	+	+			
101.	.....	+	....	+	....	90	2/3	....	+	....	....	....	....	1
102.	.....	+	....	+	....	....	....	....	+	....	+			
103.	Bacterium aerogenes	+	...	+	....	50	2/1	....	+	+	...	3	6	
104.	.....	+	....	+	....	....	....	....	+	+	+			
105.	....	+	....	+	....	....	....	+	....					
106.	....	+	....	+	....	....	....	+	....	....	+			
107.	.. ..	+	....	+	....	....	....	+	...	+				
108.	.....	+	....	+	....	....	....	+	....	+	+			
109.	....	+	....	+	....	....	....	+	+					
110.	.....	+	....	+	....	....	....	+	+	....	+			
111.	.....	+	....	+	....	....	....	+	+	+				
112.	.....	+	....	+	....	....	....	+	+	+	+			

Group VII. comprises those bacteria which ferment saccharose and adonit but neither of the other two carbohydrates. The only known organism of this group is Bacterium aerogenes (Escherich). This organism was found three times in milk and in six of the eight samples of sewage analyzed. Aside from this, another representative of the group was found in four samples of milk corresponding to No. 99. MacConkey also found the same organism in one sample of milk, besides two other representatives of this group; one of these corresponding to No. 97 and the other corresponding to No. 101.

GROUP VIII.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
113.	.....	+	....	....	+									
114.	.....	+	....	....	+	....	....	....	....	....	+			
115.	.....	+	....	....	+	....	....	....	....	+				
116.	.....	+	....	....	+	30	4/1	....	....	+	+	1		
117.	.....	+	....	....	+	....	....	....	+					
118.	.....	+	....	....	+	....	....	....	+	....	+			
119.	.....	+	....	....	+	....	....	....	+	+				
120.	.....	+	....	....	+	....	....	....	+	+	+			
121.	.....	+	....	....	+	....	....	+						
122.	.....	+	....	....	+	....	....	+	....	....	+			
123.	.....	+	....	....	+	....	....	+	....	+				
124.	.....	+	....	....	+	....	....	+	....	+	+			
125.	.....	+	....	....	+	....	....	+	+					
126.	.....	+	....	....	+	....	....	+	+	....	+			
127.	.....	+	....	....	+	....	....	+	+	+				
128.	.....	+	....	....	+	....	....	+	+	+	+			

Group VIII. comprises those organisms which ferment saccharose and inulin but neither of the other two carbohydrates. There is no known representative of this group, but one representative corresponding to No. 116 was found in milk.

GROUP IX.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
129.	.....	....	+	+										
130.	.....	....	+	+	....	....	...	....	....	....	+			
131.	.....	....	+	+	....	....	....	....	....	+				
132.	.....	....	+	+	....	25	2/1	....	....	+	+	....	1	
133.	.....	....	+	+	....	....	....	....	+					
134.	.....	....	+	+	....	....	....	....	+	....	+			
135.	.....	....	+	+	....	....	....	....	+	+				
136.	.....	....	+	+	....	....	....	....	+	+	+			
137.	.....	....	+	+	...	....	...	+						
138.	.....	....	+	+	....	....	....	+	....	....	+			
139.	.....	....	+	+	....	....	....	+	....	+				
140.	.....	....	+	+	....	....	....	+	....	+	+			
141.	.....	....	+	+	....	....	....	+	+					
142.	.....	....	+	+	....	....	....	+	+	....	+			
143.	.....	....	+	+	....	....	....	+	+	+				
144.	.....	....	+	+	....	....	....	+	+	+	+			

Group IX. comprises those organisms which ferment dulcit and adonit but neither of the other two carbohydrates. There is no known representative of this group, but one member of the group corresponding to No. 132 was found in sewage.

GROUP X.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
145.	.....	....	+	....	+									
146.	.....	....	+	....	+	....	....	....	....	....	+			
147.	.....	....	+	....	+	....	....	....	....	+				
148.	.....	....	+	....	+	....	....	....	....	+	+			
149.	.....	....	+	....	+	....	....	....	+					
150.	.....	....	+	....	+	....	....	....	+	....	+			
151.	.....	....	+	....	+	....	....	....	+	+				
152.	.....	....	+	....	+	....	....	....	+	+	+			
153.	.....	...	+	....	+	....	....	+						
154.	.....	....	+	....	+	....	....	+	....	....	+			
155.	.....	....	+	....	+	....	....	+	....	+				
156.	.....	...	+	....	+	....	....	+	....	+	+			
157.	.....	....	+	....	+	....	....	+	+					
158.	.....	....	+	....	+	....	....	+	+	....	+			
159.	.....	....	+	....	+	....	....	+	+	+				
160.	.....	....	+	....	+	....	....	+	+	+	+			

Group X. comprises those bacteria which ferment dulcit and inulin but neither of the other two carbohydrates. There are no known representatives of this group and no organisms belonging in this group were found in milk or sewage, neither did MacConkey find a representative of this group.

GROUP XI.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
161.	.....	....	.. .	+	+									
162.	.....	....	....	+	+	....	....	....	....	....	+			
163.	.....	....	....	+	+	....	....	....	....	+				
164.	.....	....	....	+	+	...	....	....	....	+	+			
165.	.....	....	....	+	+	....	....	....	+					
166.	.....	....	....	+	+	....	....	....	+	....	+			
167.	.....	....	....	+	+	....	....	....	+	+				
168.	.....	....	....	+	+	....	...	....	+	+	+			
169.	.....	....	....	+	+	....	....	+						
170.	.....	....	....	+	+	....	....	+	....	....	+			
171.	.....	....	...	+	+	....	...	+	....	+				
172.	.....	....	....	+	+	....	....	+	....	+	+			
173.	.....	....	....	+	+	....	....	+	+					
174.	.....	....	....	+	+	....	....	+	+	....	+			
175.	.....	....	....	+	+	....	....	+	+	+				
176.	.....	....	....	+	+	....	....	+	+	+	+			

Group XI. comprises those bacteria which ferment adonit and inulin but do not act on either of the other two carbohydrates. There are no known representatives of this group and none were found in milk or sewage.

GROUP XII.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
177.	.....	+	+	+	....	80	1/2	....	....	....	....	2		
178.	.....	+	+	+	...	....	....	....	....	....	+			
179.	Bacterium pneumonicum.	+	+	+	....	30	3/1	....	....	+				
180.	.....	+	+	+	....	40	3/1	....	....	+	+	....	....	1
181.	.....	+	+	+	....	75	3/2	....	+	....	....	....	....	1
182.	.....	+	+	+	....	....	....	....	+	....	+			
183.	.....	+	+	+	....	20	1/2	....	+	+	...	1		
184.	.....	+	+	+	....	....	....	....	+	+	+			
185.	.....	+	+	+	....	....	....	+						
186.	.....	+	+	+	....	....	....	+	....	....	+			
187.	.....	+	+	+	....	....	....	+	....	+				
188.	.....	+	+	+	....	....	....	+	....	+	+			
189.	.....	+	+	+	....	....	....	+	+					
190.	.....	+	+	+	....	....	....	+	+	....	+			
191.	.....	+	+	+	....	....	....	+	+	+				
192.	.....	+	+	+	....	....	....	+	+	+	+			

Group XII. comprises those bacteria which ferment saccharose, dulcit, and adonit but do not attack inulin. The only known representative of this group is Bacterium pneumonicum (Friedlander). No organisms of this species were encountered by us in milk or sewage. Two other representatives of the group were found in milk; two cultures corresponding to No. 177, and one culture corresponding to No. 183. In addition to these MacConkey found an organism corresponding to No. 180 and one corresponding to No. 181.

GROUP XIII.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
193.	.....	+	+	....	+									
194.	.....	+	+	....	+	....	....	....	....	....	+			
195.	.....	+	+	....	+	....	....	....	....	+				
196.	.....	+	+	....	+	....	....	....	....	+	+			
197.	.....	+	+	....	+	50	1/1	....	+	....	....	....	1	
198.	.....	+	+	....	+	50	2/1	....	+	....	+	1		
199.	.....	+	+	....	+	....	....	....	+	+				
200.	....	+	+	....	+	....	....	....	+	+	+			
201.	.....	+	+	....	+	....	....	+						
202.	.....	+	+	....	+	...	....	+	....	....	+			
203.	.....	+	+	....	+	....	....	+	....	+				
204.	.....	+	+	....	+	...	....	+	....	+	+			
205.	.....	+	+	....	+	....	....	+	+					
206.	.....	+	+	....	+	....	....	+	+	....	+			
207.	.....	+	+	....	+	....	....	+	+	+				
208.	.....	+	+	....	+	....	....	+	+	+	+			

Group XIII. comprises those bacteria which ferment saccharose, dulcit, and inulin but do not attack adonit. There are no known representatives of this group, but an organism corresponding to No. 197 was found in sewage, and one corresponding to No. 198 was found in milk. MacConkey found no representative of this group.

GROUP XIV.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
209.	.....	+	....	+	+									
210.	.....	+	....	+	+	....	....	....	....	....	+			
211.	.....	+	....	+	+	75	2/1	....	....	+	....	2		
212.	.....	+	....	+	+	....	....	....	....	+	+			
213.	.....	+	....	+	+	30	2/1	....	+	....	....	1	....	1
214.	.....	+	....	+	+	....	....	....	+	....	+			
215.	.....	+	....	+	+	65	1/1	....	+	+	....	2	....	1
216.	.....	+	....	+	+	....	....	....	+	+	+			
217.	.....	+	....	+	+	....	....	+						
218.	.....	+	....	+	+	....	....	+	....	....	+			
219.	.....	+	....	+	+	....	....	+	....	+				
220.	.....	+	....	+	+	....	....	+	....	+	+			
221.	.....	+	....	+	+	....	....	+	+					
222.	.....	+	....	+	+	....	....	+	+	....	+			
223.	.....	+	....	+	+	....	....	+	+	+				
224.	.....	+	....	+	+	....	....	+	+	+	+			

Group XIV. comprises those bacteria which ferment saccharose, adonit, and inulin but do not attack dulcit. There are no known representatives of this group but there were found in milk two cultures corresponding to No. 211, one corresponding to No. 213, and two corresponding to No. 215. No organisms of this group were found in sewage. MacConkey found a representative of No. 213 and one of No. 215.

GROUP XV.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
225.	.....	....	+	+	+									
226.	.....	....	+	+	+	....	...	....	....	....	+			
227.	.....	....	+	+	+	....	....	....	....	+				
228.	.....	...	+	+	+	....	....	....	....	+	+			
229.	.....	...	+	+	+	....	....	....	+					
230.	.....	....	+	+	+	....	....	....	+	....	+			
231.	.....	....	+	+	+	....	....	....	+	+				
232.	.....	....	+	+	+	....	....	....	+	+	+			
233.	.....	....	+	+	+	....	....	+						
234.	.....	....	+	+	+	....	....	+	....	....	+			
235.	.....	....	+	+	+	....	....	+	....	+				
236.	.....	....	+	+	+	....	....	+	....	+	+			
237.	.....	...	+	+	+	....	....	+	+					
238.	.....	....	+	+	+	....	...	+	+	...	+			
239.	.....	....	+	+	+	....	...	+	+	+				
240.	.....	....	+	+	+	....	....	+	+	+	+			

Group XV. comprises those bacteria which ferment dulcit, adonit, and inulin but do not attack saccharose. There are no known organisms of this group and none were found in milk or sewage, neither were any found by MacConkey.

GROUP XVI.

No.	Name.	Saccharose.	Dulcit.	Adonit.	Inulin.	% of Gas.	H/CO_2	Gelatin.	V/P	Indol.	Motility.	Milk.	Sewage.	MacConkey.
241.	.....	+	+	+	+									
242.	.....	+	+	+	+	....	....	....	....	....	+			
243.	.....	+	+	+	+	....	....	....	....	+				
244.	.....	+	+	+	+	...	....	...	....	+	+			
245.	Bacterium oxytocum.....	+	+	+	+	60	1/1	....	+	....	....	....	8	1
246.	.....	+	+	+	+	....	....	....	+	....	+			
247.	.....	+	+	+	+	90	1/1	....	+	+	....	1	....	4
248.	.....	+	+	+	+	50	3/2	....	+	+	+	1		
249.	.....	+	+	+	+	....	....	+						
250.	.....	+	+	+	+	....	....	+	....	....	+			
251.	.....	+	+	+	+	....	....	+	....	+				
252.	.....	+	+	+	+	....	....	+	....	+	+			
253.	.....	+	+	+	+	....	....	+	+					
254.	.....	+	+	+	+	....	....	+	+	....	+			
255.	.....	+	+	+	+	....	....	+	+	+				
256.	.....	+	+	+	+	....	....	+	+	+	+			

Group XVI. comprises those bacteria which ferment each of the four carbohydrates. The only known organism of this group is Bacterium oxytocum,[15] an organism which Matzuschita isolated from stale milk. This organism was not found in milk but was encountered in each of the eight samples of sewage analyzed. MacConkey has found this organism in one sample of milk. Two other members of this group were found in milk, one corresponding to No. 247 and one to No. 248. MacConkey found four representatives of No. 247.

It will be seen that we have found organisms representing only forty-three of the two hundred and fifty-six possible combinations of the eight characteristics which have been employed in the classification. Even though not another representative should be discovered we still believe that this

method of classification is of distinct value in identifying bacteria of this group. One may venture the prediction, however, that there are many more representatives of this group of bacteria in nature.

Although all our attempts to make bacteria acquire new characteristics have yielded negative results, we are of the opinion that in Nature's great laboratory such evolutionary changes are constantly in progress. The cultivation of organisms in a medium containing a carbohydrate not previously fermented did not induce fermentation after fifty generations. In the same manner an organism that did not give the Voges and Proskauer reaction did not acquire this property after fifty generations in the appropriate medium. Nor did an organism unable to liquefy gelatin acquire this property after cultivation in gelatin for fifty generations. The same is true of the power of producing indol. After fifty generations in appropriate media this power had not been acquired.

In consequence of the results of our experiments we regard the biological characters of an organism as fixed and unchangeable as far as ordinary manipulations are concerned, except in the direction of diminished or lost function. The latter form of alteration is so well known as to need no comment. Because of the facility with which some bacteria may lose their normal characters it is essential to grow them for a number of generations on appropriate media in order to definitely establish all their characteristics. A number of our tests were repeated many times in order that we might be certain of the identity of the organism under cultivation.

The organisms which we isolated from the fifty samples of milk represent twenty-seven different species, only five of which are known species and seven of the other species had been encountered before by MacConkey in his analysis of sixteen samples of milk, while the other fifteen species appear to be new.

The organisms which we isolated from sewage represent nine different species, all of which had been previously described.

The seven organisms isolated from milk which MacConkey had also found, but has not named, correspond to the following numbers in our system of classification: 3, 35, 99, 101, 213, 215, and 247.

The nineteen organisms isolated from milk which appear to be unnamed and which were not encountered by MacConkey correspond to the following numbers in our system of classification: 2, 6, 8, 18, 20, 22, 26, 38, 86, 116, 177, 183, 198, 211, and 248.

MacConkey found in sixteen samples of milk and in one sample of water eight other unknown species which we did not encounter in either milk or sewage. These organisms correspond to the following numbers in our system of classification: 1, 35, 44, 52, 97, 101, 180, and 181.

No doubt most, if not all, of the undescribed bacteria encountered had been found by other investigators, but indefinite descriptions prevent us from being absolutely certain on this point.

All bacteriologists have frequently encountered bacteria which resembled Bacillus coli in many respects, but differed from it in one or more particulars, and it has always been a matter of regret, personally, that it was impossible to give more definite classification of such organisms than to say that they belong in the "colon" group.

The studies of MacConkey and others indicate that all of the bacteria of this group are more or less constant and normal inhabitants of the intestines of man and the domestic animals, and that the discovery of these organisms in milk, water, or other food products is an indication of direct or indirect fecal contamination. There is as yet no means of determining the remoteness of the contamination, neither is it possible to determine whether the contamination is of human or animal origin. It is possible that by still greater refinement of methods we may eventually discover means of determining whether the bacteria are of human or animal origin.

CONCLUSIONS.

The results of our studies appear to indicate the following conclusions:

1. The power of fermenting the different carbohydrates affords an important means of differentiating between various species of closely related bacteria.

2. The carbohydrates which are of especial value in the differentiation of the members of the colon-aerogenes group of bacteria are saccharose, dulcit, adonit, and inulin.

3. The other biological characters of bacteria which MacConkey regards of importance for the differentiation of bacteria of the colon-aerogenes group, and which we have adopted in our work, are the presence or absence of liquefaction of gelatin, the presence or absence of the Voges and Proskauer reaction, presence or absence of indol production, and presence or absence of motility.

4. The biological characters of bacteria are fixed to such an extent that no new properties are acquired during the usual laboratory manipulations, though changes of a degenerative type are prone to occur and normal functions may be lost after a time. For this reason it is necessary to cultivate bacteria through several generations in order to be certain that their true characters are ascertained.

5. The amount of gas produced in dextrose broth and the relation of the quantity of H to CO_2 are factors that are subject to considerable variations, and consequently great care must be exercised in arriving at conclusions on these points. It will be best to carry the organisms through several generations in order to have them exercise these functions in a satisfactory manner before deciding as to their true characters. Although we have laid less stress upon the amount of gas produced than upon the other properties utilized in the classification of the bacteria of the colon-aerogenes group, we believe that this factor is one of importance in differentiating between different species. For instance, with an organism that produces from twenty-five to thirty per cent of gas, like Bacillus coli verus, we know of no

means by which such organism can be made to produce a larger percentage of gas.

6. In determining the percentage of gas produced and the relation of the amount of H to CO_2 produced it is advisable to make the observations and tests at the earliest moment when fermentation has been completed. We have found that usually forty-eight hours' incubation is sufficient for the completion of the fermentation of the dextrose, and that at this time the relation of the amounts of H to CO_2 is most constant. If more time elapses before the gas is analyzed there is reabsorption, and this takes place very irregularly, though usually the CO_2 disappears most rapidly.

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ON THE NATURE OF THE OPSONINS OF NORMAL AND OF IMMUNE SERA AND ON OPSONIC EFFECTS RESULTING FROM THE COMBINED ACTION OF IMMUNE BODY AND COMPLEMENT.*

CARL H. BROWNING, M.D.

(*Assistant in Bacteriology to the Professor of Pathology, Glasgow University.*)

(*From the Pathological Department of the University, Glasgow.*)

It has been shown by Muir and Martin [10, 11] that the thermolabile opsonins of normal serum and the thermostable opsonins of an immune serum differ widely in their combining properties. Thus, the former were shown to have the biological characters of complements, that is to say, the complex receptor immune body (red blood corpuscles, bacteria, serum plus the corresponding immune body) took up practically all of the opsonin of a normal serum. The specific opsonin of the immune serum, however, combined only with the corresponding organism. It was, therefore, concluded that the opsonic effect in the two cases depended on two distinct classes of substances; on the one hand a non-specific thermolabile body, which is taken up by complement absorbers, and on the other hand the specific thermostable opsonins which are true anti-bodies, and which persist in the serum after the absorption or destruction of complement. These results have been confirmed by numerous subsequently published observations.

Accordingly, it has been conclusively shown that, in general, for the production of opsonic effect on the part of a normal serum the complement is indispensable. Certain thermostable opsonins of normal serum, such as that of human serum for the bacillus diphtheriæ are, of course, not under discussion. By the process of immunization, bodies are produced which act as opsonins quite independently of complement. At the same time it is left an open question as to whether or not under certain circumstances an opsonic

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effect may be the result of the combined action of two substances of the nature of immune body and complement. This consideration was originally brought forward by Dean[8] who attributed the fall in opsonic power, consequent on heating an immune serum, to the loss of complement. It is obvious that one must distinguish between two possibilities; firstly, the summation of opsonic effects due to the simultaneous and possibly independent action of specific and non-specific substances; and secondly, an opsonic effect for which the action of both bodies is indispensable. In this latter instance the process would be analogous to the hemolysis resulting from the combined action of immune body and complement, where neither element by itself causes the slightest lysis.

Dean has shown that phagocytosis, due to the action of one volume of a mixture of certain dilutions of a fresh normal serum and of an immune serum, may greatly exceed the effect due to one volume of either by itself. It is clear, however, that where each component by itself has a considerable opsonic effect the conditions existing in a mixture must be highly complicated. Dean has further observed an increase of the opsonic effect when employing mixtures of one volume of a dilution of the immune serum plus one volume of a dilution of fresh normal serum, as compared with one volume of either alone. Cowie and Chapin[9] have obtained similar results. In addition, the latter workers have also studied the action of combinations of heated normal serum plus fresh normal serum deprived of its opsonin by treatment with the homologous organism at 0° C.

What follows is concerned with: (1) a further examination into the nature of normal and of immune opsonins; (2) the production of an opsonic effect by the combined action of immune body and complement.

The method of opsonic estimation followed was that of Wright and Douglass[13]; human leucocytes were employed and the opsonic mixtures were incubated at 37° C. for twenty minutes. The organism used throughout was Staphylococcus

pyogenes aureus from twenty-four-hour agar cultures, and the contents of at least fifty leucocytes were counted in every instance. The single experiments quoted are, of course, only examples of repeated confirmatory results.

I. On the nature of normal and immune opsonins.

(*A.*) It has been shown by Sachs and Teruuchi [12] that the hemolytic complement of fresh guinea-pig's serum is destroyed to a large extent by dilution with from five to ten volumes of water and incubation at 37° C. The same has been found by the author to hold good for the normal opsonin in guinea-pig's and rabbit's serum. The serum was diluted with five volumes of water and incubated at 37° C. for one and one-half hours; then sufficient ten per cent NaCl solution was added to make up to .85 per cent. The control was diluted to an equal degree with .85 per cent NaCl solution. Example:

	Phagocytic Count.
Guinea-pig's serum diluted with water	.8
Control	8.8
Rabbit's serum diluted with water	2.4
Serum heated at 57° C.; then diluted with five volumes of .85 per cent NaCl solution	1.4
Control	6.0

The same procedure was carried out with an immune serum, Rabbit V. Staphylococcus aureus. The details of two such experiments are as follows:

		Phagocytic Count.	
(*a.*)	Serum diluted with five volumes of water, incubated one and one-half hours at 37° C.; then made up to .85 per cent with ten per cent NaCl solution	6.9	9.0
(*b.*)	Serum treated as in (*a*), then heated one hour at 57° C.,	4.4	8.8
(*c.*)	Serum heated at 57° C. for one and one-half hours, then diluted with five volumes of .85 per cent NaCl solution	6.5	7.7
(*d.*)	Serum heated at 57° C. for one and one-half hours, then diluted with five volumes of water and incubated for one and one-half hours at 37° C., then made up to .85 per cent with ten per cent NaCl solution	5.8	9.7
(*e.*)	Untreated serum diluted with five volumes of .85 per cent NaCl solution	18.7	18.0
(*f.*)	Control with .85 per cent NaCl solution instead of serum,	1.0	.5

These results are in complete agreement with the conclusions of Muir and Martin.[10, 11] Thus, it is evident that after the complement of the immune serum has been destroyed by dilution with water an opsonic body remains which is not further affected by heating and vice versa. The variations noted are, according to the author's experience, well within the limits of experimental error.

(*B.*) It was found by Muir and Martin that the diminution in hemolytic, bactericidal, and opsonic action, produced by absorption at 37° C. with (1) red blood corpuscles, (2) serum, (3) bacteria, plus the corresponding immune body, bore a striking resemblance. But they concluded that this did not justify the expression of an opinion as to the identity or the nonidentity of the substances producing these effects. It remained to test the effect of varying the temperature at which the serum was treated. On treating guinea-pig's serum with large quantities of Staphylococcus aureus at 0° C. and then centrifugalizing, it was found that the opsonic effect for Staphylococcus aureus was markedly reduced, while a considerable amount of hemolytic complement remained over. A comparative test, in which the same quantities of serum and of cocci respectively were put in contact at 0° C. and at 37° C., showed that much of the opsonin could be removed at 0° C. — as has been found by Bullock and Atkin[1] — but that a large amount of hemolytic complement remained over, which was, however, taken up in the case where the mixture was incubated at 37° C. The details of such an experiment are as follows:

(*a.*) .5 cubic centimeter of guinea-pig's serum was left in contact for two hours at 37° C., with the sediment of five twenty-four-hour agar cultures of Staphylococcus aureus — killed by boiling for ten minutes — then centrifugalized at room temperature = serum treated 37°.

(*b.*) As in (*a*) but treated at 0° C. and centrifugalized at room temperature = serum treated 0°.

Phagocytic Count.	Dose of Complement for 1 cc. Ox Corpuscles + 10 doses of I.B. from the Rabbit.
Serum treated 37° 1.9	.4
Serum treated 0° 2.0	.04
Untreated Serum 18.3	.003

Accordingly, at 37° C. staphylococci remove from the serum practically all both of the normal opsonin and of the hemolytic complement. At a lower temperature, however, while the opsonin is almost entirely absorbed, there may remain over a considerable amount of complement which is efficient in causing lysis of sensitized red corpuscles. This result may be due to the existence of a natural immune body which leads to the combination of complement, and thus plays a part in the opsonin action; although by itself it has practically no opsonic effect. The complement which is left over is then ineffective in causing opsonic effect, owing to the immune body having been absorbed. On the other hand, this phenomenon may be due to the presence of multiple complements, the existence of which, in the case of hemolytic sera, has been established by the experiments of Ehrlich and Sachs[4] and Muir and Browning.[8] Thus, the complement which is most active in producing the opsonic effect has the greater affinity for the cocci and is accordingly absorbed at the lower temperature, while the additional complement which is fairly active in producing hemolysis, but which by itself has very little opsonic effect, is further taken up at 37° C.

In continuance of these absorption experiments it was found that when cocci previously treated at 0° C. with fresh guinea-pig's serum were acted on with fresh serum which had been treated with an excess of cocci at 0° C., a degree of opsonic effect could be produced which was far in excess of what resulted with treated cocci without the serum or with

treated serum plus untreated cocci. Thus, to take one example:

.3 cubic centimeter guinea-pig's serum was treated with the sediment of seven twenty-four-hour agar cultures of Staphylococcus aureus for two hours at 0° C., then centrifugalized at room temperature and the fluid made up to 1.5 cubic centimeters with .85 per cent NaCl solution = serum treated 0°.

In each of five tubes, .5 cubic centimeter emulsion of Staphylococcus aureus, plus (1) .05, (2) .025, (3) .01, (4) .005, (5) 0 cubic centimeter guinea-pig's serum. Contact one hour at 0° C., then centrifugalized at room temperature and washed twice with salt solution; finally the contents of each tube were resuspended in .5 cubic centimeter salt solution and divided in two portions, (*a*) and (*b*). To (*a*) was added an equal volume of serum treated 0°. To (*b*) was added an equal volume of salt solution. All were incubated one hour at 37°, centrifugalized, washed, and finally resuspended in salt solution.

	Phagocytic Count.				
	(1)	(2)	(3)	(4)	(5)
A.	14.8	9.5	7.5	2.8	1.8
B.	1.6	.7	.7	.1	.5

The fifth tube in series (*a*) shows the opsonic effect produced on normal cocci by serum treated 0°, which is very small. The first tube in series (*b*) shows the amount of phagocytosis with cocci treated at 0° C. with .05 cubic centimeter of fresh serum, which is also inconsiderable. When the treated cocci are acted on by the treated serum the total effect — as seen in the first tube in series (*a*) — is four times the sum of the two partial effects.

In the following experiment the effect of treating the cocci with heated serum was tested in addition:

(1.) .05 cubic centimeter guinea-pig's serum, previously heated at 37° C. for three-quarters of an hour plus .5 cubic centimeter staphylococcus emulsion.

(2.) .025 cubic centimeter of the same guinea-pig's serum unheated plus .5 cubic centimeter staphylococcus emulsion.

Contact at 0° C., etc., as in the above experiment. The resuspended cocci were then divided into two portions (*a*) and (*b*): to (*a*) serum treated 0° was added; to (*b*) salt solution. Finally the cocci were treated as in the previous experiment.

	Phagocytic Count.	
	A.	*B.*
I.	1.4	.2
II.	6.4	1.3

Control: emulsion of untreated cocci + serum treated 0° = .34.

It is seen from this that the opsonic effect with the cocci treated with heated serum previously, plus serum treated 0° is

much inferior to that produced with cocci which had been treated with half the quantity of unheated serum. Löhlein[6] noted also a decrease in opsonic effect with B. coli treated with heated guinea-pig's serum at 0° C. as compared with the organisms treated with fresh serum, on the subsequent addition of a small quantity of fresh normal serum.

In view of the fact that there might be a somewhat thermolabile immune body in normal guinea-pig's serum, the attempt was made to remove the complement in a manner less destructive than by heat. For this purpose the method of absorption by ox corpuscles heated at 57° C. and combined with the corresponding immune body from the rabbit, as previously described (Muir and Browning[9]), was employed.

As the following experiment shows, no augmented opsonic effect was produced by using one-half volume of serum treated 0° plus one-half volume treated with red corpuscles plus I.B., as compared with the effect of one volume of either serum alone:

		Phagocytic Count.
(1.)	Serum treated 0°, one volume	1.6
(2.)	Serum treated with red corpuscles +I.B., one volume .	.6
(3.)	Serum (1) a half volume + serum (2) a half volume .	.6
	Control, salt solution one volume, instead of serum .	.4

As is shown by experiment (page 211), the specimen of serum treated 0° used in the above instance contained complement, and was capable of activating a heated immune serum.

The above results showing the augmentor effects obtained with serum treated 0° plus cocci treated with fresh serum at 0° C. are similar to those obtained by Cowie and Chapin with human serum, and are cited as having been obtained independently. In view of (1) the failure to obtain such effects to any marked degree with normal sera whose complement has been destroyed or absorbed, and (2) the phenomena of summation of effect, which will be further referred to, one must hesitate to interpret such results as a definite proof that the opsonic effect of the normal guinea-pig's serum is due

to the interaction of two substances with the character of immune body and complement.

II. The production of an opsonic effect by the combined action of immune body and complement.

The experiments in this connection were all performed with a heated immune serum, Rabbit V. Staphylococcus aureus, which had been kept for more than a year, and which by itself had only a weak opsonic action. Accordingly, the results must not be unconditionally applied to any very active immune serum. At the same time the effects obtained with sera of low value, in which the molecules of immune body are either few in number or weak in their action, are likely to represent what occurs in processes of defence against infections under ordinary conditions.

(*a.*) The effect of immune serum and complement in combination.

As complement, guinea-pig's serum treated with excess of cocci at 0° C., as previously described, was employed. It was constantly found that the opsonic effect from the combination of one-half volume of a dilution of the immune serum plus one-half volume of a dilution of the serum treated 0° was greatly in excess of the effect due to one volume of the same dilution of either serum by itself. The following table shows the phagocytic counts in three such experiments:

	Immune Serum Diluted with an Equal Volume of Salt Solution. 1 Volume.	Serum treated 0° 1 Volume.	Immune Serum one-half Volume plus Serum Treated 0° one-half Volume.
1 ...	1.0	(Undiluted), 1.5.	8.0
2 ...	1.5	(Diluted with an equal volume of salt solution), 3.1.	11.7
3 ...	1.6	(Diluted with an equal volume of salt solution), 1.6.	6.5

Such results, like those of Dean, and of Cowie and Chapin, while offering presumptive evidence in favor of an opsonic

effect due to the combined action of immune body and complement, are open to the objection that we have to deal with a variety of the phenomenon of summation of effect. This possibility must be more marked where a comparison is instituted between the effect due to the mixture of one volume of each of two components and that due to one volume of either by itself. It is well known that on making successive dilutions of a serum, such that each dilution is half the strength of the preceding one (*e.g.*, 1 : 5, 1 : 10, 1 : 20, etc.), a dilution is frequently reached which gives phagocytic count much less than half of that obtained with the next higher concentration. This holds good both in the case of normal sera and of heated immune sera, and is exemplified in the tables of Wright and Douglas,[1] Hektoen and Ruediger,[5] Dean,[3] Cowie and Chapin and Marshall.[7] The last-mentioned worker finds that the effect of dilution depends on the species of serum. The possibility of this phenomenon must, however, be constantly kept in view. For this reason it has been considered advisable to deal with the question at some length here.

To take an example of the author's:

In the case of a fairly active immune serum, Rabbit V. Staphylococcus aureus, previously heated at 57° C. for one hour and diluted with salt solution. The cocci were treated with the various dilutions for three-quarters of an hour at 37° C., then washed and resuspended in salt solution.

Concentration of Serum in the Emulsion of Cocci.	Phagocytic Count.
1 : 5	26.2
1 : 10	16.6
1 : 20	5.0
1 : 40	1.0

Here, the dilution 1 : 10 gave a count of 16.6 which is three times that given by dilution 1 : 20, and dilution 1 : 40 gave only a fifth of the count due to dilution 1 : 20. It is

easy to understand that where different sera are used in combination such summation effects may readily lead to misinterpretation.

It is a fundamental fact that, in the case of hemolysis with immune sera, the lysis is due to the action of the complement on red corpuscles which have been sensitized by the immune body. If the red corpuscles are first of all treated with inactivated immune serum and are then thoroughly washed and subsequently are brought into contact with the complement, hemolysis occurs. If the procedure is reversed, no lysis takes place since complement by itself is unable to combine with the red corpuscles. Accordingly, it appeared that the question as to whether opsonic effect might be dependent on the combined action of immune body and complement would be definitely settled if it could be shown that the augmented opsonic effect due to the treated normal serum occurred only when the cocci were treated with immune serum first of all and then subsequently with the normal serum, and not when the order of treatment was reversed. That this was actually the case is shown below:

A series of tubes, each containing one volume of Staphylococcus aureus emulsion was treated as follows:

(1.) One volume of a dilution of the immune serum was added; then, after one-half hour at 37° C. the cocci were centrifugalized and washed in salt solution and resuspended in the original volume. Then one volume of a dilution of normal guinea-pig's serum treated previously with excess of Staphylococcus aureus at 0° C. was added and the whole incubated one-half hour at 37° C. Again, the cocci were centrifugalized, washed, and resuspended.

(2.) The treatment was in this instance first of all with normal serum treated 0° and then with immune serum.

(3.) Control: treatment in both instances with one volume of the dilution of immune serum.

(4.) Control: treatment in both instances with one volume of serum treated 0°. As a further control, in one instance instead of immune serum the inactivated serum of a rabbit which had not been injected with staphylococci was used. In another case the treatment was, first with serum 0°, then with immune serum, and lastly, once more with serum treated 0°, the augmentor effect occurred, of course.

The details of three such experiments are as follows:

	Phagocytic Count.		
	I.	II.	III.
Cocci treated with 1 volume immune serum twice	Serum diluted 1 : 10, 1.3	Serum diluted 1 : 10, 1.4	Serum diluted 1 : 10, .76
1 volume normal guinea-pig's serum treated 0° twice	Serum diluted 1 : 2, 3.3	Serum diluted 1 : 4, .38	1.8
1 volume immune serum, then 1 volume normal serum treated 0°	13.0	11.3	6.7
1 volume normal serum treated 0°, then 1 volume immune serum	1.9	.34	.6
1 volume serum treated 0°, then 1 volume immune serum, then 1 volume serum treated 0°	—	9.6	—
1 volume heated serum of untreated rabbit, then 1 volume serum treated 0°	—	—	1.2

Thus it is clear that in this instance the increased opsonic effect produced by the normal serum treated at 0° is directly due to the action of a complement which has little or no affinity for the staphylococci by themselves, but which is enabled to act by being brought into combination through the medium of the immune body, which has previously been anchored to the organisms. Of course, it has not been determined whether the molecules of the immune serum which act along with the complement are or are not the same as those which lead to the opsonic action in the absence of complement.

CONCLUSIONS.

1. The opsonic power of fresh normal guinea-pig's and rabbit's serum for Staphylococcus aureus, like hemolytic complement, is destroyed in great part by diluting with five volumes of water and then incubating for one and one-half

hours at 37° C. On the contrary, the thermostable immune opsonin for Staphylococcus aureus suffers no deterioration by corresponding treatment. This is in agreement with the results of Muir and Martin in regard to these two substances.

2. By treating fresh serum with large quantities of staphylococci at 37° C. practically all of the normal opsonin as well as the hemolytic complement can be absorbed. At a lower temperature, however, a proportion of hemolytic complement may remain over, while the opsonin has been almost entirely taken up. This may be due to the existence of a natural immune body which plays a part in the opsonic effect, leading to the union of complement, although of itself practically without opsonic action; or it may be due to the existence of multiple complements, those concerned with the opsonic effect combining at lower temperatures.

3. An augmented opsonic effect due to the combined action of immune body and complement has been demonstrated, when staphylococci treated with the immune serum, which by itself had very little opsonic effect, were subsequently treated with fresh serum from which the normal opsonin had been removed by treatment with staphylococci at 0° C. The possibility of summation of effect was excluded by the fact that on reversing the procedure the augmented effect did not occur.

This shows that an immune body plus complement may be concerned in the production of opsonic action; but it is left undetermined whether this immune body is the same substance as the immune opsonin which can produce an opsonic effect by itself.

[Towards the expenses of this research a grant was received from the Carnegie Trustees, for which I have pleasure in recording my indebtedness.]

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NOTE. — Galley proof corrected by the Editor.

A CLINICAL AND BACTERIOLOGICAL STUDY OF A CASE OF PYELONEPHRITIS.*

DAVID RIESMAN, M.D., AND D. H. BERGEY, M.D., OF PHILADELPHIA.

The patient, Mrs. A., is an unusually large woman of thirty-eight years, who is married for the second time. Her first husband, by whom she had two sons, now twenty and eighteen years respectively, died ten years ago. She attributes her illness to having gone automobiling after having washed her hair. The symptoms were those of an acute cold with dysuria and pain in the right flank. The urine was cloudy and bloody. Last summer she went to Europe and was treated by physicians in Berlin and Paris, the diagnosis of cystitis being made. When first seen by one of us (R.) she had pain on passing water, and had to rise several times at night. The urine was greenish yellow, and on standing deposited a large amount of pus. The appetite was good, the periods regular, the bowels constipated. Examination showed a firm mass in the lower abdomen, extending a little more to the right than to the left, and evidently a nodular fibroid tumor. There was no tenderness nor enlargement in either kidney region. The quantity of urine was large — up to ninety-six ounces (3,000 cubic centimeters) in twenty-four hours; the pus amounting by volume to from two to four ounces. The specific gravity of the urine varied from 1008 to 1020; albumin was constantly present; pus cells in large numbers; cylindroids and casts on rare occasions. Her blood count gave the following results:

Hemoglobin	65 to 70%
Red blood corpuscles	3,888,000
White blood corpuscles	4,500

Differential count:

Polymorphonuclears	57.5%
Small mononuclears	25.0%
Large mononuclears	11.0%
Transitionals	3.0%
Eosinophiles	2.5%
Basophiles	1.0%

No malarial parasites, no evident anemia, no nucleated red cells, and no abnormal cells were found.

From November eighteenth, 1907, until February twenty-third, 1908, a regular temperature chart was kept; throughout that period of over three months the temperature was above normal, hovering generally between

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99° and 100° F. The pulse was accelerated — from 76 to 96. The respiration was normal. There were no sweats and no chills. The temperature was always higher when the quantity of pus in the urine was diminished, but with a free discharge of pus the temperature would usually fall. Since the beginning of March her temperature has been normal and the amount of pus in her urine has decreased. Catheterization of the ureters demonstrated that the pus comes from the left kidney.

On December tenth, 1907, the first specimen of Mrs. A.'s urine was sent to the laboratory. It deposited a large quantity of pus, which on microscopic examination was found to consist predominantly of polymorphonuclear cells, with a small number of columnar epithelial cells. Some of the leucocytes were filled with rod-shaped bacteria, and many more extracellular bacteria of the same character were found. Cultures were prepared from the pus, and bacteria in every way corresponding to those seen in the fresh urine were isolated. There was only a single type of organism which belonged to the colon group. No tubercle bacilli could be demonstrated.

Some of the urine was centrifugalized, and the sediment injected intraperitoneally into two guinea-pigs; both were found dead in about fifteen hours. The autopsy revealed very definite reaction of the peritoneum and of the abdominal organs, with marked hyperemia of the stomach and intestinal coats and areas of local hemorrhage into the walls of the colon. The peritoneal fluid contained large numbers of the same bacteria as those found in the pus, and many of them were within polymorphonuclear cells. An organism identical with that isolated from the urine was recovered in pure culture from each of the animals. When guinea-pigs were injected intraperitoneally with one-half cubic centimeter of a twenty-four-hour bouillon culture of this organism, the same pathological effects were produced. Histological study of the tissues of the guinea-pigs killed with the bacillus revealed marked hyperemia of all the internal organs as the only pathological lesion.

On February twenty-first, 1908, a second specimen of

Mrs. A.'s urine was sent to the laboratory and this corresponded chemically and microscopically with the first sample. The same organism was present, and there was no evidence of tuberculosis. Two guinea-pigs were injected intraperitoneally with some of the pus from the urine. One of the animals died in less than three days without, however, showing the typical pathological conditions noted previously as the result of injections of cultures obtained from the first specimen, but the same bacteria were present in the peritoneal fluid. The second guinea-pig is alive and well to-day, from which fact we are inclined to exclude tuberculous infection. The survival of the one animal as well as the delayed death in the other animal would also indicate an attenuation of the organism in the patient's urine.

The organism isolated in pure culture from the second specimen of urine is identical with that obtained from the first specimen. It has the general morphological characters of the bacteria of the colon group; it is motile and Gram-negative; does not liquefy gelatin; produces indol, and reduces nitrates to nitrites and ammonia. On cultivation in the Hiss serum-water medium it is found to ferment the following carbohydrates: dextrose, levulose, lactose, sorbite, and mannite; but it does not ferment dextrin, saccharose, dulcit, adonit, nor inulin. When cultivated in dextrose broth in a fermentation tube it produces, in forty-eight hours, from forty to fifty per cent of gas, consisting of H : Co_2 : : 2 : 1. It does not give the Voges and Proskauer reaction.

This organism appears to fall within the first group of the colon-aerogenes family, as classified by one of us (B.), and corresponds with Bacillus Grünthal, an organism that Fischer isolated from a case of food poisoning occurring in Grünthal, Germany.

Several rabbits have been immunized with cultures isolated from Mrs. A.'s urine. The serum of one of these animals agglutinates the homologous organism in dilutions of 1 to 100, but the serum of this animal does not agglutinate Bacillus coli verus or Bacillus coli communior; neither does it

agglutinate an organism isolated from milk, which was identified from its cultural characteristics as Bacillus Grünthal. Neither does the serum agglutinate any of the bacteria closely related culturally to Bacillus Grünthal.

The case under consideration we believe to be one of pyelitis due to Bacillus Grünthal, member of the colon group of bacteria. As the patient seems to be improving, we have not felt it necessary to try vaccine therapy nor the use of immune serum.

A CONTRIBUTION TO THE FORENSIC VALUE OF THE MUSCULO-PRECIPITIN TEST.*

FREDERICK P. GAY.

(*From the Laboratory of Pathology, Harvard Medical School.*)

During the past winter it has been evident to the Massachusetts Fisheries and Game Commission that deer were being killed contrary to law. In the month of February a game warden in the town of A. was informed by a woodchopper that a certain X. Y., who was suspected of killing deer illegally, had been seen at the roadside nearby loading a sleigh with suspicious looking bundles. On investigation the deputy found the place where some sizable object had been dragged over the snow to the roadside and placed on a sleigh. On running the drag trail back unmistakable evidence of the killing and bleeding of a large animal was found. The sleigh track led to the house of the suspected X. Y. After a delay of a few hours, necessitated in procuring a search warrant, the house was searched and nothing found except the mutilated heart of a large animal. This heart was declared by its owner to be from a calf and to have been bought at a neighboring market. Inquiry at the market which was named failed to corroborate this story. The heart was brought to me by Dr. Field, chairman of the Fisheries and Game Commission, with the request that I determine whether it could be proved to be from a deer as suspected.

The discovery of precipitins in the sera of animals treated with the blood or blood serum of animals of another species which was made by Tchistovitch [1] and by Bordet [2] in 1899 has come to be of great practical importance. Bordet found that the serum of a rabbit immunized against hen blood was able not only to hemolyze hen red blood corpuscles but to cause a turbidity with clear hen serum. This turbidity is due to the formation of a precipitate which later falls as an

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amorphous granular deposition to the bottom of the test-tube. The important fact was noted by Bordet that whereas this serum precipitation at first seemed absolutely specific it is in reality only relatively so. Although this rabbit>hen serum gives no precipitate with serum from such animals as the horse or guinea-pig, it will cause a precipitation with the serum of an animal allied in species to the hen, the pigeon.

Uhlenhuth[3] and almost simultaneously Wassermann and Schütze[4] suggested the use of this precipitin test for the differentiation of human blood. Owing to the preëminent medico-legal value of such a test, it has since been extensively used for this purpose and, within certain limitations imposed by relativeness of its specificity, has proved very valuable. The limitations of the serum precipitin test have been discussed extensively, and useful methods of control have been suggested by various observers. Nuttall,[5] in particular, in his excellent book on "Blood Immunity and Blood Relationship," has considered these points in detail.

In addition to the blood serum precipitins, precipitins have been found for the various egg whites, milks, yeast albumins, etc., the discussion of which need not concern us. The meat precipitins described by Uhlenhuth[6] are alone of present interest. Uhlenhuth found that the serum of a rabbit immunized against pig serum would form a precipitate not only with pig serum but also with an extract from the dried organs of the pig; he obtained positive results with the antisera for pig, sheep, horse, donkey, and cat blood when these were tested with the corresponding meats. As might be expected Uhlenhuth found that these tests were not strictly specific when dealing with allied species; for example, donkey meat will give a precipitate with anti-horse serum and goat meat with anti-sheep serum. This test has been used largely to detect adulteration in such meat mixtures as sausage.

Von Rigler[7] immunized rabbits each with a watery extract of muscle from a separate animal species. The resulting precipitin sera were found in his hands to be highly specific. Of distinct interest in the present discussion is the fact that

anti-cow serum gave no precipitate with an extract of roebuck meat, and correspondingly anti-roebuck serum gave no precipitate with beef extract.

Vallée and Nicholas have gone so far as to assert that a musculo-precipitating serum active against a given meat is nearly distinct from a sero-precipitating serum active against the corresponding serum, and they therefore speak of "sero-precipitins" as opposed to "musculo-precipitins." It seems advisable to use these terms to indicate the nature of the precipitin test in question whether or not these precipitins are to be regarded as separate.

A consideration of the problem in hand, the diagnosis of the suspected heart as differential either of deer or calf, was *a priori* not promising. As already mentioned V. Rigler claimed to be able to differentiate between roebuck venison and beef by means of the corresponding antisera. On the other hand Nuttall,[8] in his careful study of the sero-precipitins, has found that of the seventeen species of the Family Cervidæ studied, fourteen reacted more or less with an anti-ox precipitating serum, although ox serum gave no reaction with antisera to the Hog deer (Cervus porcinus) and the Mexican deer (Cariacus mexicanus). In view of the assertions of Vallée and Nicholas as to the separateness of sero-precipitins from musculo-precipitins these data of Nuttall's are not strictly applicable to the matter under consideration. Diagnosis depending on a relatively specific reaction may reasonably be expected to differ in the hands of different observers in accordance with variations in the materials and technic employed. Nor can the same known antiserum be used by the same observer at different times for the diagnosis of different specimens when such diagnosis is to be regarded as differential between allied species. It is undoubtedly accurate to base a diagnosis of human blood on a voluminous precipitate in an antihuman serum of known potency, but it would not be advisable to have diagnosed this suspected deer heart as venison in contradistinction to veal, on the evidence of a precipitate formed in an anti-venison serum. Such a diagnosis

should be based rather on an experiment which is ample enough to be absolutely self-controlled.

The method employed in the diagnosis of this suspected deer heart was as follows. In addition to the heart in question the following meats were obtained:

1. A piece from a loin of venison.
2. Hearts from a young calf and a young cow respectively.

These specimens and the suspected heart were cut into convenient pieces and kept frozen in separate jars and used from time to time to prepare extracts of the meats in question. These extracts were prepared by grinding a small piece of frozen meat in a clean mortar with a little salt solution (NaCl .85 per cent) and filtering repeatedly through the same double filter paper until quite clear, or at most only slightly opalescent.

Normal rabbits were given injections of the different meat extracts as follows:

Rabbit A. — Given five injections subcutaneously of from one to three cubic centimeters of extract of authentic venison, at intervals of from four to ten days.

Rabbit B. — Given five injections subcutaneously of from one to three cubic centimeters of extract of suspected venison (heart) at intervals of from four to ten days.

Rabbit C. — Given five injections subcutaneously of from one to five cubic centimeters of extract of cow heart at intervals of from four to ten days.

Twelve days after the last injection the animals were bled from the carotid and the separated clear sera were used in the following experiments.

In the precipitin tests that follow the proportions were, antiserum 1.5 cubic centimeters, extract to be tested three drops. A positive reaction is determined by a cloudiness of the tube within two hours at room temperature, and by the

deposition of a granular amphorous sediment after conservation over night in the ice-chest.

During the immunization of the rabbits it was determined:

1. That none of the extracts employed, namely calf heart, cow heart, venison, and suspected venison gave a precipitate with normal rabbit serum.
2. That the extracts of calf heart and cow heart gave a precipitate with antisera from two different rabbits, each of which had been immunized with calf blood; neither the venison nor the suspected venison gave a reaction with either of these antisera.

These results, together with the final precipitate tests with the antisera, may be tabulated as follows:

Extract of	Normal Rabbit Serum.	S. Rabbit> Calf Blood No. 7.	S. Rabbit> Calf Blood No. 17.	S. Rabbit "A" (Anti-venison).	S. Rabbit "B" (Anti-suspect).	S. Rabbit "C" (Anti-cow).
Calf heart	o	+	+	.....	.....	++
Cow heart	o	+	+	o	o	++
Suspected deer heart	o	o	o	+	++	o
Venison	o	o	o	+	+	o

A plus sign indicates a positive reaction of greater (+ +) or less (+) intensity. Zero indicates no reaction.

From this table it is evident that the suspected heart was from a deer and not from a calf, since its muscle extract gave a precipitin reaction with venison antiserum but not with calf or cow antisera as do both calf and cow extract. Venison extract gives a positive reaction with anti-suspect serum but not with calf or cow antisera.

So far as I am aware this is the first application of the musculo-precipitin test toward conviction for infringement of game laws.

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RECURRENT LIPOSARCOMA OF THE KIDNEY.*

Guthrie McConnell, M.D.

(*Pathologist to the St. Louis Skin and Cancer Hospital.*)

Although cases of retro-peritoneal lipomata arising from the region of the kidney are, comparatively speaking, very rare, yet even more uncommon is that variety of the above that is known as recurrent lipomata or liposarcoma of the kidney. In going over the available literature on the subject there have been found eight cases besides the one here reported.

A detailed description of this present case and abstracts of those previously published follows:

F.G.Z., 44 years old; male; white. — This first examination was made on Jan. 11, 1900.

About fifteen months ago the patient noticed a lump about the size of a hen's egg situated in the right hypochondrium. Last year it increased to about the size of a baseball There was no pain or discomfort other than the inconvenience of the tumor.

Has had acid eructations and for a time the stools were soft, foul-smelling and fermenting, and gray in color. Has lost about twenty pounds in weight. Waist measure has, however, increased from forty to forty-two inches. Has passed urine frequently and in large quantities, was of a good color. Has had hemorrhoids for several years and for the past few weeks there has been some edema of the feet.

Physical examination, June 10, 1901. — There was found a large, freely movable cystic tumor filling the abdominal cavity. The intestines were pushed downwards over the lower left portion of the tumor mass.

Urine examination. — Sp.G. 1025, small trace of albumen, no sugar. Microscope showed nothing abnormal.

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June 18, 1901. — Measurements before operation: Circumference of abdomen fifty inches. From ensiform to pubes twenty-three inches.

Operation. — An incision was made in the median line from the ensiform to the umbilicus. The abdominal wall was very thin. The tumor was exposed and two attempts were made with a trochar to evacuate the mass, but with negative results. The growth was apparently soft and colloidal. The abdominal incision was continued nearly to the pubes. The colon was found lying on the left lower portion of the tumor in such a way as to indicate a retro-peritoneal origin. The peritoneum was incised and the neoplasm shelled out with but slight difficulty and little bleeding. The growth was found to surround and closely incorporate the right kidney, the ureter, which was much dilated, passing over the mass. The kidney was so lacerated that it was also removed, the ureter being tied off near its entrance into the bladder. The vein and artery were tied separately. In removing the mass two small holes were torn in the vena cava. These were tied with silk ligatures, a small portion of the vessel wall being enclosed.

The recovery of the patient was uneventful.

The growth removed at this operation was yellow in color and macroscopically resembled a lipoma. A large part of it was found to be cystic. The examination of the fluid obtained showed the following: clear amber, alkaline, gelatinous. Large amount of albumen but no sugar. Microscope showed numerous fat globules, some leucocytes and red blood cells, a few epithelial cells and some granular detritus. No scolices or hooklets. The report of the microscopic examination of the tumor was that it was a fibrolipoma with some mucoid degeneration. The weight of the growth was sixty-five pounds.

The patient recovered and was not seen again till January, 1908, at which time a large mass was found in the right hypochondrial region. He was in poor physical shape, but an operation was decided upon. On Jan. 31, 1908, the abdomen was opened and the tumor removed. The growth was quite yellowish in color, lobulated and presented the appearance of a lipoma, although some areas were rather grayish in color. In addition to the large tumor there was a smaller one about the size of a baseball that was connected to the larger by a very thin stalk that broke during the operation. The consistency of the mass varied considerably. In some places it was so soft as to be almost cystic, while in others it was quite firm. No true cystic areas were found. Weight was twenty-seven pounds.

Portions of tissue were taken from three different areas, imbedded in celloidin and stained in various ways.

Microscopic examination. — Section A: The surface of the specimen is covered by a well-developed capsule of dense connective tissue. In this are numerous small capillaries with here and there a slight infiltration of round cells. In some places a few fat cells are found in the capsule. There is then a sudden transition from the capsule to a definite fatty tissue in which there is a marked increase of the connective tissue between and surrounding the fat cells. This connective tissue is very cellular, containing many nuclei closely crowded together and differing greatly from each other. The majority are more or less spindle-shaped and stain lightly but uniformly. Besides these there are many others that are as a rule larger, quite irregular in outline and that stain very intensely. In the majority of instances several of these deeply-staining cells are grouped. In many places rather long, flat nuclei are found lining the fat spaces.

Many of the nuclei (Fig. 1), mostly those of the pale-staining type, contain vacuoles of varying sizes, with small masses of chromatin closely adjacent. In some instances the vacuoles are so large as to leave just a slight rim of nuclear substance surrounding them. As the interior of the specimen is approached the connective tissue decreases in amount and the fat cells become larger, less compressed, and consequently more circular. Capillary blood vessels are found scattered throughout the connective tissue bands, but no large vessels are found. When Mallory's anilin blue or Van Gieson's stain is used the entire specimen takes the connective tissue coloring. No traces of involuntary muscle could be found. With acid orcein a number of short elastic tissue fibers are found scattered through the capsule. About the walls of the capillaries the elastic tissue is found in quite large amounts. The elastic tissue in the rest of the specimen is very slight, except in the larger masses of connective tissue in which blood vessels are noticed. In these areas the fibrils are long, thick, and very distinct. In one portion of the specimen there is a large mass of blood

surrounded by cells containing large spindle-shaped, deeply-staining nuclei. There is no membrane between these cells and the blood and in many places there are masses of cells projecting into the blood. Isolated cells and small narrow strands are found lying free within the mass. The character of the cells enclosing the red corpuscles gradually changes as the lumen is left. Instead of the cells predominating, the nuclei become fewer and narrower and the intercellular substance increases until the outer portion resembles normal adult connective tissue. It is a very evident instance of the gradation of embryonal mesoblastic tissue to the adult.

Section B: This portion of the growth is found to consist almost entirely of solid tissue with very small collections of fat here and there. The groundwork of the specimen is composed of a slightly granular translucent material, evidently connective tissue, either edematous or myxomatous in character.

The variations of the nuclei in this section are of great interest. They differ so widely that it hardly seems possible that they can all be of the same origin. The nuclei of the matrix of the specimen are long and narrow with pointed ends. They stain deeply and in many instances long, narrow fibers extend from the poles. Gradual changes from this type are seen. The nuclei become shorter, fatter, less pointed at the poles and do not stain so deeply, till there are finally found pale, almost vesicular nuclei.

The majority of the nuclei occupy a half-way position, the ones that are fairly long, quite thick, with rounded ends and that stain lightly. These occur as a rule singly, but small clusters of from three to five members may be found.

In addition to the above forms there is a great number of cells containing nuclei that are quite irregular in shape and that stain very deeply. These are scattered throughout the tissue, individually and in groups. The latter give an impression of being giant cells, but in only a few instances were true forms of the latter seen. In one case in particular there was found a large, well-defined spherical mass of protoplasm in which were four or five deeply-staining nuclei.

Here and there were found cells resembling to some extent those of the plasma type. They were about the same size, but as a rule were more spherical, the outline of the cell was well defined and in the protoplasm was a small round deeply-staining nucleus situated most frequently a little to one end. In many the nucleus occupied the center of the cell. The protoplasm took a distinct reddish tinge with eosin and was finely granular.

Numerous cells with small round deeply-staining nuclei surrounded by very little protoplasm were found. A few polymorphonuclear leucocytes were also present.

Although many deeply-staining nuclei were found everywhere, yet in no instance was there any indication of karyokinetic figures. The multiplication of the cells would appear to take place by direct division. In the region of the larger capillaries nuclei were found that resembled those of involuntary muscle. With Van Gieson's stain it was in such places alone that the yellow color of smooth muscle was found, nowhere else in the stroma could such tissue be seen. Small capillaries are very numerous and in many areas there are slight collections of small round cells. When Van Gieson's or Mallory's stains are used the mass of the specimen takes the connective tissue color, no involuntary muscle being found. When stained with acid orcein the elastic tissue is found to be present mainly around the blood vessels, although a few isolated fibrils are seen.

Section C: This specimen closely resembles the condition found in Section A, but it is more vascular and there are many areas in which the blood has escaped into the tissues.

There is a distinct capsule covering the specimen. Beneath this the fatty tissue is compressed and the fat cells flattened. As the interior of the growth is approached the resemblance to normal fatty tissue increases until the cells become large, irregularly round and separated by thin connective tissue bands.

With Van Gieson's and Mallory's stains the specimen is shown to be composed entirely of connective tissue with no

involuntary muscle fibers present. With acid orcein a large amount of long fibrils of elastic tissue are found in the capsule. Very few fibers are found elsewhere except in the larger masses of connective tissue in which blood vessels are observed.

In the specimens taken from the three different portions of the growth there is a peculiar structure (Fig. 2) present in all instances. It is composed of a ring of cells consisting of a large amount of protoplasm in which is a large round or slightly oval well-staining nucleus. The center of the ring is clear, as a rule, and the lining of the lumen consists of the protoplasm of the above cells. In Section B this structure appears to have been cut slightly obliquely and the character of the cells is more distinct. The cell is quite large with a well-marked cell wall, within which is a slightly granular protoplasm containing a bluntly oval, quite deeply-staining nucleus. The lumen is triangular in shape and contains to one side a slightly granular substance, but no blood cells.

The appearance given is that of a true tubule lined by epithelium, or in this case more probably endothelium, considering the origin of the growth as mesothelial.

G. Heinricius[2] reports two cases of recurring retroperitoneal lipomata as follows:

Case 1. Woman, 39 years old. In January, 1899, she noticed that her abdomen was larger than normal, but did not consult a physician till September. Was operated upon on Oct. 4, 1899. The growth was found to be on the right side, was lobulated and weighed about six thousand grams. The microscopic examination showed it to consist entirely of fatty tissue surrounded by a thin capsule of connective tissue.

The patient returned in July, 1901, and was again operated upon, the growth having returned upon the right side. The mass weighed one thousand eight hundred grams, was soft and gave the appearance of a lipoma; no microscopic examination was made.

In January, 1903, the patient came back with a recurrent growth on the right side. Was operated upon during that month, but on account of her poor condition the entire tumor was not removed. The part that was taken out weighed three thousand six hundred sixty-five grams, was soft and pale reddish yellow. Patient died on Jan. 25, 1903. An autopsy was performed and it was shown that the right kidney was present and

had been pushed upwards by the tumor. The mass remaining after the operation weighed four thousand three hundred fifty grams, giving a total of eight thousand fifteen grams. No microscopic report given.

Case 2. Female, aged 63. In May, 1901, she noticed an enlargement about the size of a fist in the right side of the abdomen. Was operated upon on Oct. 12, 1901. The kidney was palpable. The growth was lobulated, the upper portion being about the size of two fists and varying in color from yellow to grayish yellow and pale gray. The consistency differed greatly in different portions. Weight not given.

Microscopic examination showed the upper denser portions to be composed of ordinary connective tissue, while the lower pole was a pure lipoma.

The second operation was performed in August, 1903. By this time the tumor had attained the size of a head and was situated in the right lumbar region. The surface was uneven. No weight or microscopical examination is given. The kidney was palpable at the bottom of the cavity made by the removal of the growth.

Heinricius concludes that neither of these growths had any connection with the sexual organs, the kidneys, pancreas, or spleen and are true retro-peritoneal tumors.

Another similar case is reported by Ullmann[3] and Lindquist.[4] Patient was a woman of 46. Was operated upon the first time by Ullmann on April 6, 1899. The right kidney, the side on which the growth occurred, was not involved. The tumor weighed five thousand five hundred grams; no microscopic examination is given.

The second operation was performed by Lindquist on Dec. 5, 1901. The right kidney was still uninvolved. "The tumor was a lipoma." The report does not state that there had been a microscopic examination nor is the weight of the tumor given.

W. Waldeyer[5] reported a case in a thirty-year-old woman of a large lipomyxoma of the mesentery with secondary sarcomatous metastases in the liver and lungs. The growth weighed sixty-three pounds (German), and was lobulated. The smaller portions were almost purely lipomatous, although towards the periphery myxomatous areas were present. In the major portion myxomatous and sarcomatous areas were present without any sharp line of differentiation. Sarcomatous metastases of varying size were found in the liver and lungs. C. Hartwig[6] quotes this case as a liposarcoma of the capsula adiposa of the kidney accompanied by a fatty degeneration of the cortex.

Dr. Lenger[7] also reports a case in a male, aet. 39. In June, 1898, the patient began losing weight, appetite became poor, and at the same time he noticed a swelling in the region of the right kidney. In August a tumor weighing five thousand five hundred grams was removed. The kidney was uninvolved. In February and in May, 1899, it was necessary to remove tumors from the lumbar and abdominal regions. In each instance the tumor was a small round-cell sarcoma. The case is reported

as a liposarcoma of the right peri-renal capsule, although there is little said about the fatty tissue.

R. J. Johnstone[8] in the British Medical Journal of Dec. 2, 1905, reports a case of a retro-peritoneal lipoma, weighing twenty-one pounds, situated in intimate relation with the right kidney and ureter. The patient, a woman aged 40, recovered rapidly and gained in weight. She remained well for about two years when pains appeared in the back and sides. On March 6, 1907, a second operation was performed, at which time a growth "consisting of a number of lobes varying in size from that of a goose egg up to that of a Rugby football, more or less pedunculated, and only united at their common site of origin, which was in the right flank in the angle between the outer border of the kidney and the iliac crest," was removed. The tumor masses altogether weighed about six thousand grams, were pinkish white in color and microscopically showed typical lipomatous tissue with no signs of malignancy. There were also "no signs of hemorrhage or degeneration, such as are almost invariably present in retro-peritoneal sarcomata which have attained any appreciable size." The author concludes from the above reasons and from the fact that there was no cachexia, no loss of weight, and no signs of metastases that the growth was not malignant. The patient recovered from the operation and left the hospital twenty-one days later.

The following is a case reported by Carl Hartwig[9]: a woman, aet. 36. 1902. For the past eight years has noticed a continuous increase in the size of her abdomen. Until July 21, 1902, she had never suffered any pain, at which time she became suddenly ill. She was operated upon immediately and a growth was found that occupied the entire abdomen and was covered by thickened peritoneum. After exploration the tumor was seen to have its origin in the right kidney. It weighed three thousand five hundred grams, was about the size of an adult head and varied in consistency, the upper portion being hard, while the lower was lobulated and soft. On section there was revealed a glistening yellowish surface, which at some spots showed a somewhat darker grayish color along with transparent portions which radiated into the surrounding structures.

The portion of the kidney connected with the growth was brownish red with a smooth surface, upon which were a number of nodules varying in size from a millet seed to a cherry stone and which protruded above the surface of the organ. In color they were yellow and translucent, the larger ones being soft and fluctuating.

The microscopic examination showed cellular areas in the general mass of fatty tissue. These cellular foci contained numerous spindle cells along with round cells and fat cells. "Without any doubt this case represents a lipoma undergoing sarcomatous transformation."

In regard to the small nodules it was found that in the centers of the growth all sections show pure sarcoma cell areas, especially such as those which have been described in the form of spindle-cell strands concentrically

arranged around the smallest arteries. They are evidently metastases from the liposarcoma.

Hartwig, in referring to the origin of this growth believes that it did not arise from the fatty tissue in the hilum of the kidney, but came from a lipoma nodule in the cortex.

Alsberg (quoted by C. Hartwig [10]) records a case where the growth occurred in a woman on the right side. The weight of the tumor is not given, but its dimensions are as follows: thirteen centimeters in length, eight centimeters in thickness, and ten centimeters in width. It was surrounded by a firm tissue envelope and the surface was smooth with the exception of a row of projecting yellow nodules. Microscopic examination showed that the areas surrounding the lipoma nodules contained well-developed strands of tissue composed of spindle cells of varying dimensions and which resembled sarcoma; there was also a very marked development of blood vessels.

The main point of interest in these lipomata is not surgical but pathological. In other words, it is the origin of these growths that attracts attention on account of its varying possibilities and the present lack of accurate knowledge.

The question as to whether or not the adrenal rests so frequently found in the kidney can give rise to these tumors has as yet not been definitely settled. After Grawitz's original article appeared it became evident that the majority of the so-called lipomata were nothing else than hypernephromata.

More careful observation showed, however, that there were true fatty tumors occurring in connection with the kidney. These might arise from the hilum, from the capsula adiposa, or from a degeneration of the renal tissue, these latter not attaining large dimensions.

The large lipomata have been generally considered as originating from the fatty capsule surrounding the kidney. By many they are held to be merely overgrowths and not true tumor formations.

In taking up the question as to origin there would seem to be certain points of importance to be gained by a study of some of the already reported cases, particularly when correlated with a finding in this new case.

As was first shown by Grawitz many of the so-called lipomata previously reported were tumors arising from adrenal rests. Since his observation many cases of hypernephroma

in which there was more or less fat present have been described. There could, however, be found enough of the adrenal tissue to establish the diagnosis.

Amongst these cases a recent one reported by Keenan and Archibold[1] is of particular value. This growth was peculiar in that, although it consisted almost entirely of fatty tissue, there were certain cells present, called the parenchymal cells by the authors, that were considered by them to be adrenal in character. After discussing in detail the various cases reported and their probable origin, the above investigators come to the conclusion that the tumor they present is a lipoma resulting from the degeneration and metaplasia of the cells of an adrenal rest. As this tissue is a mesoblastic derivative, such a process would seem quite possible.

This above-mentioned case is alluded to on account of its bearing upon the origin of lipomata, not that it was a recurrent growth, although to quote the authors there was "some evidence showing a mild degree of malignancy, with local metastases."

In the case that I am here reporting we find that the growth recurred, and that it has certain of the conditions found in sarcomata. In the cellular areas there were many transition forms from the early embryonic type to the more adult cell. In one place there was also found a large blood space formed by tumor cells and within which many isolated tissue cells were lying.

The point to be brought out, however, is the bearing that this case may have upon establishing the origin of these large recurrent fatty growths.

The tumor is composed mainly of very typical fatty areas with portions in which the cellular and connective tissues predominate. Besides these there are found in each of the three areas examined certain tubular structures that are formed by cells that closely resemble those of the adrenal.

By starting with the hypothesis that fatty changes are not uncommon in the normal adrenal we establish our premise. The presence of aberrant adrenal tissue, particularly in the neighborhood of the kidney, is a widely accepted fact. The

first condition, then, to be expected is a hypernephroma accompanied by fatty changes of varying degrees. Many such cases have been reported. In the instance published by Keenan and Archibold we have a growth in which the fatty tissue greatly predominates, but in which here and there are found cellular areas that the above authors hold as being derived from adrenal tissue. In the case now reported there is only a very slight remnant of early structures, the fatty tissue being overwhelmingly predominant. The peculiar tubular formation found in the specimen seems to resemble adrenal rather than renal tissues.

The varying degrees of malignancy of hypernephromata are well recognized, and in that way this tumor in its limited malignant effect belongs rather to that class than to the lipomata, these latter belonging essentially to the benign variety.

Granting the possibility of the degeneration and metaplasia of the adrenal elements into fatty tissue it would seem allowable to hold that this tumor was in reality a hypernephroma that had taken on a fatty change accompanied by a limited malignant tendency.

[I wish to thank Dr. H. G. Mudd for the privilege of reporting this case, as it was under his care at St. Luke's Hospital in St. Louis.]

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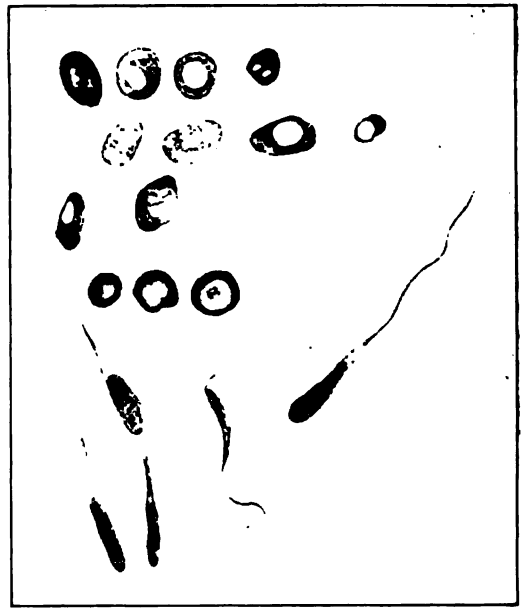
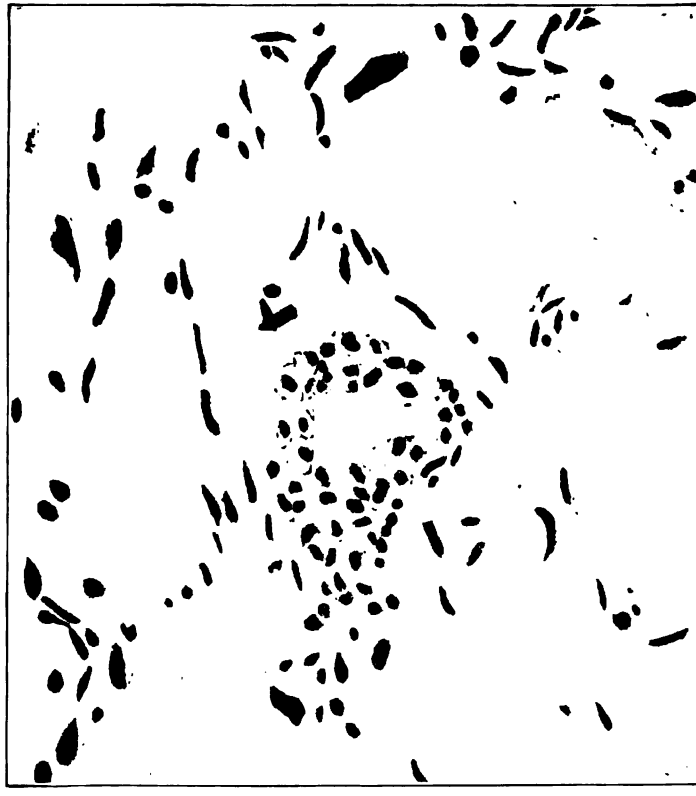


1. — Varieties of vacuolated nuclei and spindle cells. obj. 6. oc. 2.



2. — Tubular structure found in the growth (adrenal?).

OPSONIC TECHNIC.*

RALPH E. WALKER, M.D., Captain U.S.M.C., Retired.
(*Clinic of Internal Medicine, University Hospital, Ann Arbor.*)

Access to any part of opsonic investigation can properly be had only by way of the opsonic index or, to be somewhat more exact, by way of the phagocytic index.

While Wright and a few others claim that the opsonic index can be determined with practical accuracy, the general experience of most observers is certainly otherwise. Many writers during the past two years have stated that the index is liable to very large and unavoidable errors.

Others have plainly showed by the work described that their results were valueless.

The opsonic index can be determined with sufficient exactness for clinical purposes. Failure to do so signifies (*a*) improper methods of work, (*b*) lack of skill in manipulation.

It is the purpose of this paper to describe methods which, with some practice, will enable any one having fair laboratory training to do accurate work. Very naturally no originality is claimed for many of the steps to be described.

Attention is first invited to certain general considerations.

I. The basis of work is the phagocytic index which according to general usage is the average number of bacteria taken up by the polynuclear neutrophile leucocytes, under the conditions of incubation time, temperature, serum dilution, etc., observed in the work. Thus in a preparation incubated for thirty minutes at a temperature of 37° C. the first consecutive twenty-five polynuclear neutrophiles contained the following numbers of bacteria:

9-7-5-10-6-5-4-13-4-6-9-7-4-6-4-4-5-2-7-5-11-8-16-8-2;

6.68, the average for the twenty-five cells counted, is the

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phagocytic index for the serum used under the conditions mentioned.

The probable error of such an average depends upon two factors aside from the personal one, viz.:

(*a.*) The number of observations, *i.e.*, the number of leucocytes counted.

(*b.*) The variations of the counts for individual phagocytes from the average for all.

If n leucocytes be counted and the differences between individual leucocyte counts and the average or phagocytic index for the n cells be denoted by r; then, by the principles of least squares, the probable error of the phagocytic index will be $0.6745\sqrt{\frac{\Sigma r^2}{n(n-1)}}$, Σr^2 being the sum of the squares of the residuals, or differences between the average and individual phagocytic contents.

It follows from this equation that the error of the phagocytic index will be least when the number of leucocytes counted (n) is large and the variations in their contents from the average (r) least.

Since time and labor forbid counting a very large number of leucocytes, any method of work to be accurate must produce considerable uniformity in the contents of the several phagocytes, and one must count such a number of leucocytes that their average will not vary greatly from the averages of other similar groups of the same preparation.

Of course, it is entirely a different question if the phagocytic index, however accurately obtained, really represents the opsonin in the serum tested.

Properly and improperly planned work may be made to yield equally uniform phagocytic averages.

The factors concerned in producing uniform counts are as follows:

(*a.*) The use of clump-free suspensions of bacteria is the most important measure. It is possible to obtain suspensions, as will be later described, which contain practically no clumps, and none larger than six to eight individuals. Supposing that the attraction between bacteria and opsonin

follows a law similar to that of gravitation, it is readily seen that the presence of large clumps of bacteria may very markedly affect the uniform distribution of opsonized bacteria.

(*b.*) The use of warm sodium citrate and sodium chlorid solutions for washing the leucocytes, as will be later described.

The following experiment shows the effect of clumps and cold solutions on the uniformity of the phagocytosis produced.

Two bacterial suspensions were prepared from a single culture on agar of a Staphylococcus p. aureus. From one clumps were eliminated, while many remained in the other. Both suspensions in similar tubes were opaque and appeared equally dense.

Two preparations of leucocytes from the same person were made by dropping blood from a cut in the finger end into centrifuge tubes nearly full of one per cent sodium citrate in one per cent sodium chlorid solution. The temperature of one tube was 17° C., of the other 37° C.

Using the same serum (a normal person's diluted twenty times), phagocytic mixtures were made for each leucocyte preparation with each bacterial suspension. The results are found in the following table:

TABLE I.

	Suspension Clump-free.	Suspension having Clumps.	
Corpuscles washed in solutions at 37° C.	7.04	9.02	Average first 50 leucocytes.
	7.28	8.70	Average second 50 leucocytes.
	7.16	8.86	Average for 100 leucocytes.
	2.49	7.07	Average residuals for 100 leucocytes.
	.20	.60	Probable error of average for 100 cells.
Corpuscles washed in solutions at 17° C.	7.92	11.56	Average first 50 leucocytes.
	7.94	12.24	Average of second 50 leucocytes.
	7.93	11.92	Average for 100 leucocytes.
	4.05	9.20	Average residuals for 100 leucocytes.
	.33	.78	Probable error of average for 100 cells.

These figures show at a glance the bad effect of clumps on the uniformity of the phagocytosis. Cold solutions, while of less importance, still exert a very considerable effect and one well worth avoiding in the work.

It is seen that as the phagocytosis becomes more irregular the averages increase in a very proportional manner, being in order 7.16, 7.92, 8.86, and 11.92. It might be objected that the results from the two bacterial suspensions are not comparable in the absence of definite data as to the numbers of bacteria contained in them. This, however, is not true, since both suspensions were far more dense than my experience with the strain had shown necessary to satisfy the opsonin in a serum diluted twenty times, and the strain used was one for which no change in the average phagocytosis is produced by increasing the density of the suspension beyond that necessary to insure the maximum phagocytosis of which the serum is capable.

(*c.*) In addition to the use of clump-free bacterial suspension and warm solutions for washing leucocytes, our

experience with water incubation has been on the whole more satisfactory than incubation in air. While several experiments have failed to show any considerable difference in the uniformity of the phagocytosis produced by these methods, we still retain the impression that our preparations incubated in water have on the whole given more uniform results.

(*d.*) No matter how carefully the foregoing conditions are observed there will occur variations in the number of bacteria taken up by individual leucocytes. When a drop from the incubated capillary is spread on a slide or on cover-glasses and consecutive leucocytes counted, there is often observed a tendency to wave-like variations in the counts, *i.e.*, from the smallest counts one gradually gets larger ones, until the largest are found and from these the leucocytic contents progressively diminish again until the lower counts are reached, and so on. Of course as a general thing the wave-like variation is not at all mathematically exact, but the grouping of cells of like content is certainly to be observed. In such a preparation, if a large number of leucocytes be counted, a true average may undoubtedly be reached, but such a procedure takes time, to say the least, and may readily be avoided. It is only necessary to remember that, however unevenly the counts of individual leucocytes may run, it is possible by thoroughly mixing them after incubation to produce such a uniform distribution that comparatively small groups taken consecutively will show almost identical averages. If this precaution be taken groups of twenty-five leucocytes will vary but little in their average counts; though of course it is improbable that this average will be of much value as an indication of the opsonin present in the serum unless proper precautions are observed throughout in making the preparations. The following counts of groups of twenty-five cells, made from an actual preparation, indicate the point just made:

First 25 Leucocytes.	Second 25 Leucocytes.	Third 25 Leucocytes.	Fourth 25 Leucocytes.	
10	7	11	10	
8	11	9	9	
14	10	2	6	
6	7	13	15	
7	2	7	8	
6	5	10	5	
11	16	1	14	
16	6	3	16	
12	15	10	4	
12	8	7	12	
13	16	5	13	
8	9	13	5	
7	11	9	13	
9	6	13	9	
8	14	17	4	
11	10	10	14	
9	15	13	5	
10	13	16	13	
9	12	7	9	
9	8	5	13	
1	5	8	8	
7	13	8	11	
8	14	8	10	
9	10	9	8	
9	7	7	9	
25)229	250	221	243	
9.16	10.00 9.16	8.84	9.72 8.84	Averages for 25 cells.
	2)19.16		18.56	
	9.58		9.28 9.58	Averages for 50 cells.
			2)18.86	
			9.43	Average for 100 cells.

The average for the first fifty is 9.58, for the second 9.28. If we assume the average for the entire one hundred leucocytes, 9.43, to be correct, then the error, due to using either

fifty group would have been only .15, or less than two per cent of 9.43.

Attention to the foregoing points will insure uniformity in phagocytosis and very similar averages for groups of leucocytes as small as fifty or twenty-five, without which, it is obvious, we can feel little confidence in the accuracy of the phagocytic index for such groups.

II. As the writer has already explained (The Journal of Medical Research, July, 1907), no one can expect to arrive at phagocytic indices proportional to the opsonic contents of the serum used unless the bacterial suspension employed contains a sufficient number of bacteria to insure the attachment of all the opsonin in the strongest serum (and therefore, necessarily, in the others). With many bacteria an undiluted normal serum will sensitize such an enormous number (if present) that the phagocytes will be found packed with literally hundreds of bacteria. Such preparations cannot be used. It is not permissible under such circumstances to diminish the phagocytosis by using a thin bacterial suspension, because it is easily seen that, since the leucocytes are capable of taking up many more bacteria if a denser suspension be used with the same serum, the fact that fewer are taken up with the thin bacterial emulsion means only that fewer bacteria are contained therein than the serum could sensitize; and, consequently, that the phagocytosis produced represents only that part of the opsonin which the bacteria combined with, the remainder for which no bacteria were supplied being entirely lost in the estimation.

When thin suspensions of bacteria, for which much opsonin exists in the sera, are used it generally happens that both the sera sensitize all the bacteria so that the work if accurately done will produce equal phagocytic indices for both — in other terms, an opsonic index of unity — regardless of the real relation of the sera. Thus for staphylococcus a serum diluted with an equal volume of salt solution will produce just as much phagocytosis as the same serum

undiluted, with any bacterial suspension thin enough to give countable preparations.

Phagocytic indices proportional to the sera tested may readily be obtained by diluting all the sera equally to a sufficient degree, and using with these diluted sera (whose opsonic contents are exactly proportional to the same sera undiluted, but absolutely less according to the degree of dilution) a thick bacterial suspension. Thus, sera diluted thirty times with normal saline solution used with an emulsion containing ten million staphylococci per cubic millimeter will give phagocytic indices proportional to their opsonic values. The phagocytosis for a normal serum so prepared will average in thirty minutes about ten cocci per leucocyte — more or less according to the serum, the strain of bacteria, and source of leucocytes employed. By varying the degree of dilution of the sera smaller or greater phagocytic indices may be produced at will; and so long as sufficiently dense suspensions of bacteria are used these indices will remain proportional to the opsonic contents of the sera.

In order to know if a certain suspension of germs contains sufficient individuals per unit volume to satisfy the opsonin in any given dilution of serum it is only necessary to make two preparations, one for the given dilution of the serum (say 1/30), and another for a lesser dilution (say 1/15). If the phagocytosis for the last is twice that of the first, enough and probably more bacteria than are needed to satisfy both sera are present. If the phagocytosis for the stronger serum is greater than for the weaker but not twice as great, there are sufficient germs for the weaker but not enough for the stronger. By determining the necessary density of the suspension for the strain of bacteria used and afterward using a suspension considerably more rich in bacteria, there will be no trouble whatever in having always sufficient bacteria for the requirements of the serum dilution we are using in the work.

The nephylometer may be used, but so far as the opsonic index is concerned an accurately made bacterial suspension is not needed; any suspension containing sufficient germs

for the opsonin in the serum as above explained being satisfactory.

In general, American workers have found Simon's method more accurate than Wright's. Since a serum diluted so highly as to produce phagocytosis in only a fraction of the leucocytes present will obviously be satisfied by even extremely thin suspensions of bacteria, it is easily understood that the principle just explained must necessarily be observed in Simon's method, and readily explains the more consistent results obtained thereby. We believe, however, that Wright's method properly applied is much the better. Some spontaneous phagocytosis occurs in all preparations probably; and it is readily understood that the smaller the phagocytosis observed the greater will be the disturbance in the opsonic index caused by such spontaneous phagocytosis.

It is true that by Simon's method of counting, spontaneous phagocytosis, owing to its marked irregularity, is much less than by Wright's method, but it is still sufficient to cause more error in the index than follows the use of the higher phagocytic indices obtained by Wright's method.

As was before stated, the use of suspensions containing more bacteria than actually needed for the opsonin in the sera used leads to no change in the opsonic index obtained. We have found for some strains of staphylococcus, however, that as the bacterial density is increased beyond a certain point there occurs a fall in the phagocytic index. This is shown by the following experiment:

Suspensions of one, two, four, eight and thirty-two millions of cocci per cubic centimeter were used with the same leucocytes and serum (normal diluted thirty times).

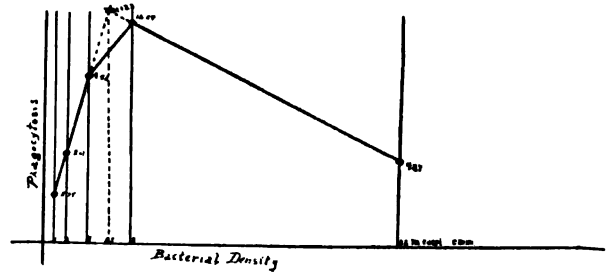
As shown in the curve the phagocytosis advances in proportion to the number of cocci until four million were used.

The next point (eight million bacteria) shows a divergence from the proportion, being 12.54. For thirty-two millions the phagocytosis was only 4.87, showing a distinct fall depending only on a greater density of bacterial suspension since all other factors were equal with the exception of the age of the leucocytes, which for the last preparation

shown were twenty-five minutes younger than for the first, the preparation of greatest density having been first used.

For other strains of staphylococci I have had a perfectly level curve from the maximum point on, so that I do not believe small differences in the age of the leucocytes at the beginning of phagocytosis to have any measurable effect.

Prolonging the converging lines as shown until they meet in the point M gives about five and one-half million cocci per cubic centimeter as the number which would have given, with the serum used, the maximum phagocytosis which it could produce under the circumstances. This maximum was about 13.3.



This fall in phagocytosis is due to a product of bacterial growth which acts on the leucocytes in such a way as to inhibit their activity.

This was demonstrated as follows:

After washing the leucocytes once in the usual way, the citrate solution was removed and the leucocytes mixed with salt solution which had been shaken in a culture tube from which a growth (twenty-four hours) of staphylococcus aureus had been removed with a wire loop. The salt solution had previously been freed from cocci by long centrifugation. The leucocytes were then washed twice again in salt solution.

These leucocytes gave twenty-five per cent less phagocytosis than other leucocytes from the same source which had been washed three times but not treated with the material from the culture tube, all other conditions being equal.

Keeping in mind the foregoing statements we proceed to describe the actual work done in obtaining the phagocytic and opsonic indices.

I. THE BACTERIA. — These should be cultivated in such manner that they may be obtained in sufficient quantity free from the culture media. For rapidly growing species young cultures are preferable because they stain best. The growth is removed from the surface of the media by means of a platinum loop and transferred to a small mortar (agate or glass). It is rubbed gently over the surface of the mortar until it forms a thin film. We seek to arrive at this point at about the time the material in the mortar becomes dry. A small drop of one per cent sodium chlorid solution is now placed in the mortar and the contents carefully rubbed up until none remains sticking to the surface. The salt solution is then added drop by drop, more rapidly as we proceed, and the trituration continued until a thick suspension results. Scraped filter paper is then loosely packed into the small end of the butt of a glass pipette (Fig. 1), and moistened with the salt solution. After moistening it is again gently packed down in the tube. The bacterial suspension is now transferred to this filter by a pipette and allowed to filter through into a centrifuge tube or other convenient vessel. The filter should be so packed as to permit the suspension to pass slowly through by gravity. The process may be accelerated by fitting a rubber bulb over the end of the filter tube and making pressure on it, but more clumps will be thus forced through.

The filtered suspension may be clump free. To determine this a small drop is spread between two cover-slips, as in spreading blood, and one of the covers stained and examined. If any clumps are found, the suspension should be refiltered one or more times through new filters.

The filtered suspension is now diluted with one per cent sodium chlorid solution to the density indicated by experience, and is thoroughly mixed by forcing it in and out of a

coarse capillary pipette fifty or more times, the position of the end of the pipette being constantly changed.

The suspension so obtained should be perfectly uniform and clump free.

Practically all workers agree that the preparation of a suspension of tubercle bacillus is a most difficult matter. When rubbed in a mortar these bacteria instead of forming a suspension tend to collect in a sticky mass on the end of the pestle, and when after much work a suspension of any density is finally produced it is found upon examination to be full of fragments of bacilli of all sizes, and consequently most unsatisfactory for work with the opsonic index.

We produce a perfect suspension of whole bacilli by the following means:

The surface of a modified Dorset egg media tube is thickly sown with tubercle bacilli. (Whole eggs mixed or beaten, decanted after the unbroken portions have settled, and diluted with forty-eight per cent of N. saline solution and two per cent glycerin are placed in test-tubes and inclined in an oven at 70°–80° C. for six hours or more. A pan of water is placed in the oven to prevent undue drying of the media.) After fourteen to eighteen hours salt solution is gently squirted over the surface of the culture until as many bacteria as possible are washed off. The surface is then gently rubbed with the platinum loop and the washing continued until all the bacteria have been removed from the surface of the media. There results a thick suspension containing many clumps of all sizes, but also numerous free bacilli.

This suspension is now filtered as before described using first a very small loosely-packed cotton filter. The filtrate thus freed from the larger clumps is further passed through one or more other filters made from scraped filter paper more closely packed down. The final suspension should be free from clumps of more than a few bacilli and even such are seldom found. When such a suspension is agitated one sees an appearance similar to that noticed on dropping potassium ferrocyanide into urine treated with acetic acid,

but this does not mean that clumps are present as might be supposed.

It is of course desirable to work with dead cultures of tubercle bacillus; and our practice is to kill them by heating the tube in water at 75° C. for twenty or thirty minutes. When heated for a short time at 100° C. in the steam sterilizer the bacilli are no longer acid fast and are consequently worthless for opsonic work.

Cultures grown as described are composed of large solid-staining bacilli up to twenty to twenty-four hours. When older than this they first show beading and later fragmentation.

Media made without glycerin produces a very small solid-staining bacillus growing in compact clumps which are exceedingly difficult to eliminate from the suspension. The morphology, however, does not change with the age of the culture.

II. THE BLOOD CORPUSCLES. — Twenty-five or more drops of blood are caused to drop freely into a centrifuge tube nearly full of warm 37° C. sodium citrate solution (one per cent sodium citrate in one per cent sodium chlorid). The tube is then filled to a mark with the warm citrate solution and the contents mixed by inverting on the thumb a number of times. The material is carefully balanced and centrifugated at high speed until the blood cells are all thrown down and the supernatant fluid is almost clear (the serum causes some discoloration as a rule).

By means of a coarse capillary pipette the citrate solution is now removed from the tube close down to the blood cells. The greater part of the serum is thus simultaneously removed. The tube is now filled to the mark with warm one per cent sodium chlorid solution and the contents thoroughly mixed as before. It is returned to the centrifuge and the blood again thrown down, which usually requires less time than for the first washing. The salt solution is pipetted off and the blood cells remaining thoroughly mixed in a pipette to break up clumps of leucocytes and evenly distribute the

white cells throughout the mass. This method gives as many leucocytes as are necessary, but if desired the upper portion of the centrifugated blood, which is richer in leucocytes, may be drawn off and mixed in another vessel. Or the lower portion may be withdrawn and discarded. In any event the blood cells to be used in obtaining the opsonic index must be thoroughly mixed. The method of attempting to draw the leucocytic cream directly from the surface of the material in the tube is subject to much error, as no two preparations are likely to contain equal numbers of leucocytes per unit volume.

III. THE SERUM. — From a prick in the finger end blood is allowed to flow down into the bent capillary end of the hook-shaped glass capsules described by Wright (Fig. 6).

When filled the straight capillary of the capsule is warmed in the flame and the end sealed. As the warm air cools, the blood remaining in the bent capillary is withdrawn into the body of the capsule. Seizing the capsule by the bend the blood is thrown down into the other end just as in shaking down a clinical thermometer. The tube is now hung on a glass or elsewhere until the blood clots, which occurs in a few minutes as a rule.

In collecting blood from one's finger it is very convenient to set the bend in the capillary of the capsule in a slit made in the top of a cover-glass box. This leaves both hands free.

After clotting, the serum capsules are hung on a centrifuge with the bodies inside the aluminum tubes, and centrifugated at moderate speed for about five minutes or until the clot is well separated from the serum. Where several sera are being used care must of course be taken to identify the capsules in some way. After centrifugalizing a nick is filed below the capillary bend just above the surface of the serum, and the bent capillary carefully broken off. The capsules are now conveniently placed by thrusting the straight end through small holes in the bottom of an inverted slide box.

In work with some bacteria, as B. typhosus, some strains of streptococcus and tuberculosis, and perhaps others, no further preparation of the serum is required. For staphylococcus, some streptococcus, and tuberculosis, colon and many others doubtless, it will be necessary to dilute the serum more or less for the reasons already given.

One should in beginning work with a new strain ascertain by the method before described the serum dilution necessary to produce a convenient phagocytosis.

For diluting sera it is convenient to employ the tubes described by Wright for making higher dilutions at a single step (Fig. 4). These are easily made and, except for comparison with others' work, need not be exactly accurate, since the same tube is used in diluting all the sera to be compared.

In order that the pipette may be in the same condition for each serum, I wash it out with one per cent salt solution before beginning and wash it also several times after diluting each serum.

To use the diluting pipette the end is introduced into the serum capsule, carefully avoiding the clot, and serum drawn up to a mark on the capillary. The end is then introduced into an Esmarch dish containing one per cent sodium chlorid solution, which is drawn in until the mark in the capillary above the body of the pipette is reached. The smallest bubble possible should be introduced between serum and salt solution. With some practice no bubble at all need be taken in. The contents of the pipette are now discharged into a small glass tube (Fig. 2) and drawn back into the diluting pipette several times to insure washing out all the serum. The material should be further mixed with a coarse capillary pipette, after which it is ready for use.

Unless perfectly fresh serum can be had, the control should be obtained at the same time as the patient's and both kept together until they can be worked. In the ice-box staphylococcus opsonin is lost at the rate of about one per cent per hour for the first ten hours. At room temperature the loss is about twice as rapid.

Having prepared bacterial emulsion, blood corpuscles, and serum as just described, one is ready to proceed with the work proper of making the mixtures from which the phagocytic indices are to be determined. If several preparations are to be made from the same serum an equal number of portions of the serum, each more than sufficient for a preparation, should be placed in separate tubes so as to avoid contamination from repeated dipping into the same tube. Likewise sufficient quantities of the blood corpuscles should be measured (roughly) into a number of tubes equal to the total number of preparations contemplated.

Since only the fresh tubes are dipped into the bacterial emulsion, a single tube of this is sufficient.

Capillary tubes having a uniform bore of at least one millimeter and a capillary five inches or more in length should be used in making the mixtures (see Fig. 3).

Each capillary is marked with a thin line about one and one-half to two inches from the end and a close-fitting soft rubber nipple of considerable capacity placed over the other end. (The same nipple may be used for each tube in turn.) Grasping the nipple between the thumb and forefinger so that its walls are closely pressed together, the end of the capillary is introduced into the tube containing bacterial emulsion. The tube is held in the left hand in a nearly horizontal position, the pipette rests lightly on the lower edge of the tube opening, with its end very little below the surface of the emulsion. By keeping the ball of the right thumb pressed firmly against the index finger and slowly raising the tip of the thumb, the emulsion is drawn into the capillary until the mark is reached. The pipette, still resting on the tube opening, is carefully slid outward, care being taken to keep the end of the capillary out of contact with the tube wall. As soon as the end has cleared the surface of the emulsion in the tube the contents are drawn up about one-fourth inch and the capillary withdrawn from the tube.

Next, blood corpuscles are drawn up to the mark on the capillary in the same way; and finally the serum is drawn up in like fashion. One now has in the capillary equal

volumes of bacterial emulsion, blood cells, and serum (diluted if necessary), each separated from the other by air spaces about one-fourth inch in length. Holding a clean tube (Fig. 2) in the left hand, the contents of the pipette are slowly expelled into its lower end. With the end of the pipette very nearly touching the bottom of the tube, the material is now alternately drawn into the capilary and forced into the tube fifty times. Care should be taken not to draw quite all of the material into the capillary nor to force quite all of it out, except occasionally when the entire contents should be emptied. Attention to this detail avoids converting the material into a mass of bubbles. Should this happen, however, the bubbles may be easily destroyed by pushing the pipette to the bottom of the tube and slowly drawing up the contents.

With a little practice the mixing just described may be done in a fraction of a minute.

After mixing the bacteria, blood, and serum the mass is drawn into the capillary until a column of about three inches is obtained. This is further drawn up until an air space of three-quarters inch remains in the end of the capillary. With the contents in this position the tip of the capillary is sealed in the flame. With a sharp file a nick is made in the capillary three-quarters inch or more above the contents and the butt of the tube broken off. The open end of the capillary is next sealed off, care being taken to withdraw from the flame the instant it closes, as otherwise the heated air will expand the soft glass and burst it. Allow fifteen seconds for the end of the capillary to cool, then place it horizontally in a pan of water in the incubator. It is convenient to have two glass rods across the bottom of the pan for the capillaries to rest on, or pans with specially constructed rests may be used.

The second preparation is made in precisely the same manner as the first except that the other serum is used. (It is well to make a practice of using either the normal or patient's serum first in every case.) The second capillary is laid in the water pan to the left of the first, and other

preparations, if any, are incubated each to the left of the one just before it.

One should keep a written record of his preparations, noting after each one the minute and nearest five seconds at which it was incubated. Other data such as the strain of bacteria, source of the sera and corpuscles, and time and temperature of incubation should also be noted on the record of work.

Our preparations are generally incubated for thirty minutes, our results having been somewhat more satisfactory for this than for the shorter incubation periods often used.

Each capillary, after incubating for thirty minutes, is withdrawn with a pair of forceps, dried quickly on a towel and the ends nicked and broken off (the tube of course being held horizontally). One end is now placed at the bottom of a clean tube and the other pushed through a small hole in a thin rubber diaphragm cemented over the end of a small soft nipple (Fig. 5). By the use of this nipple the incubated contents are rapidly mixed in the tube fifty to seventy-five times (the same for all preparations). One can mix more rapidly if the walls of the nipple are not apposed as described in the method of drawing up and making the preparation. A little practice will make it easy to avoid aspirating the material into the nipple, and the mixing need take no more than half a minute. After mixing, the contents being entirely expelled from the capillary, the nipple is almost completely relaxed when, the end of the capillary being placed in the mixture, a small quantity is drawn into the tube by the release of the nipple. A small drop is now spread on a slide, or between cover-glasses, as may be desired. On the whole we have had much more satisfactory results from cover-glass preparations and seldom employ slides.

As fast as they are made the preparations are laid blood side up in numbered squares marked out on the work sheet to correspond with the numbers of the preparations.

The method of work just described in detail is not nearly so laborious and time-consuming as might be inferred. Without special effort the time taken for making each preparation need average no more than two and a half minutes,

and this interval is ample for withdrawing the pipettes from the incubator, mixing the contents and making the spreads. I have handled thirteen preparations in thirty minutes — the incubation period. Much depends finally on properly staining the preparations. The method used will depend of course on the kind of bacteria employed in the work.

For the staphylococci, streptococci, colon bacillus, and many others, Wright's, Jenner's or some other modification of the Romanowski stain give satisfactory results. The nuclei should be faintly stained since a dark stain will conceal those bacteria contained in the nucleus. Generally the bacteria stain more deeply than the nucleus and decolor less readily.

A very satisfactory method for most bacteria is after fixation for five seconds in seven per cent nitric acid and washing clear with water, to stain lightly with methylene blue (two drops of Loeffler's mixture in ten of water for a few seconds). The protoplasm may be stained by a weak watery eosin if desired. Jenner's or Wright's stain may also be used instead of the methylene blue and often gives beautiful results. Jenner's seems more dependable than Wright's. For tubercle bacillus preparations the complicated methods of fixation and staining often described are unnecessary.

After very light fixation in a low flame the preparation is covered with carbolfuchsin and steamed for a minute and a half over a small flame. It is next washed with water decolored for five to ten seconds in seven per cent nitric acid, washed in water, stained with Loeffler's methylene blue (one drop stain in ten drops of water for five seconds), washed and dried between blotting papers. Weak watery eosin may be used before the methylene blue, but is generally unnecessary, the leucocyte outlines being sufficiently distinct without it. Instead of flooding the preparation with carbolfuchsin, excellent results may be had by immersing it in a small beaker of the stain set in a water bath at 70° C.

In many preparations one finds most of the leucocytes grouped along one or more of the edges. A moment's

search with the low power lens shows the locality where they are most numerous.

Counts should be made by good light with the oil immersion lens and a mechanical stage. In a good preparation made as before described it is sufficient to count fifty cells for each preparation, and with averages of from five to ten this should ordinarily require only about fifteen minutes to the preparation. Counts by natural and artificial light should not be compared.

If it seems that I have gone unnecessarily into detail in the foregoing work, some excuse may be found in the generally poor results obtained and due largely, I believe, to the lack of any complete detailed description extant, which would enable a beginner to do accurate work with the opsonic index.

Very much of the large amount of work so far reported should, I feel sure, be done over again with better, more accurate methods and proper controls.

It is generally assumed that the opsonic index is proportional to the patient's immunity.

Is this true for any or all organisms? Perhaps such questions may only be answered after means for measuring other factors in immunity are at hand, but as a beginning we must at least be able to do accurate work with the opsonic index.

It seems likely that in many cases experience in noting clinical symptoms may allow one to discard the laborious index; but here again accuracy in the last will be our greatest aid in correlating clinical signs and the patient's state of immunity.

The method here described can be practised by any one well trained in laboratory methods and may, I believe, be of some value in bringing some order out of the present chaotic state of opsonic work.

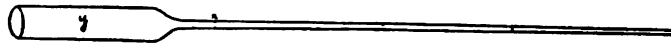
[In conclusion I wish to express my sincere thanks to those members of the Medical Staff whose sympathy and encouragement have lightened a laborious task, and particularly to Dr. George Dock, at whose suggestion the work was undertaken.]

The following articles are needed in the work described in this paper:

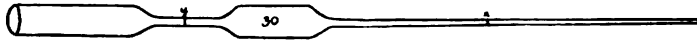
I. — Filter for bacteria.

scraped filter paper.

II. — Mixing tube also used for diluted sera, blood corpuscles, bacterial emulsions, etc. Made by sealing the end of a butt from mixing pipette.



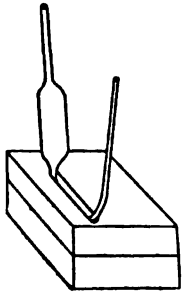
III. — Mixing pipette for drawing up bacteria, blood, and serum. Before incubating the capillary is broken off at *x* and sealed as described. The butt (*y*) is made into I. or II.



IV. — Diluting pipette for serum. Serum is drawn to the mark *x* and the tube then filled to mark *y* with salt solution. They may be easily made for any dilution as described by Wright.

V. — Nipple for mixing the material after incubation. A thin rubber cap is cemented over a soft nipple and a hole made in its centre with a hot wire.

VI. — Hook-shaped Wright tube for collecting serum. Blood runs in at *a* until the tube is filled nearly to *b*. The portion *bc* is then warmed and the end *c* sealed. The air in *b c* on cooling draws in the blood from *a*. The end *a* may be sealed if serum is not to be used immediately.



Same, set in slit in cover-glass box for getting blood from one's own finger.

SEPTICEMIA WITH ACUTE FIBRINO–PURULENT PERICARDITIS AND HYPOPYON IRITIS CAUSED BY THE MENINGOCOCCUS.*

CHARLES W. DUVAL, M.D.

(*Director of the Pathological Laboratory at the Montreal General Hospital.*)

(*From the Pathological Laboratory of the Montreal General Hospital.*)

Only in a small percentage of cases has the meningococcus been found in the circulating blood, but a careful review of the literature of meningococcic septicemia would seem to indicate that (1) septicemia due to the meningococcus is invariably secondary to meningitis, (2) when it occurs it frequently gives rise to local lesions outside the meninges.

The interest of the case here reported aside from the rarity of acute purulent pericarditis and hypopyon iritis due to the meningococcus is that it affords another example of a fatal case of meningitis with meningococcal septicemia and extra meningeal lesions.

I have been able to find in the literature only four cases of pericarditis in which the meningococcus was isolated from the lesion and proved to be the causal agent.

Again only three instances of hypopyon iritis are reported in cases of epidemic cerebro-spinal meningitis, but in no case did the author isolate the meningococcus.

Case No. 511, Montreal General Hospital, June, 1907. — W——— A———, aged seven years, was suddenly seized at 4 A.M. with vomiting. The child shortly afterward complained of frontal and occipital headache, and was brought to the hospital at noon on the same day. He became unconscious a few minutes after admission. Except for measles and scarlet fever at the age of four he had always been exceptionally healthy.

On admission there were noted in both eyes small bright red subconjunctival hemorrhages, together with an extensive petechial eruption over the trunk and arms. There was marked retraction of the head and typical clinical signs of acute cerebro-spinal meningitis.

The heart sounds were feeble and the apex beat neither visible nor palpable. Temperature, 102; pulse, 152. Leucocytes, 44,000. The pupils

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were equal and reacted to light. At the time there was no evidence of pus in the anterior chamber.

A lumbar puncture was performed and thirty-five cubic centimeters of very turbid fluid removed under tension. The fluid on microscopical examination showed large numbers of polymorphonuclear leucocytes and many intra and extra cellular biscuit-shaped cocci decolorized by Gram's method of staining. The meningococcus was isolated in pure culture from a portion of the fluid transferred to blood agar.

The patient grew progressively worse and died forty-eight hours after admission, or sixty-four hours after the first symptoms appeared. The autopsy was performed twelve hours after death and the following conditions were found: Acute epidemic cerebro-spinal meningitis; acute fibrino-purulent pericarditis; right hypopyon iritis; subcutaneous hemorrhages.

The anterior chamber of the right eye contained approximately .5 cubic centimeter of thick greenish-yellow pus which occupied the dependent part of the chamber. The cornea, which presented the normal transparency, was seared with a flat instrument and the pus withdrawn through a sterile hypodermic needle introduced into the anterior chamber.

Smears prepared from the pus and stained by Gram's method and counterstained with pyronin showed innumerable polymorphonuclear leucocytes, fibrin, and many cocci of meningococcal morphology. Occasionally the leucocytes contained a pair or more of cocci, but the organisms were for the most part extracellular. A portion of the material which was immediately distributed over a blood agar slant and placed in the incubator at 37° C. yielded a pure growth of meningococcus.

On opening the pericardial cavity it was found to be filled with thick yellowish-white fibrino-purulent material. The heart was bathed in this exudate. Over the base of the organ and also the greater part of the parietal pericardium the fibrinous element of the exudate was thickly adherent and presented the characteristic "bread-and-butter" appearance. The exudate was easily peeled from the epicardium; there was no evidence of organization. The heart weighed one hundred and thirty-five grams, but aside from the epicardium showed little worthy of special note. The myocardium was pale in color, though of normal consistence. The endocardium and valves were negative. The culture medium inoculated with the heart's blood yielded at 37° C. a few colonies which proved to be the meningococcus. Every care was taken to avoid the contamination of the heart's blood with the exudate from the pericardium.

The head was opened by the usual procedure. On removing the dura mater there was noted a marked injection of the pial vessels and flattening of the cerebral convolutions. Over the cortex and for the most part within the sulci, there was a large amount of rather dry, thick, yellowish-white pus. The purulent exudate did not extend over the base of the brain, except for a small area immediately surrounding the peduncle of the hypophysis. The absence of sub-pial edema and the extreme dryness of the exudate were most remarkable.

The brain weighed fourteen hundred and nineteen grams, and on section was tough, dry, and of the consistence of putty. The ventricles were free from exudate or fluid of any kind. The mid-brain, pons, medulla, and basal ganglia showed no visible change.

Stained smears of the exudate showed pus cells and fibrin, but no microörganisms. Transplants on culture media, however, gave a scanty growth of the meningococcus in pure growth.

The middle ears, nasal and frontal sinuses were negative.

The spinal cord was covered in places with an exudate similar in every respect to that met with covering the brain and pericardium from which the meningococcus was cultivated in pure growth.

The fact that in this case the meningococcus was isolated in pure culture from separate sources, eye, pericardium, heart's blood, meninges, and cerebro-spinal fluid, leaves no room for doubt that the meningococcus may cause extensive lesions outside the central nervous system.

Where special culture media are employed the isolation and identification of the meningococcus is comparatively easy.

It is well known that first generations of pathogenic bacteria on artificial foodstuffs are less vigorous than subsequent ones. This fact is often strikingly exemplified by the meningococcus; often a scanty growth on the isolating medium is followed in later generations by a profuse growth of the same strain accustomed to a saprophytic existence.

In smear preparations from cultures the meningitis coccus may be confused with the gonococcus or the so-called micrococcus catarrhalis, as they all possess the same morphological features, whereas upon suitable culture media, to one who has made a comparative study, definite growth differences can be distinguished. Certainly the meningococcus shows more variation in size and staining intensity than the gonococcus, and this peculiarity is early manifested in culture. A twenty-four-hour growth will show large and small cocci, some stained intensely, whilst others are scarcely stained at all. With the gonococcus this is only noted in much older cultures. The gonococcus is more difficult to decolorize by Gram's method than the other two, often retaining the stain after repeated alcohol washings.

The micrococcus catarrhalis is a larger coccus than the others of this group, is readily decolorized, and stains uniformly.

Culturally the organisms are readily differentiated. The micrococcus catarrhalis may be dismissed with a word since it produces a profuse dirty white growth on all solid media, and will live for months without sub-cultivation.

Some cultures of the gonococcus that are several generations removed from the animal body will grow well on ordinary glucose agar and blood serum. It is here that the difficulty arises in distinguishing the gonococcus from the meningococcus, since the latter also flourishes at times on these media. The gonococcus at either 37° C. or room temperature will remain viable for weeks, while the meningococcus dies out rapidly in three to four days.

During the past two years over fifty cultures of the meningococcus and the gonococcus have been isolated in the course of routine work at the pathological laboratory of the Montreal General Hospital, and it has been found that a blood agar mixture gives the most satisfactory results. It not only affords in itself a macroscopic means of differentiating between the meningococcus and the gonococcus, but is more certain to give a growth than any other medium employed.

To tubes containing .5 per cent dextrose agar medium there is added approximately .5 cubic centimeter of defibrinated human blood. It is essential, however, to prepare the medium at least a week before using, as freshly prepared blood agar does not afford the maximum growth of either the gonococcus or the meningococcus and may exert a decided inhibiting influence.

Ordinary sterile dextrose agar should be heated, then cooled to 42° or 41° C. and .5 cubic centimeter of defibrinated human blood added, thoroughly mixed and slanted. The reaction is corrected to .6–.8 per cent acid to phenolphthalein. The growth of the meningococcus upon this medium attains its maximum in fourteen to eighteen hours at incubator temperature.

When colonies develop instead of a profuse growth they are, in the case of the meningococcus, two to four millimeters

in diameter and possess a characteristic opalescence, whilst the gonococcus colony is much smaller and translucent. This difference in the size and color of the two colonies on blood agar is quite distinctive.

The first case of meningococcic septicemia to appear in the literature was reported by Gwyn.[1] This was a fatal case of epidemic cerebro-spinal meningitis complicated by purulent arthritis. He recovered the meningococcus in pure culture from the blood and joints during life and post-mortem from the meningeal exudate.

Warfield and Walker[2] reported the first case of meningococcic septicemia with acute endocarditis caused by the meningococcus. They isolated the specific organism from the blood before death and from the heart valve post-mortem. The clinical signs at no time pointed to meningitis; a high intermittent fever and other symptoms of general septicemia dominated the clinical picture. Nevertheless, as permission could not be obtained to open the head, the existence of a meningitis could not be excluded.

A similar case to that of Warfield and Walker's is the one reported by Weichselbaum[3] two years later, in which he isolated a pure culture of the meningococcus from an acute vegetation on the heart valve. Here the brain showed a well-marked purulent meningitis from which the specific coccus was cultivated. Although Weichselbaum failed to recover the organism from the heart's blood, he believed that its isolation from the heart valve and meninges was sufficient proof of the existence of a septicemia secondary to the infection of the meninges.

Lenhartz[4] reports two fatal cases of meningitis in which he recovered the meningococcus from the circulating blood. In both cases there was an acute exudative pericarditis and arthritis from which he also isolated the meningococcus and demonstrated them in smear preparations of the exudate.

Salomon[5] twice isolated the meningococcus from the circulating blood of a patient suffering with meningitis complicated by acute arthritis. He was at first inclined to regard the case as one of primary blood infection without

meningitis; but the presence of meningitis was subsequently established at autopsy, and the meningococcus was isolated from the meninges.

Robinson[6] examined the circulating blood in six out of a series of sixteen cases of epidemic cerebro-spinal meningitis and cultivated the meningococcus in only one. This case subsequently came to autopsy and the meningococcus was isolated from the heart's blood and conjunctiva. Since this was the only instance in six autopsies where he obtained the meningococcus outside the meninges he concludes that this organism is probably only an occasional invader of the circulating blood. It is remarkable that in five of these autopsies an acute bronchopneumonia was found, and from all the ordinary pyogenic cocci were isolated, but the meningococcus from none. Numerous cocci which decolorized by Gram's method of staining were found in the smears from the pneumonic exudate, but the meningococcus could not be grown from this material.

Councilman, Mallory, and Wright[7] in their study of the Boston epidemic (1898) made cultures from the heart's blood at autopsy in thirty-five cases with negative results. They demonstrated Gram negative diplococci in smears from the joints and from the pneumonic exudate of a number of cases, and although they concluded from the morphology of the organisms that these were meningococci, they did not succeed in cultivating the organism from a single case outside the meninges and conjunctiva. They conclude with regard to the meningococcus that, "so far as can be learned from cultures of blood, liver, spleen, and kidneys at post-mortem, septicemia is never produced."

The failure of these observers to cultivate the meningococcus from the heart's blood in a single instance was propably due to the fact that too little blood, that is, only a few loops full, was planted. There is no proof that their cases of bronchopneumonia complicating meningitis were caused by the meningococcus; they did not isolate the organism in a single instance, but based their opinion upon the finding of cocci of meningococcal morphology in the pulmonary

exudate together with other bacteria, hence there is every reason to believe that these bronchopneumonias were terminal infections.

All bacteriologists who have worked with the respiratory diseases agree that it is common to find cocci in the pneumonic exudate which decolorize after Gram's method of staining.

My own experience with pneumonias has convinced me that no reliance whatever can be placed on morphological characteristics uncorroborated by culture. It is rare to find a case of bronchopneumonia in which the exudate does not contain saprophytic forms identical with the meningococcus in size, shape, and staining reaction.

Elser,[8] employing special media, attempted to isolate the meningococcus from bronchopneumonia complicating meningitis, and although smears from the exudate showed innumerable cocci corresponding to the meningococcus in morphology, from none could he isolate the specific organism.

Jager[9] states that he found the meningococcus in pneumonic sputum of a meningitis case, but his statement is strongly discredited by Weichselbaum.[10]

Kischensky,[11] A. Frankel,[12] and G. Mayer[13] also demonstrated in smears from the pneumonic exudate of meningitis cocci which they thought to be the meningococcus alone or with what they regarded as the streptococcus and the pneumococcus. In no case, however, did they isolate the meningococcus.

Bernheim[14] is the only writer who claims to have cultivated the meningococcus in a bronchopneumonia complicating meningitis, though Weichselbaum[15] concludes from the description that in all probability Bernheim's culture was the micrococcus catarrhalis.

In two cases of epidemic meningitis complicated by bronchopneumonia, I found that the cocci in the exudate which decolorized by Gram's method of staining were the micrococcus catarrhalis.

Elser[16] examined the blood during life in forty-one cases

of meningitis in which a positive diagnosis had been made from the cerebro-spinal fluid, with positive findings in ten cases. He is inclined to believe that invasion of the circulation occurs more frequently than his results show. The inadequate method of cultivation and poor media are held by him to be responsible for the discrepancy. From the scarcity of colonies in the culture media he concludes that only a small number of cocci enter the circulation, and that these do not afterward multiply. Elser also states that the presence of the meningococcus in the blood of patients indicates an unfavorable prognosis. In five autopsies he found an acute purulent pericarditis complicating meningitis; the meningococcus was isolated in pure culture from the pericardium in but one of these, though organisms resembling the meningococcus were demonstrated in smears from three. Bronchopneumonia he found in four cases; purulent bronchitis in fourteen; congestion and edema in all. It is of interest to note that the meningococcus though searched for was not obtained in any of these lung conditions.

Bettencourt and Franca[17] undertook to cultivate the meningococcus from the circulating blood in six cases of epidemic meningitis with negative results. They describe, however, three cases of meningitis complicated by an acute purulent pericarditis; in two of these they demonstrated the meningococcus in smears and culture from the pericardial exudate. The third case yielded no organism of any kind either in smear or culture. In another fatal case of meningitis they isolated the meningococcus from the pleuritic exudate.

Möller[18] reports one case in which he isolated the meningococcus from the circulating blood the day before death.

Martini and Rohde[19] recovered the meningococcus from the blood on the second and fourth day of the meningitis.

Fronz[20] reports a case of meningitis in which the meningococcus was found in the joints and blood before death.

Wintersteiner[21] describes a fatal case of meningitis complicated by irido-cyklitis in which he found intracellular cocci resembling the meningococcus in smears. He failed to

cultivate the organism from the eye but did, however, grow it from the meninges. This fact led him to conclude that the cocci found in the smears from the eye were in all probability the meningococcus.

Tooke[22] also describes a fatal case of meningitis complicated with hypopyon iritis in which the meningococcus was cultivated from the meningeal exudate, but not from the eye, though cocci of meningococcal characters were seen in smears from the eye exudate.

Schotmuller[23] in his study of epidemic cerebro-spinal meningitis found acute arthritis as a complication in eight cases; the knee joint was involved five times, the hip joint once, and the wrist joint twice. In one of these he isolated in pure culture the meningococcus from the joint exudate. He mentions the fact that only a few colonies developed on the medium, though more than one cubic centimeter of pus was used. In his reported series of meningitis the meningococcus was cultivated from the circulating blood in two cases, both of which were complicated with extra-meningeal lesions and subsequently proved fatal.

Schotmuller holds that the meningococcus only seldom enters the general circulation, and when it does occur it is always secondary to the meningitis.

SUMMARY.

A review of the literature shows that the meningococcus has been isolated from the blood during life in a comparatively small number of cases. This fact when considered with the negative blood findings in the large percentage of the autopsied meningitis cases would seem to indicate that the organism is only an occasional invader of the circulating blood. Even in the authentic cases of meningococcal septicemia it is noteworthy that only a few colonies developed on the medium, though large quantities of blood were employed.

Almost in every instance where the meningococcus has been found in the circulating blood it has proved fatal, and the body showed some form of extra-meningeal lesion.

These associated lesions have invariably occurred in the endothelial lined cavities, namely, the joints, anterior eye-chamber, pericardial, endocardial, and pleural cavities.

The broncho-pneumonias complicating epidemic cerebro-spinal meningitis are in reality "terminal infections" caused by the ordinary pyogenic microörganisms. Certainly there is no proof to show that the meningococcus is the etiological factor.

It would appear that a general infection with the meningococcus is always secondary to disease of the meninges, since in all the reported cases of meningococcal septicemia where complete post-mortem examinations were made inflammation of the cerebral or spinal meninges was found. Other lesions occur, but always as complications of the focal infection. There is no authentic case where the meningococcus has produced lesions outside the meninges in the absence of a preëxisting meningitis.

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CONCERNING THE PRESENCE OF NEPHROTOXIC SUBSTANCES IN THE SERUM OF ANIMALS WITH EXPERIMENTAL NEPHRITIS.*

RICHARD M. PEARCE, M.D.,

AND

H. P. SAWYER, M.D.

(*From the Bender Laboratory, Albany, N.Y.*)

During the period of the active investigation of cytotoxic immune sera many attempts were made to utilize the results of the study of nephrotoxic sera in the explanation of various complications of chronic renal disease. It was assumed that if the injection into an animal of kidney parenchyma caused the appearance in the serum of that animal of a substance toxic, upon injection, for the kidney of another animal (heteronephrotoxin) it should be possible also to produce toxic substances in the serum of the same animal by so injuring its kidney as to cause degeneration of the renal cells. The production of such a serum (autonephrotoxin) by injuring one kidney, as by ligation of ureter, vessels, or entire pedicle, was attempted by several investigators, who claimed to be able to thus produce histological changes in the opposite kidney with the appearance of albumin in the urine. The serum of such animals also, it was claimed, had a similar effect if introduced into a normal animal of the same species (isonephrotoxin). Upon such experiments was based the theory that in chronic nephritis the continual degeneration of renal parenchyma was accompanied by an equally constant formation of autonephrolysin, the one dependent on the other, and that there was thus brought into play a vicious circle capable of explaining the course of this disease. Unfortunately for this and other theories based on similar observations, much of the early work on immune cytotoxins, on account of faulty methods, has been discarded; the theory of specificity has been set aside, and the

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observation concerning the production of autonephrotoxin by experimental ligation cannot be confirmed.

There is, however, a phenomenon brought out by the investigations along this line which remains unexplained and which is of peculiar interest in connection with any discussion of the pathology of nephritis. This is the observation of Lindemann[1] that the serum of an animal suffering from an experimental chromate nephritis has the power to produce lesions of the kidney when introduced into a normal animal and also my own[2] observation of similar results when the serum of dogs with spontaneous nephritis, or of those with lesions due to nephrotoxic serum, as had also been noted by Bierry,[3] are introduced into normal dogs. Such injections cause the excretion of albumin and casts and histological changes in the kidney. These observations which, as far as I am aware, have never been questioned, indicate the presence in the serum either of a substance formed anew during a nephritis or an accumulation of toxic substances (metabolites) normally eliminated by the kidney. The phenomenon is quite distinct from that of the action of a substance produced by immunization, as it represents presumably the action of a substance or substances resulting from tissue destruction, or faulty function, or both. If found to be a constant attribute of the serum of experimental nephritis it is a matter of extreme importance in connection with the pathology of chronic nephritis. We have, therefore, largely on account of the interest of one of us in various problems of chemical correlation as applied to the kidney[4] re-opened the subject in the hope of determining upon what factors the observation in question rests.

Methods. — From the outset the object of our investigation has been not so much the accumulation of positive evidence in support of this phenomenon as a thorough control study of the possibilities of error. As we have taken the appearance of coagulable protein in the urine as the criterion of kidney injury, we have attempted to control every other possible source of such protein. For this reason and

because the phenomenon has been frequently quoted and generally accepted by writers on diseases of the kidney, we present our methods and results in considerable detail. As this study is largely a control investigation of a definite phenomenon and not of the methods of producing it, and as Bierry and Pearce are in accord concerning the action of the serum of an animal in which nephritis has been produced by a nephrotoxic immune serum, we have not repeated the experiments of the former, but have adopted, instead, Lindemann's simpler method of utilizing the serum of animals with chromate nephritis. A study has also been made of the effect of the serum of animals with uranium nephritis.

The demonstration of the possible toxic action of a serum on the kidney has been attempted in two ways; first by the examination of the urine after the injection of the serum of animals with experimental nephritis and second, the action of such serum, in vitro, upon renal cells. Dogs and rabbits were used. In experiments of the first type the urine was examined for several days or a week for albumin and casts. This is very important in both dogs and rabbits, but especially in the former on account of the frequency of spontaneous chronic nephritis. The animals which were to furnish the serum received injections of either potassium chromate or bichromate or of uranium nitrate. The former were given subcutaneously to rabbits in doses .03 gram and to dogs .06 gram daily or every other day, and the latter in like manner in doses of .0075 gram to rabbits and .015 gram to dogs. An estimation of the degree of kidney injury was obtained by daily examination of the urine. The animals were bled from the femoral artery after periods varying from two to five days. The serum thus obtained was injected into normal animals, always of the same species, as soon as possible after bleeding, usually within two to four hours, though occasionally on account of the slow separation of the clot, the serum stood over night before being used. The injections into rabbits were made into the ear vein without anesthesia. Dogs received the serum either in the abdomen without anesthesia or, after incision of the skin, in a small subcutaneous

vein opposite the second joint of the hind leg under light ether anesthesia.

In these experiments the presence of coagulable protein and of casts in the urine has been taken as evidence of an influence of the serum upon the kidney. All possibilities of contamination of the urine were avoided. The animals were kept in metabolism cages which were scrupulously cleaned daily and the food limited to dog biscuit, which could not readily become mixed with the urine. In those experiments with dogs in which the skin was incised before the injection the wound was carefully dressed in order to prevent oozing of blood or serum. Dogs developing a diarrhea, as occasionally happened, were discarded.

Owing to the almost constant presence of mucin in the dog's urine, as shown by Jackson and one of us [5] elsewhere, and the necessity of controlling this in the test for albumin, the following procedure was adopted: Four portions of absolutely clear filtered urine were placed in test-tubes; the first served as control, to the second acetic acid only was added, to the third acetic acid and potassium ferrocyanide solution, and the fourth was heated and acetic acid then added. The amount of acid added to each tube was always the same and approximately the same quantity urine was used daily. By this method the clouding or precipitate due to mucin was, we think, accurately controlled. This clouding of normal dog's urine by the presence of mucin may be expressed thus o, +, +, +. This we have taken as a normal standard and when clouding in both third and fourth tubes has been greater than that in the second we have considered it evidence of the presence of protein, and the degree of clouding we have indicated by multiplying plus signs.

The centrifugalized sediment of the urine has been examined with special regard to the presence of casts, renal cells, and red and white blood corpuscles.

Results. — Eight dogs received the serum of dogs with chromate nephritis. Of these four were injected intravenously. In one, receiving the serum in dose of one cubic

centimeter to five hundred and seventy-eight grams of body weight, no change in the urine occurred. In a second and a third, doses of one to four hundred and ninety-three and one to three hundred and fifty respectively, a trace of protein was present in the urine on the day following the injection. In the latter of these a few fine granular casts, renal cells, and leucocytes were found in the sediment. The fourth experiment is given in detail, as follows:

Dog 61. — February 28, female, weighing fifteen thousand three hundred thirty grams, placed in metabolism cage.

February 29 to March 3, urine contains no albumin and sediment is negative.

March 3, received in small vein of leg under light ether anesthesia, thirty-two cubic centimeters (dose 1 to 480) of serum of chromate dog.

March 4, cage urine (seventy cubic centimeters, Sp. Gr. 1034) contains a considerable amount of coagulable protein which unfortunately was not estimated. Sediment shows numerous fine fat globules and cells of the type of renal cells and a few polymorphonuclear leucocytes; no casts, no red blood corpuscles. Urine from bladder at 11 A.M. amounts to seventy cubic centimeters with a specific gravity of 1020 and contains .5 per cent protein by the Esbach method. Sediment contains numerous cells of renal type and a few leucocytes. Urine from bladder at 3 P.M., sixteen cubic centimeters in amount, Sp. Gr. 1034, protein 2.5 per cent, sediment unchanged.

March 5, cage urine amounts to one hundred fifty-four cubic centimeters, specific gravity is 1042 and Esbach method gives 2.5 per cent protein; sediment as on the fourth. Urine from bladder at 9 A.M. contains but a trace of protein with elements of sediment fewer in number.

March 6, protein .25 per cent, sediment contains few renal and pus cells but no casts.

March 7, ditto.

March 8, urine, one hundred sixty-five cubic centimeters, specific gravity 1038, color amber, protein .25 per cent, sediment contains a small number of renal cells and leucocytes and fat globules and for the first time a few finely granular casts.

March 9 and 10, protein and sediment as on the eighth.

March 11, two hundred and seventy-five cubic centimeters of urine, specific gravity 1029, protein in traces which cannot be estimated by Esbach. Sediment negative except for an occasional leucocyte.

March 12 to 15, urine normal. Weight of dog on March 15 fourteen thousand four hundred twenty grams.

In order to control the possible toxic action of ether on

the kidney and also any accidental admixture of protein to the urine, by oozing of blood or serum from the incision over the injected vein, four dogs received serum injected directly into the peritoneal cavity without ether and without incision. The dose varied from one to two hundred and eighty-seven to one to four hundred and ninety. In all of these the injection was immediately followed by the appearance in the urine for a period of two to five days of traces of protein and small numbers of finely granular casts, but in none was the amount of protein equal to that in the urine of dog 61 previously described. The following experiment is typical of this group:

Dog 63. — February 28, male puppy, weighing four thousand nine hundred and seventy grams, placed in metabolism cage. February 29 to March 3, urine normal. Results of tests for protein give the mucin reaction (o, +, +, +). Sediment negative.

March 4, ten cubic centimeters of chromate serum (dose 1–490) injected into peritoneal cavity.

March 5, protein tests, o, +, 3+, 3+; sediment, few renal cells and leucocytes; no casts.

March 6, protein tests ditto, sediment as above with addition of a few finely granular casts.

March 7, no urine.

March 8, as on sixth.

March 9, 10, and 11, urine normal.

It is worthy of note that the lesions produced were not as severe or as constant as those described by Lindemann.

The experiments with uranium serum include one dog injected intravenously and four intraperitoneally. The intravenous injection (dose 1:1000) was followed by an elimination of casts without albuminuria. The casts were present in small numbers and were of a finely granular type with an occasional hyaline or epithelial cast and a few renal and white blood cells. The casts appeared on the first and third days after injection and could not be found on the fourth and fifth days. Casts had not been present in the preliminary examination covering a period of six days.

On account of the peculiar result of this experiment the animal was injected intraperitoneally on the fifth day. The

same serum, which had been kept on ice, was used (in the dose of 1:370). Casts of the finely granular and hyaline types and renal cells and leucocytes reappeared in the urine for two days, but no protein could be detected.

Of the three experiments in which uranium serum was injected intraperitoneally, one (dose 1:408) was negative and the other two (doses 1:400 and 1:635) showed a transient elimination of small amounts of coagulable protein with, in one, a few hyaline and finely granular casts and in the other no casts.

Although these experiments with dogs give a fair proportion of positive results, a small number of similar experiments with rabbits were absolutely negative. Three rabbits received uranium serum and two chromate serum in doses of five to twelve cubic centimeters (1:110 to 1:350) with no change whatever in the urine.

Although this investigation has to do mainly with the effect of the serum of experimental nephritis, the detection in the course of our routine examinations of several instances of spontaneous nephritis has permitted the testing of such serum. In an earlier communication, one of us[2] describes the occurrence of albuminuria with the elimination of casts in three of seven dogs receiving such serum intravenously. Of the present experiments, two in number, one confirms this earlier experience. The serum of a dog with spontaneous nephritis, eliminating from two to five per cent protein and an abundance of casts throughout the month during which it was under observation had a definite nephrotoxic power (in the dose of 1:210). The serum of a second dog with a less severe nephritis had no effect in the same dose.

The occurrence of spontaneous nephritis in the rabbit was observed but once. An animal eliminating .5 to 1.25 per cent protein daily was bled and its serum injected in doses of ten cubic centimeters (1:150) into two rabbits with no effect.

In three instances dogs with spontaneous nephritis were utilized in another way. It occurred to us that if the serum of an experimental nephritis has a toxic action on a normal

kidney it might have the power to aggravate an existing spontaneous nephritis. One of these dogs therefore was injected, intravenously, with uranium serum (dose of 1 :400) and a second with chromate serum, intraperitoneally (dose of 1 :460), but without effect on the elimination of protein or casts. In a third experiment of this type a dog weighing nine thousand ten grams, with a very severe nephritis, received at the same time forty cubic centimeters of uranium serum, intravenously, and twenty cubic centimeters in the abdomen, without result.

A word may be said about another phase of this subject. Bierry and also Pearce have shown, apparently conclusively, that the serum of a dog suffering from a nephritis caused by a heteronephrotoxic serum (prepared by injecting the rabbit with dog's kidney) has a definite nephrotoxic power when injected into the blood stream of a second dog. We have not repeated these experiments, but in the course of our work on another problem we found it necessary to procure the reverse type of immune nephrotoxic serum, that is, one toxic for the rabbit's kidney (prepared by injecting rabbit's kidney into the dog). This serum had a definite toxic action on rabbit's kidney, but the serum of rabbits so treated when injected into normal rabbits (dose 1 :100 to 1 :200) had no nephrotoxic effect, as is the case in the dog.

It is noteworthy that by no method of experimentation have we been able to demonstrate a nephrotoxic action of the serum of rabbits with kidney injury.

The question arises as to whether the toxic action of the serum of an experimental nephritis in the dog may be due, not to some peculiar substance developing as the result of injury to kidney cells, but to metabolites retained as the result of imperfect kidney function. We have performed no experiments, such as the injection of the serum of animals with complete nephrectomy, to test this point, but have found that the serum of animals with experimental reduction of the kidney substance to one-fourth the normal amount has no effect on the kidney of normal dogs. This conclusion is based on experiments with the sera of two dogs, the serum

of one obtained one month and of the other two months after extirpation of three-quarters of the kidney substance. Thirty cubic centimeters of the first serum was injected in a dog weighing five thousand four hundred forty grams and thirty-two of the latter in a dog six thousand grams in weight. In neither did protein or casts appear in the urine.

The power of the serum of experimental nephritis to agglutinate renal cells in vitro was tested with seven sera. A mixture of renal cells in salt solution was prepared from the washed kidney, and to one-half cubic centimeter lots in test-tubes were added five cubic centimeters of each of the sera to be tested. Cell mixtures and sera were used within a few hours after they were obtained. Observations were made every hour for six hours. As a control, normal dog serum was used. Three sera of dogs with uranium, three with chromate, and one with spontaneous nephritis were tested in this way. In no instance did agglutination occur, and the degree of precipitation did not differ from that of the control. No difference in cell structure could be detected by microscopic examination.

In so far as this method of testing may be of value these results point to the absence of an isonephrotoxic activity of the serum of experimental nephritis and the results are not in accord with those obtained by injecting the same sera into the living animal.

In addition to the various methods of control which we have described, there is naturally the question of the elimination by the kidney of an excess of protein injected into the blood stream. In view of the fact that the injections have always been made into an animal of the same species and also that the amounts injected have been comparatively small, this factor could be set aside at once if it were not for a curious observation of Weiss.[6] This investigator as the result of a very extensive series of experiments came to the conclusion that all foreign sera cause albuminuria of some degree, but that homologous sera do not except when the serum of one sex is injected into the opposite sex. His evidence on the latter point, however, rests on a single experiment

in which traces of protein resulted from injecting the serum of a male into a female rabbit and his quotation of Favaret's single observation of a like result upon injecting the serum of a bitch into a dog. In our control experiments, we have kept this point in mind, but have not been able to confirm it. In the dog, injections of normal dog serum in doses as high as one to three hundred, and in the rabbit of normal rabbit serum in doses of one to one hundred and fifty have failed to produce albuminuria, irrespective of the sex of the animal furnishing or receiving the serum. As we have always used homologous sera, the toxic effect of an alien serum does not come into question.

We have also controlled the possibility of a periodic albuminuria in the dog. For this purpose four dogs were set aside, and the urine of each examined daily for periods of ten days to two weeks. In none was albuminuria found nor were casts present during this time. Each gave the mucin reaction but nothing more. During this period two of these dogs were etherized and small quantities of blood taken from the jugular vein without any effect on the urine. These latter served therefore as controls also of our operative procedure.

The possibility of carrying over in the serum minute amounts of the salts injected must be considered. Attempts to detect, by the usual tests, the presence of chrome and uranium salts in the filtrate of the serum concentrated after coagulation have failed. Such tests, however, are not very sensitive and it is possible that traces may have been present. If present, the amount must have been so minute that it seems very improbable that they could have anything to do with the lesion described. Certainly they appear to have had no effect in the experiments with rabbits, and on this evidence alone we feel justified in ruling out the possibility of carrying over these salts in the serum.

In addition to the various controls described, we have by careful selection and elimination ruled out the possibility of an admixture to the urine of protein substances from local

inflammatory conditions, as balanitis in the dog, or lesions of the uterus or vagina in the bitch. The surface of the entire body as well as the mouth of each animal has also been carefully examined for lesions capable of allowing a discharge of blood or serum. As the food has been limited to dry dog biscuit and the cage carefully screened to prevent particles dropping into the collecting bottle, we believe the presence of protein from the food can be ruled out.

In short, we have investigated every possible source of error which suggested itself, but have been unable to explain the presence of albumin in the urine except by a toxic action of the serum on the kidney.

CONCLUSIONS.

The positive results here described confirm the observation of Lindemann, Bierry, and Pearce that the serum of dogs with various types of nephritis has a toxic action on the kidney manifested by the appearance, for a short time, of protein and casts in the urine.

A very complete series of control observations offer no explanation for the appearance of protein other than as the result of injury to the kidney.

It has not been possible to demonstrate a nephrotoxic power for the serum of rabbits with experimental nephritis and it is therefore manifestly improper to assume that this nephrotoxic power is a constant characteristic of the serum of all animals suffering from nephritis.

For this reason, although the phenomenon observed is suggestive and worthy of investigation on a larger scale, it should be utilized with caution in any theoretical explanation of the pathology of chronic nephritis.

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THE HEMOLYTIC REACTIONS IN CASES OF HUMAN CANCER.*

RICHARD WEIL, M.D.

(*From the Loomis Laboratory, Department of Experimental Pathology, Cornell University, New York, and the Pathological Laboratory of the German Hospital, New York.*)

It is the purpose of the present paper to give a brief survey of work done during the last twelve months on the subject of hemolysis in cases of human cancer. In a previous paper, on the hemolytic reactions of the serum in dogs affected with lymphosarcoma,[22] it was shown that there were certain characteristic features distinguishing the serum and the corpuscles of such animals from healthy individuals of the same species. The extension of the research to the human subject was a natural corollary, and has occupied the attention of the writer more or less constantly for the past year.

The history of the problem is of considerable interest. The attempt to discover in the blood a reaction characteristic of the existence of malignant new growths is by no means new. Every fresh method of hematological research which has been developed in the laboratory has in turn been made subservient to this object. The staining methods of Ehrlich gave rise to a considerable literature, now largely forgotten, the aim of which was to demonstrate certain morphological characters of the blood cells, supposed to be pathognomonic of the presence of malignant tumors. At the present time it is generally admitted that this attempt has failed. This phase was succeeded by the study of the resistance of the red cells in various pathological conditions to anisotonic solutions of salts. As a result of these researches it has become established that the resistance of the red cells of human beings varies considerably,[4, 22] and that it is as a rule increased in far advanced cases of malignant disease.[21] The clinical applicability of this finding is unfortunately very slight. With the advent of the modern methods of studying

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the agglutinating and the lytic effects of serum on corpuscles, a literature of no small volume has accumulated as the result of the application of these methods to human pathology. A number of observers asserted that the serum in certain diseases, including cancer (Ascoli,[1] Grünbaum,[13] and others), was capable of agglutinating the corpuscles of normal individuals, while the corpuscles of another individual with the same disease were immune to this action. This is the phenomenon of so-called isoagglutination. The Italian observers claimed that this biological reaction was a safe diagnostic criterion of malaria.[23] The careful researches of Landsteiner,[18] [19] since verified by Donath,[6] Gay,[35] and many others, threw grave doubts on all these results by proving definitely that there was a certain series of formulæ, three in number, to which the agglutinating powers of all human sera — normal and pathological — could be shown to conform, and that there was nothing in any manner characteristic of the reaction in any form of disease.

Partly contemporaneous with this work on agglutination, but mostly later, came a number of researches which dealt with the lytic power of human serum on the red cells of the blood. This work, as bearing more directly on the subject matter of this paper, demands special attention. Certain observers studied the action of serum in various diseases on the corpuscles of alien species. One of the most noteworthy of these researches, unfortunately cut short by his early death, came from J. M. Polk,[25] who succeeded in clearing up a great many of the baffling intricacies of the technic. Kelling[15] tested the serum of human cancer cases upon the corpuscles of sheep, cows, and chickens, and found that in forty-three per cent of the cases its hemolytic power greatly exceeded that of normal human serum. His theory of the specificity of this reaction in cases of cancer was attacked by v. Dungern,[8] and recently by Fischel.[11] The latter author has, within the current year, tested the sera in a variety of human diseases upon the red cells of chickens. He finds that in about fifty per cent of the cases of malignant tumors the blood has a markedly increased hemolytic power as compared with the

normal. On the other hand, he finds the same reaction to be present in some cases of diabetes, pernicious anemia, chronic endocarditis, and tuberculosis. It is, therefore, not specific in tumors. It is an interesting fact, as is pointed out by Fischel, that his observations are in perfect accord with those which I reported to the American Association for Cancer Research in 1907,[38] although in his experiments the human serum was tested against alien corpuscles, and in mine against human red cells.

Recently the tendency has been rather to study the lytic effect of human serum on the corpuscles of other human individuals. Ascoli,[1] in 1901, published the results of such a study in one hundred and fourteen human individuals, of whom seventeen were normal, ninety-seven diseased. He found that the normal sera were, as a rule, not hemolytic at all, or at most only slightly so.

He found no hemolysis in five cases of chlorosis, two cases of anchylostomiasis, one of liver abscess, three of acute rheumatism, three of exudative pleurisy, two cases of plumbism, one of acute Bright's, two of chronic Bright's, and a large number of cases of bronchitis and of gastritis. On the other hand, he found that hemolysis was pronounced in two cases of gastric cancer, one of pneumococcemia, and in a large number of cases of tuberculosis, and of pneumonia. Kreibich[17] (1902) found that the serum had no hemolytic power in six cases of pemphigus, ten of erysipelas, five of lues, three burns, one purpura, and one staphylococcemia. Eisenberg[10] found that of fifteen cases of typhoid, ten had hemolytic sera; of eight cases of scarlatina, seven hemolyzed; and of eight syphilitic subjects, five hemolyzed.

The net result of these and of some other less extensive researches was to demonstrate that human serum in various diseases is occasionally hemolytic towards the corpuscles of other human individuals. The mechanism of this hemolytic action of serum was beautifully illustrated by the researches of Landsteiner and Donath[7] on cases of paroxysmal hemoglobinuria. At the same time, it was clear that this hemolytic phenomenon, interesting and striking as it was, could not be considered of diagnostic value, inasmuch as it was present in so many and such varied conditions of disease.

In 1907 I described a new method of studying hemolysis in disease, based not only on the reaction of the serum, but also on the degree of resistance of the corpuscles to that serum. It is of importance for the understanding of that method and of the results which it has yielded, not only in my hands, but in the hands of other investigators, to describe in some detail the work which has led up to it. The material on which the earlier work was based consisted of a large number of dogs affected with the type of tumor known as infectious lymphosarcoma, which were put at my disposal by S. P. Beebe. The character of these tumors and their mode of growth have been fully described by Beebe and Ewing.[2] The first step in the study was to investigate the action of these tumors, when removed from the body and extracted in salt solution, on the red cells of the dog. The same method had been previously employed by a number of observers in the study of human tumors, but necessarily under far less satisfactory conditions. As a result of a large number of experiments,[29, 30] it became clear that the tumors of dogs, as well as most of their normal organs, when freshly extracted, possess the power of destroying to a very slight extent the corpuscles of other dogs, and that this power can be slightly enhanced by adding to the tissue extract an extract of red blood cells. The latter phenomenon has been described by Sachs[26] in the case of hemolysis by cobra venom, the red cell constituent of the reaction, which in my paper was called " red-blood-cell-derivative," or R. B. C. D., being designated by him as endo-complement. Far more striking, however, was the effect of necrosed or broken down portions of these same tumors upon the red cells; under these conditions the red cells were rapidly deprived of their coloring matter and even completely destroyed. On the theory that this result might be due to the presence of autolytic products in these broken down tumors, organs of the dog were autolyzed antiseptically outside of the body, and a number of intra vitam necroses were produced by tying off the vessels of the kidney. It was found that these autolyzed tissues produced hemolysis in exactly the same

fashion as did the necrotic tumors. This work has recently been repeated and verified by Fukuhara.[12] It is therefore quite evident that the broken down tumors contain a material very poisonous to the red cells of the blood. The clinical aspect of this fact is revealed in the observation that cachexia, as Beebe has found in his dogs, is associated not with the progressive growth of these tumors, but with the process of necrosis and softening. It was the theoretical anticipation of this fact which led F. Müller,[24] in 1889, to attribute the cachexia of cancer to a "toxogenous disintegration of protoplasm, independent of nutrition," in other words, to a specific toxic effect of the broken-down tumors.

The next step was to determine whether this supposed toxic material, or any characteristic effect of its activity, could be traced in the circulating blood of dogs affected with the growth.[31, 32] Blood was therefore taken from a large number of dogs, including some with tumors and some without, and the effect of the sera upon corpuscles was determined in a series of experiments, the results of which were published during the past year. These experiments showed conclusively that a considerable proportion of dogs with tumors, especially when in the stage of necrosis, possessed a serum which is hemolytic in some degree for all dog corpuscles. Normal dog serum is very rarely hemolytic, and, if so, generally in a very slight degree. In the second place, the corpuscles of dogs with tumors are far more resistant to this hemolytic activity of the serum of tumor dogs than are the corpuscles of normal animals. In other words, the serum of tumor dogs frequently contains an "isolysin," and their corpuscles are relatively immune to its action. The phenomenon is precisely comparable to the existence of isoagglutinins and the immunity of the corpuscles to these agglutinins in certain groups of human individuals, as demonstrated by Landsteiner.

The existence of such a toxin in the circulating blood of dogs affected with these tumors has gained additional support from the observation of Wade[34] that the growth of the tumor is associated with the production of an interstitial

nephritis. The supposed "toxin can be isolated from it (the tumor) by filtration, and produces interstitial nephritis" of the same character experimentally.

The existence of a double reaction of the type just described is evidently a very different matter from the simple hemolytic reaction described by Ascoli or Eisenberg. It is conceivably capable of assuming diagnostic importance, inasmuch as there appears in dogs, at least, to be something specific in the relative resistance of the corpuscles to the hemolytic activity of the serum. This consideration led, upon the completion of the work on dogs, to the study of the blood of human subjects suffering from new growths. (These results have already been reported in brief at the first and second meetings of the American Association for Cancer Research.[31,33]) I have had at disposal the material of two large general hospitals, the German Hospital and the Sydenham Hospital, and a few cases in Bellevue, kindly furnished by Dr. Coleman. The work was done on a selected set of eighty-two cases, in all of which the diagnosis was fairly certain, being confirmed by microscopic examination of autopsy or operative material in the group of tumors. There were thirty-one cases of malignant tumors, of which fifteen were in an early stage and sixteen were in an advanced condition, using the terms "early" and "late" in a broad clinical sense. There were three cases of benign new growth. There were forty-two cases of other diseases. Six of the cases were apparently normal. The method was that previously described. From ten to twenty cubic centimeters of blood was aspirated from the median basilic vein; part of this blood was allowed to clot and the serum was poured off, while the rest was defibrinated, and the washed corpuscles made up into a two per cent emulsion in normal salt solution. In every experiment, the serum was tested both against its own corpuscles and against the corpuscles of another individual described as the "control;" after some experimentation it was decided to select as a "control" either a normal individual or one with a different type of disease. The results obtained in all these experiments are given in the accompanying table (Table I.).

TABLE I.

The hemolytic reactions in eighty-two human cases.

Diagnosis.	Pathological Examination.	Remarks.	Hemolysis of	
			Own Corpuscles.	Alien Corpuscles.
I. Malignant tumors:				
Tumor of lip	Carcinoma.	Early.	−	−
Tumor of lip	"	"	−	+
Tumor of tongue	"	"	+	+
Tumor of tonsil	"	Late.	−	−
Tumor of larynx	"	Early.	−	+
Tumor of esophagus	"	Late.	+	−
Tumor of stomach	"	Early.	+	−
Tumor of stomach	"	"	+	+
Tumor of stomach	"	"	−	+
Tumor of stomach (4 cases)	"	Late.	−	+
Tumor of colon	"	"	−	+
Tumor of sigmoid	"	"	±	−
Tumor of rectum	"	Early.	−	−
Tumor of prostate (2 cases)	"	"	−	−
Tumor of penis	"	"	−	−
Tumor of breast	"	"	−	+
Tumor of breast (3 cases)	"	Late.	−	+
Tumor of breast	"	"	+	+
Tumor of cervix	"	Early.	−	−
Tumor of vaginal scar	"	"	−	−
Tumor of kidney	"	Late.	+	+
Tumor of face	Epithelioma.	"	−	−
Tumor of scalp	"	Early.	−	+
II. Benign tumors:				
Tumor of uterus	Fibroid.	.....	−	+
Tumor of uterus	"	.....	−	−
Tumor of breast	Adenoma.	.....	+	−
III. Other diseases:				
Plumbism (3 cases)	.....	.....	−	−
Pneumonia	.....	.....	−	−
Pneumonia	.....	.....	−	+
Pernicious anemia (2 cases)	.....	.....	−	−
Leukemia	.....	.....	−	−

TABLE I. — *Continued.*

Diagnosis.	Pathological Examination.	Remarks.	Hemolysis of	
			Own Corpuscles.	Alien Corpuscles.
Hodgkin's			+	−
Malaria (2 cases)			−	−
Tuberculosis of lungs (2 cases)			−	+
Tuberculosis of joints			−	+
Tuberculosis of cervical glands			−	−
Syphilis			−	+
Syphilis			−	−
Typhoid (2 cases)			−	−
Acute rheumatism			−	−
Chronic rheumatism			−	−
Gout			−	+
Cerebral embolism			−	−
Chronic endocarditis (2 cases)			−	−
Abscess of liver		Amebic.	−	−
Obstructive jaundice			−	−
Cirrhosis hepatis		Ascites, no jaundice.	−	−
Pancreatitis			−	−
Chronic gastritis			−	−
Chronic colitis			−	−
Chronic mastitis			−	+
Appendicitis		Abscess.	−	−
Hernia			−	−
Neurasthenia (2 cases)			−	−
Senility			+	−
IV. Normal (6 cases)			−	−

It is apparent that the results may be grouped under four headings, as follows:

1. Serum fails to hemolyze its own corpuscles, but hemolyzes alien corpuscles.
2. Serum hemolyzes both its own and alien corpuscles.
3. Serum hemolyzes its own, but not alien corpuscles.
4. Serum hemolyzes neither its own nor alien corpuscles.

The results have therefore been summarized according to these four categories, in Table II., in which the first group is indicated as — +, the second as + +, the third as + —, and the fourth as — —.

TABLE II.

(*Summary of Cases.*)

	I.	II.	III.	IV.
	−+	++	+−	−−
1. Early malignant tumors	6	2	1	6
2. Late malignant tumors	9	2	2	3
3. Benign tumors	1	0	1	1
4. Other diseases	11	0	2	29
5. Normal cases	0	0	0	6

Group I. (— +) conforms to the type of reaction described in the lymphosarcomata of dogs, and may be called a "positive" reaction. Of the early malignant tumors, six (forty per cent) conformed to this type; of the late malignant tumors, nine (fifty-six per cent) conformed to it; of the benign tumors, one (thirty-three per cent), and of the other diseases, eleven (twenty-six per cent) showed this type of reaction. It is evident, therefore, that the reaction is not pathognomonic of malignant tumors, early or late, that it occurs in a considerable proportion of other diseases, and that a large proportion of tumors present an altogether different type of reaction. On the other hand, it must be admitted that a much larger percentage of malignant new growths present this type of reaction than do cases of other disease. The results of the experiment are, on the whole, far less characteristic than in case of the tumors of dogs, and do not lend themselves at present to diagnostic application. There are a number of obvious explanations for these differences between dogs with lymphosarcoma and human beings with malignant new growths. In the first place the former

present a single and sharply demarcated type of growth of which all the cells are of one stock and belong to one family. In human beings, however, the group of cancers comprises a collection of growths which differ very markedly, not only in their morphology, but also in their biological characters. A thyroid cancer, as is shown by the well-known case of v. Eiselberg, may save the body from myxedema; an epithelioma of the face, even though far advanced and inoperable, often produces hardly any constitutional effects; a tumor of the stomach, even though of slight extent, may destroy its subject (without producing pyloric stenosis). Striking differences such as these between the various types of new growth grouped as "cancer" might easily be multiplied, showing that the action upon the organism as a whole may, on the one hand, be intensely destructive, or, on the other, even physiologically compensatory in some respects, with every gradation between these two extremes of action. It is therefore unreasonable to expect that such different conditions should all produce a uniform alteration of the serum. In spite of this difficulty I believe that some importance must be attached to the fact that the sera of individuals with tumors present a far larger proportion of "positive" (— +) reactions than do other individuals, whether normal or diseased. It seems very possible, therefore, that something of value may eventually come of the use of this method in human disease. But it will have to come with a refinement of method which will correspond to the complexity of the factors involved.

The importance of the method in its application to human disease has been recently given considerable prominence by Crile.[5] He studied hemolysis and corpuscular resistance, according to the method described by me, in cases of human cancer, and with slight variations he exactly confirms my conclusions in the study of the blood of dogs. He states that all early cases of malignant new growth have a hemolytic serum, and that the corpuscles of such cases are immune to the destructive action of their own serum, or of the serum of other cancer cases. As the tumors progress, he says that

the hemolytic power of the serum disappears, and with it the resistance of the corpuscles. This reaction he finds to be characteristic of cancer. The only apparent exception is found in cases of tuberculosis. He finds that the reaction is so delicate as to betray the very earliest beginnings of malignant new growths as, for example, in cases in which the "cancerous transformation" of a luetic scar or the "sarcomatous transformation" of a fibroid of the uterus were evident only on microscopic examination. It is evident that a method as delicate and at the same time as unerring as this would be of great use in practice. My own observations do not permit of such a sweeping generalization, and I believe that it will hardly stand the test of further investigation. The method yields results of such a character that great caution and reserve must be exercised in their application to human diagnosis.

The theoretical aspect of this type of hemolysis is of great interest, and has not hitherto been presented. It is, on first thought, somewhat difficult to conceive that a substance destructive to the life of red blood cells — a hemolysin — should circulate in the blood of the living animal. Evidently, if such a substance does exist, the cells of the animal in which it occurs must be immune to its action. Otherwise, there would be progressive destruction of the red cells and death. Now the observations of many careful workers show that just these conditions do actually exist. Landsteiner and many others have demonstrated the presence of isoagglutinins in human blood, and the existence of a specific immunity to their action on the part of the cells of the individual in which they occur. Yet these same cells may be very susceptible to agglutinins present in the blood of other individuals of the same species. Do the same laws hold of hemolysins? Until recently it was not known that hemolysins for the red cells of the same species could normally occur. Ehrlich[9] had indeed shown that such hemolysins (iso-hemolysins) could be artificially induced in goats by injecting the red cells of other goats. In these injected animals, however, the serum never became hemolytic

for their own cells. Ehrlich called this phenomenon the "horror autotoxicus," which is simply another way of stating that their own red cells had become immune to their own hemolysin. In 1906 Theobald Smith[27] showed that isolysins normally occurred in a considerable number of horses, which were capable of destroying fifteen per cent of the corpuscles of other horses. In 1907 I showed the same to be true in some dogs not affected with tumors. There is, therefore, no doubt that animals may have in their blood hemolysins for the red cells of their own species, but not toxic for their own cells. The question now arises, can the red cells of an animal become immune to the action of a hemolysin introduced into the blood from without during adult life? For example, could the red cells become immune to a toxin circulating in the blood and derived from a disintegrating new growth? There is ample evidence to show that red cells may change in their resistance to anisotonic salt solution during the course of disease.[4, 21] This, however, is not a specific response. There is, as far as I know, only one condition in which it has been abundantly shown by independent observers that there may be such a specific immune response on the part of the red cells. If eel serum be injected a number of times into rabbits,[3, 16, 28] their red cells become relatively immune to its destructive action in the test-tube. The mechanism of this response need not be very complex. New red cells are being constantly manufactured, and it is only necessary to imagine that those less resistant to the poison die off, leaving only those more resistant. There is, therefore, no inherent difficulty in the conception of an isolysin due to disease and a corpuscular immunity thereto. On the other hand, we are very far from having proved that this is the actual explanation of the hemolytic reactions of the blood in certain forms of disease, specifically in the lymphosarcoma of dogs. It is conceivable that the phenomenon is due to an entirely different set of factors. This is a problem which only much labor and care can hope to solve.

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THE ETIOLOGY OF AN EPIDEMIC OF INFLUENZA. RELATION OF THE INFLUENZA BACILLUS AND OTHER ORGANISMS TO THE RECENT EPIDEMIC IN BOSTON (1907–08). COMPARISON WITH AN INTER-EPIDEMIC PERIOD (1902–04).*

FREDERICK T. LORD, M.D.

(*Physician to Out-Patients, Massachusetts General Hospital.*)
(*Assistant in Clinical Medicine, Harvard Medical School.*)

From a clinical point of view, influenza may be classed as (1) Pandemic Influenza, (2) Endemic-epidemic Influenza, and (3) Sporadic Influenza. If the influenza bacillus be regarded as the sole cause of true Influenza, the disease may be designated as Influenza Vera, when this organism is present, while those cases with a similar clinical picture, but in which the influenza bacillus cannot be found, may be classed as Influenza Nostras or "Grippe." The specific relation of Pfeiffer's influenza bacillus to Influenza still needs confirmation, however, and the question may not be settled before another pandemic. Until the etiology of the disease is finally determined, it seems best to continue to use the term Influenza to designate a complex of symptoms, without regard to the apparent bacterial cause.

From about the last week in November, 1907, to the middle of January, 1908, this community suffered from an epidemic of acute respiratory infection which must be regarded, clinically, as Influenza. In general, the symptoms of the infection were less severe than in the epidemics and pandemics of Influenza in 1889–90, 1891 and 1892.

MORBIDITY. — Though exact reports are lacking, it can be stated that the disease was widely distributed throughout the United States. It appears to be certain for New England, at least, that a larger proportion of the population was attacked than in any previous outbreak of acute respiratory

* From the Clinico-Pathological Laboratory of the Massachusetts General Hospital, J. H. Wright, Director. Received for publication Aug. 15, 1908.

infection since 1889–92. The morbidity in Boston may be judged from an inquiry by circular in one of Boston's department stores, in which ninety-nine (16.5 per cent) of about six hundred employees replied that they had been affected in the three weeks preceding December twelfth, 1907. The epidemic was at its height at this period. As it did not subside until three or four weeks later, the total number must have been considerably greater.

BACTERIOLOGY. — A study of the bacteria found in the fresh material was made in twenty cases, comprising the more severe types of the disease.

Of the twenty cases, the infection was tracheo-bronchial in seventeen and nasal in one. The material collected for examination in these cases was muco-purulent or purulent. The two remaining infections were predominantly tonsillar; and in these cases smears were made and cultures taken from the inflamed tonsil. The material from fifteen cases was examined within the first week of the illness. The previous duration in five tracheo-bronchial cases was ten days in two; sixteen days and four and five weeks, respectively, in the remaining three.

The specimens were studied by smear preparations and by cultures. The method was similar to that described in a previous publication.[1] A special search was made for the influenza bacillus. The presence of other organisms was also noted.

The research was undertaken for the purpose of comparing the bacteriological findings in the cases from the epidemic with similar studies on the respiratory infections during an inter-epidemic period from August, 1902, to January, 1904.[2]

Comparison of the bacteriological findings in the epidemic cases with the results in the inter-epidemic cases.

1. Mixed infections. — In the twenty epidemic cases an infection with more than one group of organisms was present in eleven (fifty-five per cent).

In the one hundred eighty-six inter-epidemic cases previously studied, mixed infections also comprised a large proportion, being noted in one hundred twenty (sixty-four per cent).

These one hundred eighty-six cases were unselected, except to exclude from the investigation those patients in whose sputum tubercle bacilli were found. There was no epidemic of influenza in Boston during the period covered by the investigation, and the cases merely represented the normal number constantly seeking admission to the Out-Patient Department of the Massachusetts General Hospital. The series comprised cases of acute or chronic disease of the respiratory tract, for the most part bronchitis.

Of the more important bacteria identified in these mixed infections, influenza bacilli, pneumococci, micrococcus catarrhalis and the pyogenic cocci may be mentioned.

2. Pure infections. — The remaining cases in the two series may be classed as pure infections. They were thus regarded when one organism was found in overwhelming numbers to the practical exclusion of other bacteria.

Among the twenty epidemic cases one group of organisms predominated in nine (forty-five per cent). In this class the influenza bacillus was present as a practically pure infection in three, the pneumococcus in two, the micrococcus catarrhalis in one, and the staphylococcus pyogenes aureus or albus in three.

In the one hundred eighty-six inter-epidemic cases, one group of bacteria was found in practically pure culture in sixty-six (thirty-five per cent). Of this number, the influenza bacillus was demonstrated in forty-seven, the pneumococcus in eight, the micrococcus catarrhalis in five, while few or isolated examples of practically pure infection with the pyogenic cocci, the streptococcus mucosus capsulatus, and the bacillus mucosus capsulatus were also noted.

A comparison of the number of cases showing influenza bacilli in the two series may be emphasized in the following table:

	No. of Cases.	Influenza Bacilli.	
		Practically Pure Culture.	Mixed with other Organisms.
Inter-epidemic period, 1902–04	186	47 (25%)	110 (59%)
Epidemic period, 1907–08	20	3 (15%)	7 (35%)

Investigation of excised and other tissue. — In the hope of finding some single and constant factor acting to incite the growth of such bacteria small pieces of tissue were excised from the inflamed pharyngeal wall or the tonsil in six cases. These were hardened in Zenker's solution, in formalin or in a saturated, aqueous solution of mercuric bichloride, in normal salt solution. They were stained by the ordinary hematoxylin eosin stain, by Wright's modification of Leischmann's stain, applied to sections, and also with silver nitrate and pyrogallic acid, according to Levaditi's method. Aside from degenerative products, probably due to their removal under cocain, nothing abnormal could be found.

Smears of blood and serum from the pharynx or tonsils of patients with the disease were likewise negative, as was also a search of smears from the fresh exudate, stained with Wright's stain.

Cause of the epidemic. — In view of the importance with which we have been led to regard the relation of the influenza bacillus to pandemic influenza, it is especially significant that while twenty-five per cent of unselected cases with cough and expectoration, during an inter-epidemic period, showed this organism in overwhelming numbers, it was similarly present in only fifteen per cent of cases with the clinical features of influenza from this epidemic. The influenza bacillus must, therefore, be excluded as a principal cause. It appears to bear the same relation as other common respiratory organisms both to the epidemic and inter-epidemic cases.

Etiological unity appears to be lacking in the epidemic cases, not only as regards the influenza bacillus, but also for other organisms, since no one group of bacteria could be demonstrated as a constant invader in the specimens. The investigation of tissue excised from the involved regions likewise failed to disclose any principal cause to which the bacteria could be related as secondary invaders. Furthermore, no striking differences could be demonstrated in the

various kinds of bacteria or in their proportions in the epidemic and inter-epidemic cases. In both series, the same organisms were present and in nearly like proportions.

In the absence of other discoverable causes, we are forced to the conclusion that such various organisms as the influenza bacillus, the pneumococcus, the micrococcus catarrhalis, the pyogenic cocci, and others singly or combined, are responsible not only for the inter-epidemic but also for these epidemic cases with the clinical picture of influenza.

[I am indebted to Dr. A. Coolidge, Jr., for pieces of tissue from the pharynx and tonsil; to Dr. A. H. Crosbie for his constant interest and assistance in obtaining cases for study; and to Dr. E. P. Joslin for placing material from one of his cases at my disposal.]

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THE MICROSCOPIC ANATOMY OF TRICHINELLA SPIRALIS.*

CHANNING FROTHINGHAM, JR., M.D., BOSTON.

(*From the laboratory of the Department of the Theory and Practice of Physic, Harvard University.*)

The gross anatomy of Trichinella spiralis in its different stages has been frequently described. Among the later authors are Neveu-Lemaire,[1] MacQueen,[2] and Braun.[3] The object of this paper is to describe the microscopic anatomy of this parasite. The parasites were studied at two ages, one three days after the ingestion of the infected meat, the other about ten days. By three days both male and females are developed. At ten days the male remains the same, except for possibly a slight increase in size. The female at this time shows the changes incident to impregnation, and also has increased in size.

The trichinella studied were in the intestines of rats which had been fed muscle from an infected cat. The tissue was fixed in Zenker's fluid, mounted in paraffin, cut in serial section and stained with eosin and methylene blue. Mallory's connective tissue stain and the phosphotungstic acid hematoxylin stain were also tried, but did not show anything that was not brought out by the other stain.

The accompanying figure shows diagrammatically the gross anatomy of the male and female trichinella. In it no attempt has been made to keep the proportions of the length and breadth of the parasite, but an attempt has been made to represent correctly the relative amount of space occupied by the different internal parts of the parasites. The generally accepted sizes for the female are three to four millimeters long and sixty microns thick; for the male 1.4 to 1.6 millimeters long and forty microns broad.

In order to avoid repetition, I shall describe once and for all a certain type of cell which is common to several parts of the parasite. This type of cell in the rest of the paper

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will be referred to as "special cell." The cytoplasm of this cell has no distinct limits, but fuses with that of the neighboring ones into a homogeneous mass without granules. With the low power the nuclei are not or only barely visible. With high powers nuclei are seen scattered irregularly throughout the cytoplasm. They are represented as round or oval clear zones two to three microns in diameter. They have no nuclear membrane or reticulum. Within the nucleus usually near the center is a small round solid mass two-thirds to one micron in diameter, presumably a nucleolus. These cells usually take the eosin stain in the cytoplasm and nucleolus, while the nucleus is unstained. In some places they take on a basic tinge.

The male trichinella fully developed has an integument which covers it entirely, except for a break at the anterior end for the mouth and at the posterior end for the opening of the cloaca. With low power the integument appears as an eosin-staining dense capsule, two microns thick. It is sharply defined on the outer side. Within it is less dense and the edge is less sharply defined. In some places this is seen to be composed of the "special cells." In other places no cellular elements are visible, even with high magnification. The appendages at the posterior end are similar in structure. Beginning near the head on the inside of the integument are two clumps of these "special cells" (see Fig. 1) which extend in towards the body cavity of the parasite. They are situated opposite to each other and measure eight by ten microns. They take the eosin stain as a rule, sometimes a slight basic tinge. In this same relation they run posteriorly to a short distance beyond the posterior limits of the anterior cells, gradually decreasing in size until they disappear.

The intestinal tract is composed of a tube which is chiefly round or oval. It varies in size, and in the thickness of its walls in different places. Starting at the anterior end it runs in the middle of the parasite. Passing the anterior cells it lies near the periphery, in a groove (see Fig. 1) formed by these cells. Posteriorly to the anterior cells it is crowded to

the periphery by the genital apparatus, and finally ends in the cloaca. Anterior to the anterior cells the intestinal tract is seven microns in diameter and the walls three microns thick. (In all the measurements of diameters the walls are included.) In some places the "special cells" can be seen in the walls of the intestinal tract, in other places only eosin-staining columns. Passing the anterior cells the intestinal tract is four microns in diameter, and takes the eosin stain. No distinct lumen or cellular structure can be made out at this level. Posterior to the anterior cells the intestinal tube enlarges (see Fig. 3). It is twelve microns in diameter and the walls are three microns thick. The walls are composed of these special cells which here take a basic tinge. On the lumen side of the wall is a thin layer of homogeneous substance taking a faint purplish stain. Whether this is intestinal contents or is part of cells or their secretion could not be decided. From here on to the cloaca the tube gradually becomes more narrow, averaging six microns in diameter with walls two microns thick. The walls are composed of these special cells with an eosin stain.

The cloaca is a slight enlargement of the tube, which is lined also with these special cells. It opens posteriorly between the two appendages.

The anterior cells start a short distance from the mouth and extend back about one-half the length of the parasite. At first they do not fill up the body cavity, but soon do (see Figs. 1 and 2). They vary in size from thirty-two to twenty-two microns in transverse diameter and six to four microns in longitudinal diameter. They have a fairly distinct cell membrane, a cytoplasm that is faintly reticulated and contains fine granules. Eccentrically placed, they have a clear oval nucleus six microns in longest diameter, with a suggestion of a membrane. Near the center of the nucleus is a solid small round nucleolus two microns in diameter. The cytoplasm alternates in stain between red or blue, the granules all take the basic stain, the nucleus is colorless, the nucleolus always takes the basic stain. Along one edge and opposite to one of the cell masses of the integument

already referred to the intestinal tube makes a groove in these cells. In the body space between the anterior cells and the integument anterior to where the cells fill up the entire body cavity are crowded masses of small clear rings three microns in diameter which have a faint eosin-staining circumference.

The genital tract in the male consists of a tube which starts with a blunt end near the tail of the parasite and then runs forward to the anterior cells, where it turns and runs posteriorly until it empties into the cloaca. With the intestinal tract the genital apparatus practically fills up the body of the parasite (see Fig. 4). Its diameter in the first part is twenty-eight microns. At the turn it is somewhat less and soon after the turn it narrows into a tube of eight microns in diameter whose walls are two microns thick. The external coat of the tube at the start is represented by a thin line. Later, as it becomes a narrower tube, the walls are composed of these "special cells." The tube from the start, including the bend and for the first part of the return, is lined upon the inside by masses of cells. These cells have no distinct outline, but their protoplasm presents a purplish staining homogeneous mass, which in places appears to be finely granulated. Scattered through this are clear oval nuclei without a nuclear membrane which measure six microns in their longest diameter. They take a pink color. Within the clear nucleus is a small round mass one micron in diameter which stains purple at the periphery of the tube, but also takes a bright eosin stain as one approaches the lumen. The lumen of the tube has many of these nuclei with their nucleoli in them. Scattered through these masses of cells is an occasional round body three microns in diameter, which takes a basic stain, and which is homogeneous, but varies somewhat in parts in intensity of the stain. A short distance behind the bend all these cellular elements disappear and the tube leading to the cloaca is seen to be filled with these clear nuclei with bright red nucleoli. These latter are the spermatozoa.

The body cavity, not occupied by the genital and intestinal

tracts, shows no special cellular structure. Occasionally a few fine eosin-staining fibers are seen or a few of the small clear rings mentioned above in the description of the anterior end of the parasite.

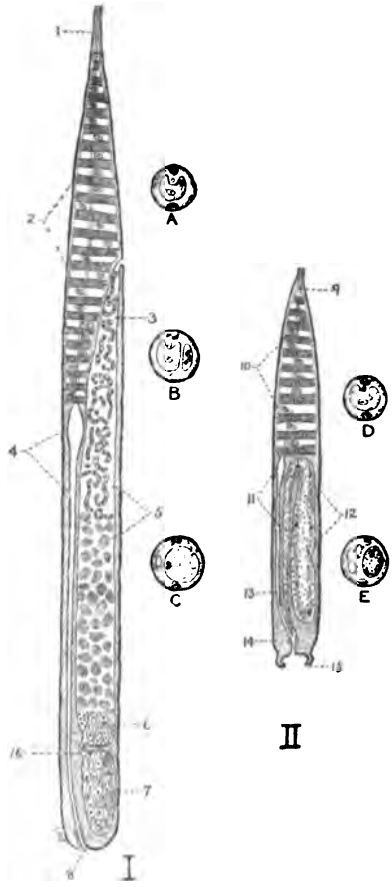
The female trichinella when ten days old has an integument similar to the male parasite, except for an extra opening for the vagina. The intestinal tract is also similar except that it opens at the posterior end of the parasite, directly through the anus, which, like the rest of the canal, is lined by these special cells. The anterior cells vary in the female only in the size and shape of their posterior ones, which are crowded or replaced in part by the vagina.

The genital tract in the female consists of a tube which starts blindly near the tail and runs up to the anterior one-fifth of the body, where it ends in the vaginal opening. At one point shortly after its start there is a constriction of the tube, and beginning with the posterior end of the anterior cells it gradually diminishes in size. Otherwise it practically fills up the body of the parasite. The walls of this tube are composed in chief of a fine eosin-staining line. In the oviduct and vagina this thickens up and shows a few cells of the "special type." On the inside of the wall in the ovary for a part of its circumference cells seem to grow. Near the base they are small, but in the lumen of the canal they attain the size of eighteen by ten microns, and are roughly oval. These cells fill the ovary and oviduct (see Fig. 5). The cytoplasm of these cells takes a purple stain and is homogeneous. Scattered through it are granules some one to two microns in diameter, which take a darker purple stain. Near the center of this cytoplasm is an oval nucleus six to eight microns in diameter. It has a faint membrane, and is filled with a homogeneous eosin-staining mass of material. Near the center of this material is a deeply basic-staining round solid mass one to two microns in diameter. At the posterior end of the uterus (see Fig. 5) in impregnated parasites are a great number of bodies similar to the spermatozoa described in the male trichinella. Scattered through these are a few ova. Passing anteriorly from

here the ova undergo progressive changes, until the embryos are fully formed. The first changes in the ova are that the outline becomes less distinct, the nucleus less distinct, and the nucleolus has gone. Then the purplish granules disappear from the cytoplasm and the cytoplasm becomes filled with pale pinkish small round bodies, which are without membrane, markings, or nucleoli. Next the ova assumes irregular shape, increases in size, and these pale bodies disappear. A few deeply basic-staining granules now appear in these ova. Then these become more and more shaped like an embryo, until finally the fully developed embryo is present, lying coiled up within the tube (see Fig. 3). The embryos are one hundred and fourteen microns long, and five microns broad. They have a distinct blue-staining periphery. The body takes a light pink stain, and scattered over it are small basic-staining granules, which, upon high magnification, resolve themselves into circles. Usually the vagina and considerable of the uterus posterior to the anterior cells is crowded with embryos (see Fig. 3).

Some of the female trichinellæ only three days old have not become impregnated. In them there are no spermatozoa in the uterus, but both uterus and vagina as well as the ovary are crowded with ova. In others that have just become impregnated the spermatozoa are present, but no developed embryos. In these cases the ova filling the uterus and vagina show different stages in the development of the embryos.

In concluding, let me repeat that this description is limited to the histology of the adult male and female Trichinella. Although Virchow,[4] Chatin,[5] and Graham[6] all mention the cellular features of certain parts of these parasites, their complete microscopic anatomy has not been described before. It still remains for the histology of the encysted trichinella parasites to be described.



I. Female adult Trichinella.
II. Male adult Trichinella.

1 and 9. Head end showing beginning of intestinal tract.
2 and 10. Anterior cells.
3. Vagina filled with embryos.
4 and 11. Intestinal tract.
5. Uterus filled with embryos and developing eggs.
6. Spermatozoa in uterus.
7. Ovary.
8. Anus.
12. Testicle.
13. Vas deferens.
14. Cloaca.
15. Appendages.
16. Oviduct.

A. Cross section through female.
Anterior cells with intestinal tract in groove.
Masses of cells on inside of integument.
B. Cross section through female.
Same as A, with addition of vagina and embryo.
C. Cross section through female.
Uterus filled with developing eggs and intestinal tract.
D. Cross section through male.
Same as A.
E. Cross section through male.
Testicle. Vas deferens, intestinal tract.

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DESCRIPTION OF PLATE XI.

FIG. 1. — Cross section of anterior end of male trichinella. Integument with cell masses. Anterior cell not filling body cavity. Intestinal tract in groove of anterior cell. x 1,000.

FIG 2. — Longitudinal section of female parasite. Anterior cells. Integument. x 750.

FIG. 3. — Just posterior to anterior cells. Longitudinal section of female. Embryos in uterus and beginning of vagina. Posterior end of anterior cells. Intestinal tract. Nucleolus of special cell seen in wall of intestinal tract. x 1,000.

FIG. 4. — Longitudinal section of male. Testicle showing spermatozoa in lumen. Integument. x 1,000.

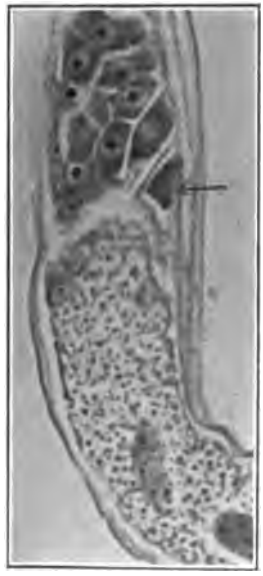
FIG. 5. — Female longitudinal section. Ova in ovary. Spermatozoa in posterior end of uterus. Arrow points to ovum in oviduct. Integument. Intestinal tract crowded to periphery. x 1,000.

(I am indebted to Mr. L. S. Brown for the photomicrographs.)

1.

2.

3.



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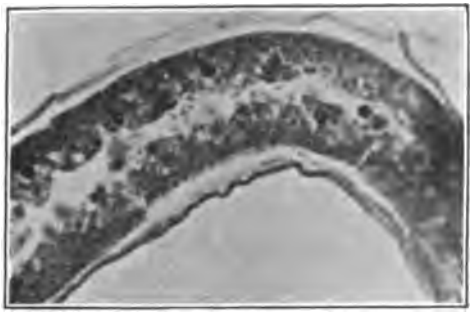

4.

THE PROTEOLYTIC ENZYME OF HUMAN MILK.*

A. E. AUSTIN.

(*From the Medical Chemistry Laboratory of Tufts College.*)

Since 1900, when Babcock and Russell[1] first claimed to have discovered its presence, a proteolytic enzyme in cows' milk has been generally accepted. The proof of this rests upon the labors of Spolverini,[2] who did not attempt to differentiate it from trypsin, upon those of E. Moro,[3] who employed the method of Mette, using coagulated tubes of egg albumen, as well as that of Bidder and Schmidt[4] upon well dried and weighed fibrin flakes; these last when placed in two hundred fifty cubic centimeters cows' milk weakly acidified with hydrochloric acid lost .0009 gram, while the same amount of human milk caused a loss of .0006 gram (pepsin). In weak alkaline solution two hundred fifty cubic centimeters cows' milk dissolved .0019 gram, and the same amount of human milk .0013 gram. Furthermore, Friedjung and Hecht[5] by using the method of Fermi,[6] when the digestion was protected by thymol and had a strictly amphoteric reaction, determined that there was a very slight proteolytic action, and since the reaction remained amphoteric the enzyme must be distinct from either pepsin or trypsin.

According to Neumann Wender,[7] the enzyme which he calls galactose is not a single but a group of ferments possessing proteolytic properties upon casein.

Vandervelde, de Waele, and Sugg[8] also found an enzyme in milk which acted upon both the albumen and the casein, but notwithstanding the possible action of bacteria which they prevented as far as possible by peroxide of hydrogen, which was found to have no action upon enzymes, and the slight amount of the ferment, their results seem to have been the first to be fully established by careful experimental safeguards. The only objection to their mode of procedure is that peroxide alone when added to cooked milk is able to

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digest fourteen per cent of the albumen in fifteen days. Still deducting this they found that twenty-four to fifty-five per cent of the protein would be digested by an inherent protease.

Freudenreich [9] has also found that a protease exists in cows' milk, but says that it only dissolves the casein without carrying the process farther, as is shown by the slight increase of the amid nitrogen.

From this brief summary of the work accomplished on this subject it will be seen that outside of the results of E. Moro but little attention has been paid to human milk, and it seems to me that his deductions are based upon such minute differences that they are not beyond the possible limits of error. Then, in connection with another matter, this possible presence of a proteolytic enzyme is of great importance, the so-called rest nitrogen, that is, that portion represented by the difference between the total nitrogen in human milk and the protein nitrogen. This has been variously regarded as due to urea or amino acids, or possibly both. Hans Rietschel [10] has found that this amounts to fifteen to twenty per cent of the total nitrogen. That it cannot be in the form of ammonia to any great extent he has shown by making estimation of this substance and finds that not over two per cent of the rest nitrogen is in the form of ammonia. A small portion of the former can be removed by shaking with naphtylisocyanate, indicating that it is the form of amino-acids, of what nature he was unable to determine. Even the presence of this small portion must indicate one of two things — either that they are remnants of other and larger portions used synthetically to form the proteins of milk or they are the products of proteolysis of the proteins of milk and indicate the presence of an enzyme. Again, the mere determination of the constituents of milk has never been able to show us why a breast milk often disagrees with an otherwise healthy infant. Is it due to the presence or absence of an enzyme, to the increase or diminution of the rest nitrogen? In our endeavor to settle this question I have examined the milk of twenty-one women at different periods of lactation,

which I can group as nutritive and non-nutritive in that the nursing infant was badly nourished or had persistent diarrhea, with reference to the presence or absence of an enzyme and with reference to the amount of total and rest nitrogen, and any change in these relations occurring after digestion.

At first the method of Volhard was employed, which is fully described in its adaptation for trypsin determination by Walter Löhlein.[11] By this method, however, the addition of five cubic centimeters cooked and uncooked human milk in three experiments with three different milks to the stock solution after periods of fourteen, fifteen, and sixteen days' digestion in the brood oven at 37.5° C. produced no difference in the solutions, and there was no difference between these and an equal amount of stock solution without milk placed in the oven for the same time, which was used as a control; hence we are forced to conclude that the milk possesses no enzyme or that it is too weak to produce any change in the Volhard mixture or that the environment (alkaline reaction) is not favorable for its activity. In all these cases the mother and child were perfectly well and the child well nourished. For the next attempt a modification of Rietschel's procedure for the determination of rest nitrogen was employed. Three portions of human milk of five grams each were weighed out; one portion was destroyed by the Kjeldahl method and its nitrogen determined; to each of the other portions ten cubic centimeters distilled water were added, one cooked thirty minutes in a boiling water bath, cooled, toluol added to each in excess so that a layer stood over the fluid after thorough shaking, and both placed in a thermostat at a temperature of 38.5° C. for forty-eight hours. Then they were removed, twice as much phosphotungstic acid added and after standing a few hours filtered with a suction pump. The precipitate was then placed in a Kjeldahl flask and its nitrogen determined as before. By deduction of the protein nitrogen from the total nitrogen and comparison of the cooked and uncooked milk, an opportunity was given to determine whether any digestion had taken place or at least whether the digestion if

present had carried the destruction of the protein molecule beyond the peptone stage. All specimens were but a few hours old and were always tested for their reaction before beginning to manipulate; they were always found amphoteric or slightly alkaline.

No.	Total Nitrogen (Grams).	Rest Nitrogen Uncooked (Grams).	Per Cent.	Rest Nitrogen Cooked (Grams).	Per Cent.	Remarks.
First group, mother and child well.						
1. ...	.0102	.0016	15.6	.0013	12.7	Lactation five weeks.
2.....	.0081	.0013	16.2	.0007	8.7	Lactation five weeks.
3.....	.0081	.....	.....	.....	.....	Lactation two weeks.
4.....	.0081	.0016	19.7	.0016	19.7	
Second group, mother ill.						
5.....	.0081	.0009	11.1	.0003	3.7	Left breast, temp. 104.
6....	.0071	.0008	11.2	.0013	18.3	Right breast.
7.....	.0128	.0003	2.3	.0022	17.1	Aborted six months, loss of lactation.
8.....	.0114	.0008	7.0	.0016	14.0	Septic.
9.....	.0196	.0021	10.7	.0044	22.7	Eclampsia, delivery at six months. First milk.
10....	.013	.0058	44.6	.0073	56.1	
Third group, mother well, child badly nourished.						
11....	.0051	.0014	27.4	.0011	21.5	
12....	.0075	.0014	18.6	.0015	20	
13....	.0122	.0003	2.4	.0027	22.1	
14[1]..	.0102	.0022	21.5	.001	9.8	Fourth week, lactation.
15....	.0105	.0019	18.0	.0027	25.7	
16....	.0115	.0025	21.7	.0017	14.7	

[1]The casein removed by the Schlossman method with alum gave .00225 gram nitrogen, leaving .00595 gram distributed between lactglobulin and lactalbumen.

Any ferment existing in milk is probably of the autolytic variety, which from analogy is most active in a neutral or amphoteric reacting mixture, hence the natural reaction of

the milk ought to be most satisfactory for its activity. Again, if this enzyme varies in different breast milks, it ought to be found in the largest amounts and hence most effective in mothers whose health is perfect, and whose children are thriving; hence the first group may be taken for a standard or normal. We find in our list that in two instances there are some evidences of slight digestion, since the uncooked milk after staying in the brood oven has more rest nitrogen than the cooked milk; in one, the two are equal, while in another there is none whatever. Before drawing any conclusions, however, we must consider the effect of thirty minutes' cooking on a water bath. This may either denaturize a portion of the protein or it may break up some of the bodies containing the rest nitrogen and drive it off in the shape of ammonia either from amino-acids or from urea, both of which are suspected in milk, but whose presence has not yet been proven (Rietschel).

The effect of heat (98° C.) upon the acids I find nowhere stated, so that it is reasonable to suppose that they will withstand this degree of temperature. It is well known that cooking will denaturize the proteins, but I find no evidence that unaided by alkali or acid this change passes beyond the stage of albumoses or peptones, and, as these are both precipitated by phosphotungstic acid, this can have no effect upon the rest nitrogen. With urea, however, the case is different; Weyl[12] states that by cooking urea in water, under pressure, ammonia can be split off, but it is quite improbable that thirty minutes' cooking on a water bath could split any appreciable amount of urea. Hence we may conclude that the cooking to which the milk was subjected could do no more than drive off the ammonia, which does not form more than two per cent of the total rest nitrogen.

That some change takes place by heating is evident from the fact that phosphotungstic acid does not so thoroughly precipitate the milk proteins, and it is so difficult to obtain a clear filtrate that filtering with the suction pump has to be done with great care. Another great difficulty in separating the proteins from the rest nitrogen is

due to the mechanical adherence of the latter to the precipitate; while this was always thoroughly washed with the same phosphotungstic acid there was no assurance that the precipitate was free and it is possible that this separation would be much more complete if the precipitate were rubbed up with the reagent and again filtered. If a proteolytic enzyme exists in milk, it is quite unexplainable why the amount so varies; if we may rely on these results taking the rest nitrogen of the cooked portion as that preëxisting in the milk, we have a digestion equivalent to 7.5 per cent of the total nitrogen in one case, and 2.9 per cent in another, while in the third no enzyme is discernible. The period of lactation can have nothing to do with it in the first two instances, as it is the same.

In the second group we have the converse true, that is, the cooked portion has the greater rest nitrogen; this occurs so frequently (in all but one instance) that it cannot be a coincidence. How then are we to explain it? Since no enzyme can resist the action of cooking, it must be the heat alone which produces this change, and as it was not produced in the former group, we must have an increase of some substance here which cannot resist the influence of heat and, becoming split off, is not precipitable by phosphotungstic acid. The two substances to be thought of are of course urea and amino acids. Do these ever unite with proteins or ammonia by which they are precipitated by the reagent, and are they separated by heat? Urea is not precipitated by this reagent in solutions containing less than three to four per cent; allowing that eighty per cent of the rest nitrogen is in the form of urea, we have approximately fourteen per cent of the total nitrogen in this form. This, based upon an average of .12 gram for five grams milk, would give .07 per cent urea for the undiluted milk, and of course much less for the diluted, so that the precipitation of urea by this means is out of the question. Mörner and Sjöquist [13] have shown that phosphotungstic acid may throw down urea in urine, but here we are dealing usually with concentrations of two per cent.

The first possibility is, of course, that urea is combined with sodium chloride, a combination which is known to be very unstable, decomposing by the mere recrystallization from water. Whether the behavior of this compound to phosphotungstic acid differs from that of urea alone could only be learned by actual test, so that equal molecular portions of urea and sodium chloride were dissolved in the smallest possible amount of water, evaporated to a syrup and allowed to crystallize in a desiccator. To a solution of each containing one part to a hundred of water twice as much phosphotungstic acid solution, acidified with hydrochloric acid, was added. No precipitate occurred nor did one form when the original solution had been cooked thirty minutes before the reagent was added. Such an explanation, therefore, cannot be maintained, and we are left in doubt as to the cause of this phenomenon.

The second possibility is that urea may combine with lactose. The union of the former with dextrose is a well-known compound, and from analogy at least we may have a similar union with milk sugar; in fact it is stated in Thierfelder's Hoppe-Seyler that urea pairs with all reducing sugars except fructose, but does not specifically state whether double sugars are included. It is also stated that the glucose ureid is decomposed by heating with weak sulphuric acid, but does not state the effect of heat and water alone. Hence a similar equimolecular solution of lactose and urea in water was prepared to which a few drops of sulphuric acid were added and the whole evaporated to a syrup on the water bath and allowed to crystallize in a desiccator. A one per cent solution was made of this and to five cubic centimeters twice the volume of water was added and then thirty cubic centimeters phosphotungstic acid solution was added. No precipitate was produced, nor was one obtained after the solution of lactose-urea was cooked thirty minutes upon the water bath. Hence this possibility is untenable.

Next we have the possibility that the amino acids which are supposed to be present in milk may be combined with ammonia; such combinations are readily precipitated by

phosphotungstic acid as shown by Bergell and Feigl;[14] the objection to this supposition is the difficulty with which such amino bodies are split; the above authors found it necessary to use alkalis and heat, by means of which they are able to split off only about two-thirds of the nitrogen in the form of ammonia. Hence it seems very improbable that heat at 98° C. would be liable to accomplish this feat. Were it so, however, we have an analogous condition, for while diglycinimid, for instance, as stated above is precipitated by phosphotungstic acid, glycocol is not.

In the third group nothing distinctive was found; three times the rest nitrogen was found greater in the digested portion and three times less. There certainly was no evidence that any deeply destructive proteolysis had taken place nor did the heating produce the results which it did in the second group. It still remains in doubt whether any substance appears in the second group which does not in the third and second. Furthermore, these results offer no explanation of the fact that the milk did not nourish the child. At least there is not the slightest evidence that the presence or absence of a proteolytic ferment has anything to do with this feature. The variations in the amount of rest nitrogen extend to much wider limits than Rietschel's, though leaving out a few that are palpably the result of error, the limits are practically those established by him.

Before the subject here treated can be fully settled we must know more of the character of this rest nitrogen, and the most attractive feature is the possibility of the presence of amino acids. These may be either the products of the decomposition of protein material or, what more readily suggests itself, that they are primary and from them the breast builds its various kinds of protein found in milk. This appears still more probable from the labors of Abderhalden and others to show that practically all protein in the body is the synthetic product of these acids. While those found in the fluids (blood and urine) of the body are catabolic products, yet from the fact that nitrogen equilibrium can be maintained, in fact, nitrogen can be increased by

feeding the cleavage products of protein, it is not improbable that milk protein is produced from the same substances and those present simply a remnant remaining uncombined. If this hypothesis be true, we have an example of this synthesis such as has been sought in vain elsewhere.

In order to verify the presence of amino acid a combination of the methods of Ackermann[15] and P. A. Levene[16] was employed. Six hundred cubic centimeters of fresh breast milk obtained at three intervals from the same individual were immediately precipitated by tannic and phosphoric acid, and after filtration, the filtrates united, the tannic acid removed by barium hydrate solution, the barium by sulphuric acid and the latter by plumbic oxide. The lead was then removed by sulphuretted hydrogen, the filtrate concentrated to a liter and, while hot, picric acid added to saturation. Upon cooling a copious crystalline yellow precipitate was thrown down. This was removed by suction filter, lightly washed with ice water, dissolved in distilled water and after the addition of sulphuric acid was shaken with ether as long as any picric acid could be removed. The solution was then treated with barium carbonate in excess, filtered and concentrated; a slight precipitate was thrown down which proved to be barium; after its removal the solution was cooked with an excess of copper oxide, filtered and the filtrate allowed to cool. This should have contained glycocol were it present, but after long standing not the slightest suspicion of a crystal could be found. Hence we may say that no free glycocol is present; as to the possibility of the presence of a polypeptide containing glycocol, nothing can be said.

Two more attempts, one with one hundred twenty cubic centimeters, and the other with two hundred seventy cubic centimeters human milk were equally fruitless. Furthermore, on the same evidence alamin also showed itself absent, since, if present, it is also found in the picric acid precipitate. The picric acid was removed from the filtrate by ether and sulphuric acid and the latter by calcium carbonate with heat.

After filtration, it was concentrated and attempts made to obtain crystals in a desiccator. After three weeks no crystallization was found which resembled leucin, which should be found at this stage of this process. As none of the more common amino acids were found it did not seem worth while to carry the process farther, but I hope to do so with a larger portion of milk at an early date. We can summarize very briefly the results of this effort in the following:

(1.) There is no evidence of auto-digestion of human milk, at least under the conditions pertaining to such digestions in organ tissues.

(2.) The digestive disturbances of infants fed on human milk can have no relation to such an enzyme, as the milk of both healthy and sick women was examined.

(3.) The rest nitrogen is still a riddle, though if amino acids compose a part of it, they are remnants of a synthesis to protein, and not products of digestion of milk protein.

(4.) Urea when present is probably not free, but combined with some of the other ingredients of milk; but with which all my efforts so far fail to solve.

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EXPERIMENTAL AND CLINICAL STUDIES ON THE CURATIVE ACTION OF LEUCOCYTE EXTRACTS IN INFECTIONS.

A Series of Papers by

PHILIP HANSON HISS, JR., M.D.

(*Professor of Bacteriology in the College of Physicians and Surgeons, Columbia University, New York.*)

AND

HANS ZINSSER, M.D.

(*Instructor in Bacteriology in the College of Physicians and Surgeons, Columbia University, New York.*)

ANALYSIS.

I.

THE CURATIVE INFLUENCE OF EXTRACTS OF LEUCOCYTES UPON INFECTIONS IN ANIMALS.*

PHILIP HANSON HISS, JR., M.D.

I. THEORIES AND FACTS OF INFECTION AND IMMUNITY.

Before passing to the experimental part of our work, it may be well to consider certain matters of fact and theory relating to the subject of immunity. It would be a task, however, entirely apart from the requirements of this paper, to give even a fragmentary review of the literature of immunity and to attempt to sift the observations, experiments, data, and conclusions which have been educed in the study of the processes of protection against invading microörganisms, and of the establishment of cure and of post-infection immunity.

To those familiar with these questions, it may be taken for granted, certain fundamental conceptions and facts stand prominently forth—That, on the one hand, the theory of phagocytosis has been strengthened by the test of time, and the recent work on opsonins has but added a stimulus to further observations and experiments explanatory of the stimulation and mechanism of this well-known phenomenon. On the other hand, that the immense amount of work in elucidation of the part played by the fluids of the body has furnished an invaluable set of data in explanation of the method of production and action of antitoxins and the so-called immune bodies of the serum and plasma.

In spite, however, of the mass of data thus collected in the study of the processes involved in meeting infection and the establishment of cure and immunity, actual advances in serumtherapy and biological methods of treatment during this period have not kept pace with theory; and the successful production of antisera for diphtheria and tetanus and a few other diseases in which strong diffusible toxins play the major part stands out in bold relief against the practically

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universal failure when the same methods are attempted in the production of sera for the treatment of other germ diseases, notably those whose symptoms are not so definitely referable to the action of soluble toxins.

It is to these diseases, in which the bacterial poisons are supposed to be principally endotoxins, that our attention is chiefly directed in this paper.

Attempts to produce curative sera by the repeated inoculation of animals of various species with the inciting micro-organisms of these endotoxin diseases, as well as the injection of various strains of a given microörganism into the same animal to produce the so-called polyvalent sera, have thus far failed to lead to satisfying results. No more have the extracts of these microörganisms, obtained by pressure, freezing, and breaking up, or by osmosis or autolysis, when used for immunization instead of the intact organism, given results which have so far led to the practical use of such sera in the treatment of disease in man.

These sera have not apparently been antitoxic in the usually accepted sense and depend, so far as theory goes, for any curative value they may be supposed to possess upon their germicidal and bacteriolytic power and possibly, in the light of the opsonin theory, upon the opsonins they may carry and thus stimulate phagocytosis. That these sera are capable of protecting an animal from a many times lethal dose of an infecting organism, when mixed with it in surprisingly minute quantities is, of course, known to all, but that any consistent curative effects, other than merely local, have been definitely established as due to such germicidal action, after an infection has once been established, may justly be questioned. Something must, therefore, be lacking in these sera, either a toxin neutralizing body, or, as has been suggested, a complementary body necessary for the activation of the bactericidal or bacteriolytic immune bodies. In experiments in vitro, complements may, as is well known, be furnished by fresh normal sera of various kinds, but in the body of infected animals and man must be furnished, if at all, by the plasma or cells.

Even if experiments had demonstrated conclusively that bodies existing in drawn sera were present in the same condition and amounts in the blood plasma — a condition which we have no right definitely to postulate — we have reason to believe that the complement, at least so far as it is free in the plasma, is not present in sufficient amount to render efficient the intermediate body; and, so far as our knowledge of complement in sera is concerned, it is not, as is the immune body, increased during disease or in response to inoculations. Certain incomplete immune bodies, therefore, even if they are produced in excess and exist free in the plasma, are, in the absence of activating bodies, powerless against the invading or infecting organism. We have no convincing evidence, then, that the complementing body or cytase is normally at liberty in the plasma, and likewise we have no evidence that the opsonins are free in the circulating blood. Metchnikoff, in fact, claims that the cytases — micro and macro — are not normally free in the plasma, but are contained in the leucocytes and only given up by them under stress of death or injury, such as may occur from unusual osmotic conditions or in the formation of serum, and that, if the immune sera are directly destructive of organisms in the body they are so only by virtue of their combination with the products of injured or destroyed leucocytes.

However this may actually be, test and experiment have shown that animals and man suffering from a true infection running to a fatal termination may and often do furnish sera capable of strong bactericidal and bacteriolytic action (when combined with normal sera containing complement) if the disease has run a sufficiently long course, and yet, in spite of this fact, they succumb.

In the light of these and other facts which might be cited it has long seemed to me that one must give pause in attempts to produce beneficial effects by injecting still further amounts of bacteriolytic or similar bodies, and must seek further for an explanation of the exact methods and processes of the cure effected in those animals and man who do

survive an infection. Failure to solve these problems on lines hitherto followed should not discourage us, however, while we know that the mechanism of the animal body suffices to protect the animal even against enormous doses of injected organisms, without serious histological changes or marked systemic symptoms, if these organisms be given at proper intervals and in gradually increasing amounts. The conclusion that this power must reside in increased digestive and neutralizing or poison-destroying powers of the animal organisms cannot well be avoided, and these functions of the animal mechanism will probably be found to reside in some group of cells which are not only able to take up and digest the introduced or infecting microörganisms but are also able to neutralize the poisonous products resulting from the metabolism or destruction of the microörganisms and to thus protect the more sensitive and specialized cells from the action of such poisons.

Bearing directly on this point the brilliant researches of Metchnikoff have focussed attention upon phagocytosis and the actions of the various cells taking part in the scavenging and removal of foreign bodies and organisms from the infected animal and man. These researches have followed faithfully the steps involved in infection and the gradual return of the body to the normal state; in other words, have disclosed the story of infection and cure as seen under the microscope and revealed by staining reactions, especially as these processes unfold themselves in localized or local infections such as those going on in the peritoneal or pleural cavities or under the skin. Conclusions of the utmost importance have been drawn from these phenomena as to the active participation and functions of the white cells in protection of the animal or human body against infection.

In addition to these controlled animal experiments we have knowledge of cellular activities in man gained from morphological investigation of inflammatory exudates and of infected tissues and organs, and also a knowledge, in many diseases, of the changes and fluctuations in the white cell content of

the blood as influenced by the stage and character of the disease, all of which factors point a guiding finger to the forces acting for the protection and relief of the infected animal or man.

It is our belief that if one studies intelligently in each disease the character of the exudates and cellular changes, no matter where found, and in the different stages of the disease, that these will give strong clues to the major cellular forces acting in defence of the invaded organism, and may thus lead to a logical course of biological treatment. In determining this course, however, one must recognize that mere morphological studies are simply indicative, and that imagination and interpretation of function, backed by experiment, must really supply the key to the invisible physiological forces at play, and give us access to those storehouses of energy and supply whereby the ailing and losing animal organism may be artificially reinforced.

Possibly the animal body ideally protected in the time of bacterial invasion is one in which some set of cells — phagocytes — are immediately ready and able to take up the bacterial invaders and destroy them, and within their own bodies to neutralize any poisons secreted by such invaders or arising from their destruction by digestion, and this without serious harm to the ingesting cells, or — failing this full immunity from serious harm — that these ingesting cells should in their turn be taken up and with their noxious contents be digested by other scavenging cells, with a minimum liberation of the substances which could injure the body cells dedicated to specialized functions. And this is apparently what does occur in the case of the diseases which we are considering, for in experimental peritoneal infections not severe enough to cause death, the bacteria are sooner or later ingested, usually by polynuclear leucocytes, and these when injured by their bacterial contents are, in their turn, ingested by the larger mononuclear cells, which thus aid in the process of digestion and absorption or fixing of the harmful agent.

OBSERVATIONS AND THEORETICAL CONSIDERATIONS LEADING DIRECTLY TO THE EXPERIMENTAL WORK UPON LEUCOCYTE EXTRACTS AND INFECTIONS.

An apparently valid observation made in my laboratory* during the early part of 1907, that there was a difference between the phagocytic power of corpuscles from certain persons suffering from infection and the phagocytic power of corpuscles from normal man, led me to conclude that it was not a matter of indifference, as assumed by A. E. Wright, what might be the source of corpuscles used in opsonic tests, and from this I formulated a set of experiments which I thought might demonstrate that corpuscles of an infected animal, especially one suffering from systemic infection following intravenous inoculations, passed through a gradual change independent of the so-called opsonic content of its serum — a change, first of depression in phagocytic function as the height of the infection was reached, and then, of gradual exaltation of functions as the animal regained normal and passed into the immune state.

Unfortunately, although such experiments were actually undertaken by Dr. North and myself, they were interrupted.

In thinking over this work I came to the conclusion that in many diseases we are probably dealing with an immunity a large part of whose mechanism is individually cellular, not only in the sense of phagocytosis and digestion, but in the neutralization of poisons given rise to by the disintegration of the bacteria — a mechanism in which the protecting cells *must* intervene and, unaided by bodies in the plasma, neutralize within themselves the poisonous products of the invading microörganisms.

It was this thought that gave rise to the further idea of aiding the leucocytes by furnishing them as directly as possible with the weapons which were being taken away from them in their fight with invading microörganisms, and to thus protect them from destruction and give them an opportunity to recuperate and carry on successfully their struggle against the invading germs. These weapons, whatever might

* Dr. Charles E. North — personal communication.

be their nature, I assumed might possibly be furnished by an extract of the active substances of the leucocytes themselves — substances not ordinarily given up to the plasma or serum — and I also assumed that extracts would be more efficacious than living leucocytes themselves, introduced into the infected animal, since being diffusible they would probably be distributed impartially to all parts of the body by the circulatory mechanism and, as quickly as absorption would permit, relieve the fatigued leucocytes and protect, by any toxin-neutralizing or other power they might possess, the cells of highly specialized functions.

This idea of immunity differs from one that simply assumes the cells as the source of all immune bodies — which logically seems to be the case — in that it takes into consideration the presence and production in the leucocytes of agents, which are not normally given up to the plasma, but which are constantly able to reproduce themselves and carry on the functions of digestion or neutralization simply for the benefit and protection of the individual cell, while not being secreted or excreted by the cells for the more general benefit of the cell community at large.

Thus we have a differentiation of immune agents into those which by virtue of their liberation and overproduction by the cells, such as the antitoxins, amboceptors, and agglutinins, etc., are free in the plasma and thus, when active, are immediately available for the protection of all the body cells; and into those agents by which certain cells primarily nourish and protect themselves, and are only of benefit to the cell community at large by virtue of the direct intervention of these cells between the invading germs and their products and the highly specialized cells requiring protection.

It seems, then, that when these sources of protection are overtaxed or fail to act efficiently on account of some inherent weakness or untoward circumstance of location, that the most reasonable course is, if possible, to support the chief army of attack as indicated by a study of the exudates and pathological changes in the disease in man and animals, and to endeavor to supply those products which are

most heavily taxed in the fight, in other words, to introduce into the infected animal or man the *substances* composing the chief cells or all the cells of an exudate in the most available and diffusible form, and as little changed by manipulation as possible. Such substances, if they become free from the cells by extraction, might serve to neutralize poisons in the blood, might alone or in combination with bodies already present in the blood act deleteriously on the bacteria, and thus protect and augment the activities of the flagging leucocytes by supplying them with their own weapons in the fight against the invading organisms. And further, the extracts of such exudates from previously immunized animals might even better serve this purpose, since their cells probably have in their own fight against the same organisms gained increased powers, as is evidenced by the ability of such immunized animals to safely dispose of immense numbers of organisms without serious harm or loss of weight. Also, as a further adjuvant, immune sera might be found serviceable in some cases, especially early in the disease and when non-immune cells are being used, although it is our belief that sufficient immune bodies (bactericidal or bacteriolytic) are often present, and in sufficient amount even early in the disease, if the animal economy has not been entirely overwhelmed by an enormous primary dose of the infecting organisms or their poisons.

The leucocytes seem to lend themselves more than any other body cells to these more generalized functions, and no further exposition of the reason for selecting them for such experimentation is necessary.

The complete scheme of experimentation mapped out was as follows:

To determine,

(*a.*) The effect of extracts of leucocytes of normal animals on infections.

(*b.*) The effect of extracts of leucocytes of immune animals on infections.

(*c.*) The effect of immune serum alone, and combined with (*a*) and (*b*), on infections. And

(*d.*) The effect of extracts of the blood forming organs — bone-marrow, spleen, and lymph nodes — and of mononuclear leucocytes of normal and immune animals, alone or combined with immune serum.

Extracts of spleen and lymph nodes, and of mononuclear leucocytes were included not only because they might be found to have a toxin neutralizing effect, but because Metchnikoff has stated that mononuclear leucocytes are more active in phagocyting certain organisms than the polynuclears, such for instance as the bacilli of tuberculosis and certain organisms giving rise to chronic infections.

It is obvious that such a scheme of experimentation is a broad one, and that some of its phases have been attacked from various sides by other writers, notably Petterson, to whose excellent papers the reader is referred.

Petterson, however, has been chiefly interested, apparently, in elucidating the direct destructive action of certain leucocyte extracts upon bacteria, and in bringing out differences between the serum alexines (complement) and the bactericidal and bacteriolytic bodies of the leucocytes, in other words, with the visible mechanism of immunity as shown in vitro and in the peritoneal cavity. Our work, on the other hand, has had as its immediate object the practical determination of the *curative* effects of such extracts, the best method of extracting, etc., and the most available animals to use for material not only as to supply and ease of handling but as to the character of the extract obtained. In this connection it was apparent, from the first, that not all species may serve this purpose, since distinct differences have been shown to obtain not only in bactericidal power of serum from different species of animals, but in their leucocytes, and it was only fair to suppose that the functions of leucocytes from different species, such for instance as the rabbit and dog, might differ,

so that conclusions drawn from experiment with one might not be applicable to the other.

And again it might well be that experiments successful in certain animal species might not succeed in others, for instance, rabbit extracts might protect rabbits, but not other animals, since the reactions given might not be simple toxin neutralizing ones, but might require certain definite complementing actions to take place before the desired result could be obtained, and any of these might only obtain in certain species combinations.

That these and other points require investigation before the full value of the work thus outlined may justly be determined even from its more empirical side, is evident to the writer, and the different phases of investigation are being taken up as rapidly as may be, and will be treated of in separate papers. In the present paper, however, only certain fundamental experiments will be brought out that they may serve as a demonstration of certain facts and as a basis for our further work.

EXPERIMENTAL WORK.

The investigations set forth in the present paper were undertaken to determine the influence, and especially the curative influence, if any, of extracts of leucocytes upon infections.

Practical work. — Animals used: The animals used for obtaining the leucocytes and for most of the experiments were rabbits, although dog leucocytes have also been experimented with, and guinea-pigs have served in some instances as test animals.

Preparation of extracts. — The leucocytes have been obtained for the most part by double pleural inoculations with aleuronat, and the amount obtained after twenty-four hours from rabbits has usually been from thirty to sixty cubic

centimeters of turbid, often blood-stained fluid. This has been quickly centrifugalized and the serum decanted. The cells then at times washed in normal NaCl solution, or directly subjected to the extracting fluid, which is added in amounts about equal to the fluid poured off.

It is evident that the extracts must vary in strength, as there is no means by which they can be exactly standardized, principally on account of the red blood cells which are often present in the exudates.

Although extractions were first attempted by rapid freezings and thawings in .85 per cent NaCl solution, this was abandoned, and in the work detailed in this paper the extracts were obtained by thoroughly emulsifying the cells in distilled water, and allowing them to stand for a few hours at 37.5° C., and then at ice-box temperature until used. In most instances not only the clear supernatant fluid has been injected into animals, but also the cell residue which may easily be emulsified by shaking. We have done this so that the animal would have the benefit of all the cell products, since our methods of extraction are as yet too crude for us to feel certain that all active substances are freed from the cell by them.

It is a fact worthy of note that rabbit leucocytes and those of the dog act differently in the presence of distilled water. Rabbit leucocytes are not markedly disrupted, morphologically, by their new environment of distilled water, and no matter how small or large the quantity of water added, there is no tendency to a gelatinization. Dog corpuscles, on the other hand, seem to go into solution in distilled water, for after emulsifying in small amounts of water and then adding up to ten to fifteen cubic centimeters to one cubic centimeter volume of cells and placing at 37.5° C., a gelatinous clot-like mass forms, which soon, however, seems to dissolve and leave only a comparatively slight residue. Rabbit cells do not show this phenomenon unless placed in alkali or in strong NaCl solutions, and then do not tend to go again into solution when water is added. This solution of dog polymorphonuclear leucocytes is extremely interesting, and

renders the preparation of extracts from dog exudates one of great ease. It however seems to indicate a physiological as well as physical difference from rabbit leucocytes. This question will be discussed later.

2. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON STAPHYLOCOCCUS INFECTIONS IN RABBITS.

Our first experiments were made with Staphylococcus pyogenes aureus. The culture was a very virulent one for rabbits, and the doses of leucocyte extract were small. Brief protocols are given.

Experiment I. — March 13, 1907. At noon two rabbits, weighing respectively one thousand two hundred and one thousand four hundred grams, were inoculated subcutaneously in the abdomen with one-half of a twenty-four-hour agar culture of staphylococcus Pr. II. The one-thousand two-hundred-gram animal died within eighteen hours. The one weighing one thousand four hundred grams still survived and was given small doses — 1.5 cubic centimeters of NaCl leucocyte extract obtained by freezing and thawing — at 3 P.M. and at 4 P.M. of March 14. The animal's temperature was falling at the time of injections and no reaction was noted. Animal probably moribund at time. Died within thirty-six hours.

The experiment was without value except in establishing the virulence of our culture, since treatment was commenced very late, and after the death of the control.

Experiment II. — March 15, 1907. 11.30 A.M. Two rabbits, a gray weighing one thousand five hundred grams, and a maltese weighing one thousand five hundred and fifty-five grams, received subcutaneously in the ear one-fifth of a twenty-four-hour culture of staphylococcus Pr. II.

The gray, one-thousand-five-hundred-gram rabbit, was left without treatment. Its temperature reached 105.9° F. by 6 P.M. On March 16 at 8 A.M. its temperature was 102° F. There was no local lesion of note and the animal died at 1 P.M.

The maltese rabbit reached a temperature of 105.4° F. at 4.20 P.M., when it was given two cubic centimeters of an aqueous extract of rabbit corpuscles intraperitoneally. At 5 P.M. temperature was at 105° F. On March 16 at 8 A.M. its temperature was 105°, and at 12 M. 104° F. There was extensive local edema; animal otherwise in good condition. At 12.30 P.M. two cubic centimeters more extract were given. Temperature 1.30 P.M. 103.2°, then a gradual rise to 103.8° F. at 5 P.M. March

17, animal in good condition, eating. March 18, good general condition. Local infection a distinct abscess with discharging pus. Temperature normal. Animal practically normal on fifth day in temperature, appearance, and weight.

This experiment seemed to indicate that possibly the extract had some influence in localizing the infection and thus saving the animal.

Experiment III. — March 18, 1907. In this experiment three rabbits were inoculated subcutaneously in the ear with one-fifth of a twenty-four-hour agar culture of staphylococcus Pr. II. None of the animals died. The control weighing two thousand four hundred grams lost most weight, being one hundred and thirty grams lighter at the end of eight days, while the treated animals, weighing two thousand two hundred and eighteen grams and two thousand one hundred and forty-four grams, respectively, were normal in weight at the end of eight days. The treated animals received only small doses of extract, the first doses being given five hours after infecting — two cubic centimeters subcutaneously in one case and one cubic centimeter intraperitoneally in the other, — and at the end of twenty-four hours the same doses were repeated. Little or no effect was noted on the temperature, and there was little difference in the local lesions, the conservation of the weight of the treated animals being the only noteworthy feature of the experiment.

Experiment IV. — March 22, 1907. 10.15 A.M. Two rabbits, one thousand nine hundred and forty grams and one thousand nine hundred and thirty grams, were each given subcutaneously in the left ear one small twenty-four-hour agar culture of staphylococcus Pr. II.

The one-thousand-nine-hundred-and-thirty-gram rabbit received treatment — two cubic centimeters aqueous extract after five hours, and one cubic centimeter after seven hours — intraperitoneally. A drop of about one degree was noted after each injection at the end of one hour.

On March 23, as the animals did not seem particularly sick, another staphylococcus inoculation of one-half of a twenty-four-hour agar culture was given to each, in the right ear. Two hours afterward the rabbit undergoing treatment received two cubic centimeters of aqueous extract intraperitoneally — no drop in temperature was noted. On March 25 both rabbits were given an intravenous dose of a one-fifth agar culture of staphylococcus. The control died at 6 P.M. of March 28, six days after the first inoculation and three days after the intravenous inoculation. The treated animal received small daily intraperitoneal and subcutaneous injections of extract, which temporarily lowered the temperature, but from the twenty-eighth of March to the first of April ran a continuously high temperature, 104° to 105° F., which then dropped subnormal and

afterwards assumed a swinging character, the animal gradually emaciating. Chloroformed on April 5, having survived the control by eight days.

At autopsy the control animal showed on gross examination no visible abscesses in the voluntary muscles. There were abscesses in the kidneys and in the liver, and in the pericardium purulent fluid, and sanguineous fluid in the pleural cavities.

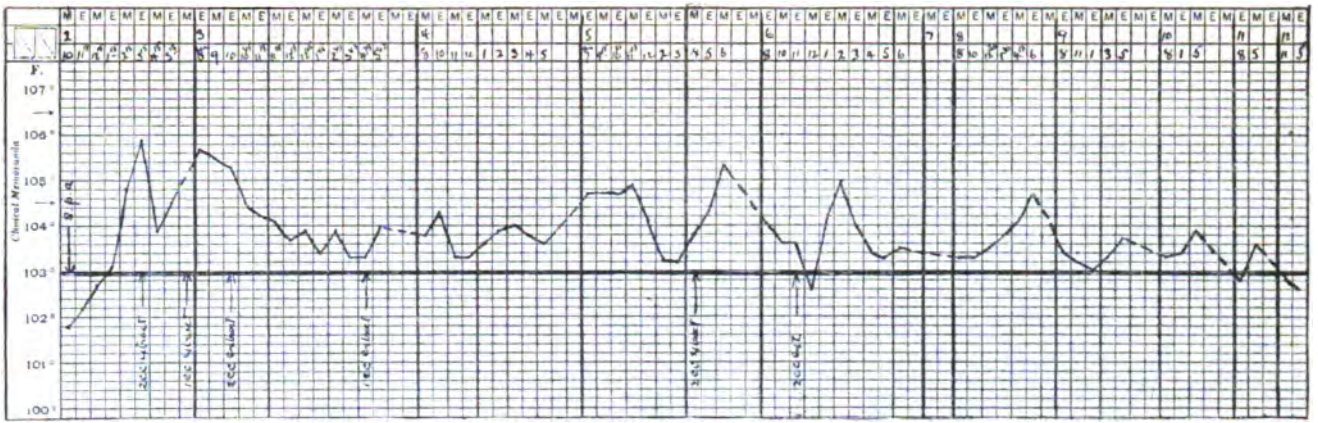
The treated animal had abscesses in the ventricles of the heart, small amount of pericardial fluid, no fluid in pleural cavities; liver, no gross lesions; kidneys, masses of abscesses. Large abscesses in voluntary muscles. Peritoneum normal. Pus in joints of front paws.

Here again the treatment with leucocyte extract apparently had a marked effect on the course of the infection, although given in small amounts and not earlier than five hours, even after the intravenous injection of the staphylococci. The disease was apparently changed from a rather acute septicemia to a fairly chronic pyemia, as evidenced by the anatomical picture at autopsy.

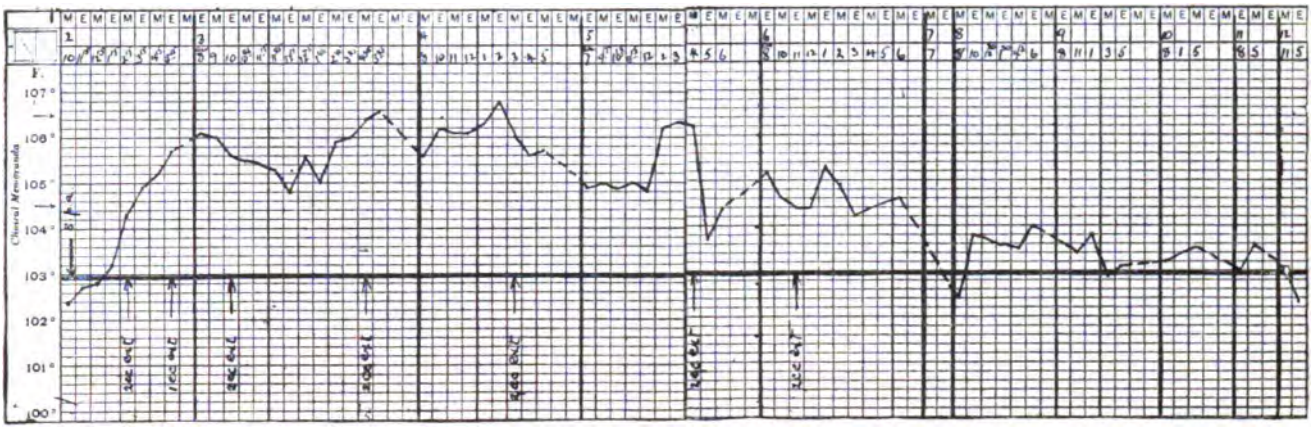
Experiment V. — April 2, 1907. As the culture used in the foregoing experiments showed signs of attenuation, the culture employed in this experiment was one whose virulence had presumably been increased by passage through a rabbit.

Four rabbits were given subcutaneously in the ear one-fifth of a twenty-four-hour agar culture Pr. II. R. I. at 10 A.M. The controls weighed one thousand three hundred and twenty grams and one thousand two hundred and seventy grams. The smallest control died in fourteen hours, and the largest in forty hours. The treated animals weighed one thousand one hundred and sixty grams and one thousand one hundred and five grams, and both survived. The smallest received intraperitoneally two cubic centimeters of extract after four hours, the largest after five hours. The largest animal showed a remission of temperature of two degrees within an hour after being treated, and then the temperature slowly rose again; the smaller animal did not show a remission, but its temperature was not so high as in the animal showing the remission. After seven hours each rabbit again received extract, and then daily for three or four hours. Neither animal lost much weight, the smallest one losing the most, about seventy-five grams. At the expiration of about eight to ten days even their temperatures, always apt to be above normal when animals are frequently handled, had reached normal. (See appended charts.)

Apr. 2, '07. R.P. 1,160 gm. Staphylococcus subcut.

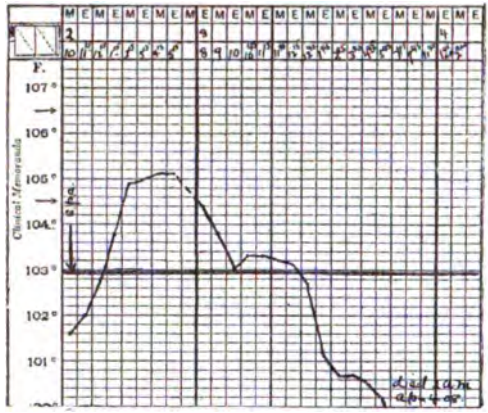


Apr. 2, '07. R.Q. 1,105 gm. Staphylococcus subcut.

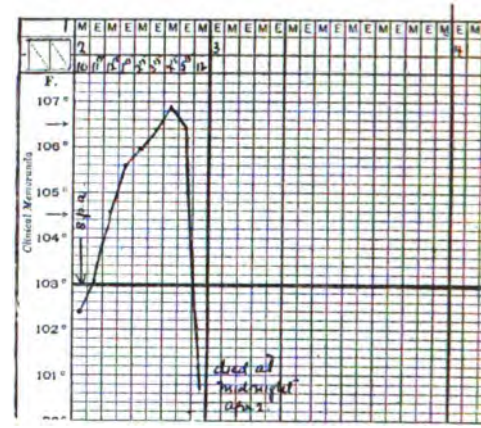


Staphylococcus. Exp. V. Temperature charts of treated rabbits. Animals survived.

Apr. 2, '07. R.N. 1,320 gm. Staphylococcus subcut.



Apr. 2. '07. R.O. 1,270 gm. Staphylococcus subcut.



Staphylococcus. Exp. V. Temperature charts of control, untreated rabbits.

The results of this experiment are self-evident. The test, as indicated by the rapid death of the controls, was a severe one. The doses of leucocyte extract, although small, were given intraperitoneally and were probably quickly absorbed, as indicated by the change in temperature in one of the animals. They were probably efficacious in preventing a rapid systemic infection, although some systemic invasion probably took place.

Experiment VI. — April 4, 1907. 5 P.M. Four rabbits received one-fifth agar culture of staphylococcus Pr. II. R. I. subcutaneously in the ear. The controls weighed one thousand seven hundred and forty grams and one thousand five hundred and seven grams. The one-thousand-seven-hundred-and-forty-gram control died in eighteen hours. There had been some hemorrhage at the point of inoculation and it was thought that some of the injection had gone intravenously. This may account for the death of this animal while the lighter one did not show severe infection and was in apparently good condition on the fifth day following.

The treated animals did not receive any protection until the lapse of sixteen hours (April 5), and then only two cubic centimeters of extract intraperitoneally. These animals weighed one thousand four hundred and seventy grams and one thousand four hundred and fifteen grams. The heaviest showed a drop in temperature of three degrees (105° to 102°), while the lighter one's temperature fell only about one degree. The temperature rose slowly again and after five hours the protective dose

was repeated, the temperature again falling about one degree. On the following day the treatment was repeated, the temperature in one case dropping to below normal, but rising again within three hours. As the remaining control animal did not die, and was running nearly a normal temperature on the fifth day, a fresh infection was attempted, one-fourth of a twenty-four-hour agar culture being given each animal *intravenously* at 10 A.M. By 3 P.M. the temperatures of all the animals were above 106° F. The control and the one-thousand-four-hundred-and-seventy-gram-treated animal died during the night, in spite of the fact that the latter animal received two cubic centimeters of aqueous extract intraperitoneally five hours after the staphylococcus inoculation, and dropped two and one-half degrees in temperature within three hours.

The other treated animal, one thousand four hundred and fifteen grams, was treated with small doses intraperitoneally and survived until the twenty-fifth of April, when it was chloroformed, having lost six hundred grams in weight and being evidently in a lethal condition. This animal survived the control fifteen days.

At autopsy the control animal showed a few small abscesses in the voluntary muscles and some large ones in the kidney. In the ventricles of the heart were numerous pin-head abscesses. The treated animal, which died at the same time as the control, instead of showing evidences of prolonged infection, as did the control, which was evidently systemically infected from its first dose, gave a picture of acute septicemia. There were no macroscopic lesions in the heart, kidneys, or liver, or in the voluntary muscles. Organisms were cultivated from the blood.

In the case of the animal which survived fifteen days and received continuous treatment, the picture was one of great emaciation. The heart, lungs, and liver were free from abscesses. No fluid in the body cavities. Spleen seemed normal. In the left kidney was a fibrous patch possibly from an abscess. The right kidney was swollen and contained many abscesses. There was an abscess posterior to the kidney apparently becoming encapsulated. Organisms were recovered from the kidney pus, but not from the heart's blood.

It is worthy of note that this animal, following the intravenous inoculation, had shown signs of involvement of the joint of one of its front paws. This disappeared, however, during treatment.

This experiment seemed very instructive to us and almost to warrant the conclusion that the animal was saved from a rapid septicemia and generalized pyemia. The lesion was practically confined to the kidney, the organ most susceptible to staphylococcus infection, and the animal probably succumbed to disturbed metabolism and elimination, and, possibly, to chronic poisoning due to the localized infection.

Experiment VII. — May 1, 1907. This experiment does not properly belong in this series, but is given, not only because the animals really serve as further controls for the immediately preceding experiments, but as indicating possibly a slighter efficiency of dog corpuscle extract as compared with that of rabbits, at least when used in the treatment of rabbits. The infecting dose was, however, very severe and the experiment was not controlled with rabbit leucocyte extract so that little weight is to be attached to the outcome of the experiment.

Three animals, weighing one thousand six hundred and fifty grams, one thousand four hundred and ninety grams, and one thousand three hundred and eighty grams, were given one-fifth of an agar culture of staphylococcus Pr. II. R. I. (same doses as in immediately preceding experiment) intravenously at 10 A.M. At 11 A.M. the one-thousand-three-hundred-and-eighty-gram rabbit was given two cubic centimeters of dog leucocyte extract subcutaneously, which was followed by a four-tenths drop in temperature, and at 3 P.M. (five hours) two cubic centimeters more, followed by a six-tenths drop. Temperature, however, steadily climbed thereafter, and was over 106° F. at 6 P.M. The one-thousand-four-hundred-and-ninety-gram animal was treated with two cubic centimeters at 3 P.M. (five hours). Its temperature had not risen over one-half degree, but showed a remission of five-tenths and then began to rise steadily to 104.6° F. at 6 P.M. At 6 P.M. the control's temperature, after an initial fall at two o'clock, had risen abruptly to 105.8° F.

All of the animals were found dead in the morning (about eighteen hours).

No conclusions can legitimately be drawn directly from this experiment but, compared with the preceding, one might suppose dog leucocyte extract less efficient than that of rabbits.

No further staphylococcus experiments were undertaken at this time. Further experiments with this special infection are reserved for a later paper, in which the effect of treatment with immune leucocytes and immune serum will also be considered. Here we are chiefly interested in the effect of leucocyte extract (from normal animals) on various infections.

If we analyze our whole series it immediately becomes apparent that animals receiving subcutaneous injections of rapidly fatal doses of Staphylococcus pyogenes aureus can generally be saved by treatment with the extract of normal leucocytes of rabbits even in small doses, especially when

these are given intraperitoneally. Thus, in Experiments II., V., and VI., we find four control animals out of five dying in twenty-six, fourteen, forty, and eighteen hours, while the five treated animals survived, although never receiving treatment before the lapse of four hours, and in Experiment VI. not before sixteen hours. In Experiment VI. one control, however, also lived. The animals, however, in VI. were observed only five days before being given an intravenous injection of staphylococcus.

When intravenous injections were practiced the results were different, but treated animals usually survived the controls many days, and presented modified histological pictures.

Thus, in Experiment IV. we find our control dying in three days, while the treated animal survived eleven days.

In Experiment VI. the control died in eighteen hours, as did also one of the treated animals, while the other lived twenty days and showed an extremely favorable histological picture.

Such encouraging results from a novel method of treatment naturally led to tests on animals suffering from other infections.

3. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON TYPHOID INFECTIONS IN RABBITS.

Typhoid infections, if indeed we may really call them such, in rabbits are essentially different from infections caused by such organisms as staphylococci, streptococci, and pneumococci. The animals seem rather to suffer an acute intoxication, from which they either die within a very limited time and organisms may then be recovered from them, or they recover completely, or go into a state of cachexia, with gradually increasing emaciation, followed by death, but without organisms in the blood or organs. Death apparently is caused by cellular changes and disturbed metabolism induced by the primary violent poisoning, which is due, probably,

to the rather abrupt dissolution of the injected organisms by bacteriolysis and a liberation of their body poisons.

In man a hypoleucocytosis is characteristic of the typical fever, and changes indicating an activity of mononuclear cells are to be observed upon histological examination of involved tissues. The typhoid bacillus, however, in the semi-immune or immune (*i.e.*, patient following typical typhoid fever) is, nevertheless, associated with local purulent (polynuclear) inflammations and abscesses, which must, it would seem, be interpreted as endeavors to rid the system of these lingering invaders.

Such considerations would naturally lead one to hesitate in prophesying as to the effects of polynuclear leucocyte extracts upon the course of the disease, and it is fully recognized by the writer that experiments with mononuclear leucocytes or with spleen or lymphoid tissue extracts, either alone or combined in treatment with immune serum, might well have a more decided influence than polymorphonuclear leucocyte extracts alone.

Experiment I. — April 8, 1907. Three rabbits, weighing one thousand one hundred and twenty-five, one thousand one hundred and eight, one thousand one hundred and four grams respectively, received at 10.20 A.M. one-third of a twenty-four-hour agar culture of typhoid "70" intravenously. The one-thousand-one-hundred-and-four-gram rabbit received two cubic centimeters of aqueous extract of leucocytes at 11 A.M. and at 5 P.M. intraperitoneally; and the one-thousand-one-hundred-and-eight-gram animaltwo cubic centimeters intrap eritoneally at 3 P.M. Little or no drop in temperature followed these injections. The control animal, one thousand one hundred and twenty-five grams, showed, however, a temperature of 106.5° F. by six o'clock, while the temperatures of the other animals were only 104.8° and 104.3° F.

April 9. No treatment given. The control's temperature fell fairly steadily throughout the day from 105.8° at 8 A.M. to 104.6° at 6 P.M. The one-thousand-one-hundred-and-eight-gram rabbit (treated five hours) was at 105.7° at 8 A.M., 106.3° at 1 P.M., and 105.7 at 6 P.M.

The one-thousand-one-hundred-and-four-gram animal (treatment after one hour) was at 105.5° at 8 A M., 105.1° at 1 P.M., and 104.4 at 6 P.M.

April 10. At 8 A.M. the control was at 104° F., the one-thousand-one hundred-and-eight-gram at 104.7° F., and the one-thousand-one-hundred-and-four-gram at 103.3, which was practically normal.

At 10.20 A.M. the animals all received an intravenous injection, one-third of an agar culture of typhoid "70."

The control's temperature reached 106° F. at 1 P.M., then fell gradually to 105° at 3 P.M., and was 105.5 at 6 P.M. From this time on its temperature ranged continuously between 104° and 106° F. until April 15 when it fell to 103.4° at 6 P.M.

The one-thousand-one-hundred-and-eight-gram rabbit's temperature reached only 105° by 3 P.M., when it received two cubic centimeters of cell extract intraperitoneally, temperature remitting about a half degree, and returning to 105° F. by six o'clock. From this time on the temperature ranged between 104.4° and 106.2° at 4 P.M. on the twelfth of April, when two cubic centimeters more of the extract was given intraperitoneally, the temperature dropping a degree in one hour and remaining between 104° and 105° until the fifteenth, when it dropped below 104° F.

The temperature of the one-thousand-one-hundred-and-four-gram rabbit rose abruptly from 103.3° F. to 105.1° within one hour, when two cubic centimeters of extract were given intraperitoneally. An hour later the temperature fell to 103°, over two degrees, and then rose gradually to 105.7° F. at 4 P.M., steadily falling from this time on and reaching 102.8 (normal) the following morning at 11 A.M. The range was then principally between 103° and 104, touching 103° on the fifteenth, no treatment being given.

On April 16 the dose of typhoid bacilli given intravenously was repeated, it being our hope in this way to simulate the typical infection of man by giving such apparently sub-lethal doses at comparatively frequent intervals.

Up to this time a careful record of the weights of the animals showed the following:

Control.	Treated within 5 Hours.	Treated within 1 Hour.
April 8. 1,125 grams.	1,108 grams.	1,104 grams.
" 11. 1,035 "	1,114 "	1,135 "
" 12. 1,030 "	1,160 "	1,200 "
" 13. 1,010 "	1,125 "	1,190 "
" 15. 1,085 "	1,148 "	1,225 "

Here it is seen that both of the treated animals had actually gained weight, the one receiving the treatment within one hour after infection having gained one hundred and twenty grams and the other one forty grams, while the control, although apparently picking up by the fifteenth, had lost fifty grams.

After the injection of the typhoid bacilli the control's temperature went up to 106° F., then gradually down to 103° F. on the nineteenth. The

one-thousand-one-hundred-and-eight-gram animal's temperature reached 106.5° F. by 3.30 P.M., at which time three cubic centimeters of cell extract were given. At 4.30 P.M. the temperature was 104.1°, and it ranged between this and 103.8° until the nineteenth.

The temperature of the one-thousand-one-hundred-and-four-gram animal (always treated within one hour) shot abruptly to 106.4° from 103.2° within one hour, when three cubic centimeters of extract were given and the temperature fell to 104.2° by one hour, and then gradually climbed to 107° F. by 4.30 P.M., but returned to 103.2° (normal) the next morning, and then ranged mostly below 104° F. until April 19, that is, remained practically flat.

April 19. Again the animals were given a dose of typhoid bacilli, this time one-third of an agar culture of a different strain, "Mallon." Following this inoculation the temperature of the control fluctuated and was even below 103° F. two hours after inoculation. From this time on the temperature rose gradually, reaching 106° F. on the twenty-first, then ranged between 104° and 105° F. till the twenty-fourth, when it commenced to fall, at times ranging higher, the animal dying on April 27, nineteen days after the first inoculation. Autopsy showed extreme emaciation, kidneys large and anemic, spleen of normal size. Clear fluid in peritoneal cavity. No organisms on cultivation.

The one-thousand-one-hundred-and-eight-gram rabbit showed a rise after the injection of two degrees within three hours, and after four and one-half hours received one and one-half cubic centimeters of extract intraperitoneally with little influence on the temperature. The following morning the temperature was as low as 102.8° and from then on ranged practically between 103° and 104°, the animal remaining in good condition, weighing one thousand one hundred and forty grams on May 1 when records were discontinued.

The one-thousand-one-hundred-and-four-gram rabbit's temperature rose from 103.2° at 12 M., the time of typhoid injection, to 107.5° at 2 P.M., when the animal received two cubic centimeters of extract intraperitoneally. At 6 P.M. the temperature was 106.4° F., but the following A.M. touched 103° (normal), and ranged between 103° and 104° until May 1 when records were discontinued.

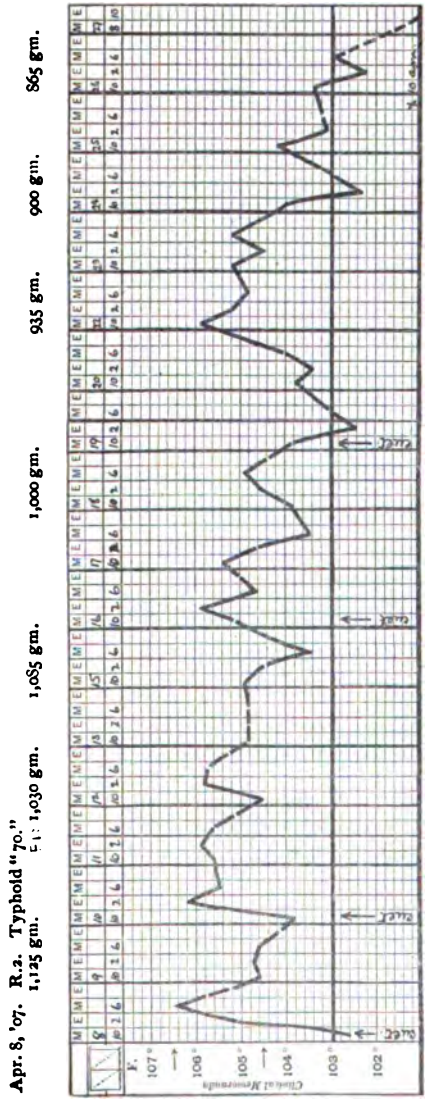
The weight records from the fifteenth on were as follows:

Control (1,125 Grams).	Treated within 5 Hours (1,108 Grams).	Treated within 1 Hour (1,104 Grams).
April 15. 1,085 grams.	1,148 grams.	1,225 grams.
(Injection.)	(Injection.)	(Injection.)
" 17. 1,020 grams.	1,130 grams.	1,185 grams.
" 18. 1,000 "	1,188 "	1,250 "
" 19. 968 "	1,160 "	1,228 "
(Injection.)	(Injection.)	(Injection.)
" 22. 935 grams.	1,100 grams.	1,135 grams.
" 24. 900 "	1,100 "	1,170 "
" 26. 865 "	1,085 "	1,210 "
" 29. Dead.	1,070 "	1,265 "
May 1. —	1,140 "	1,305 "

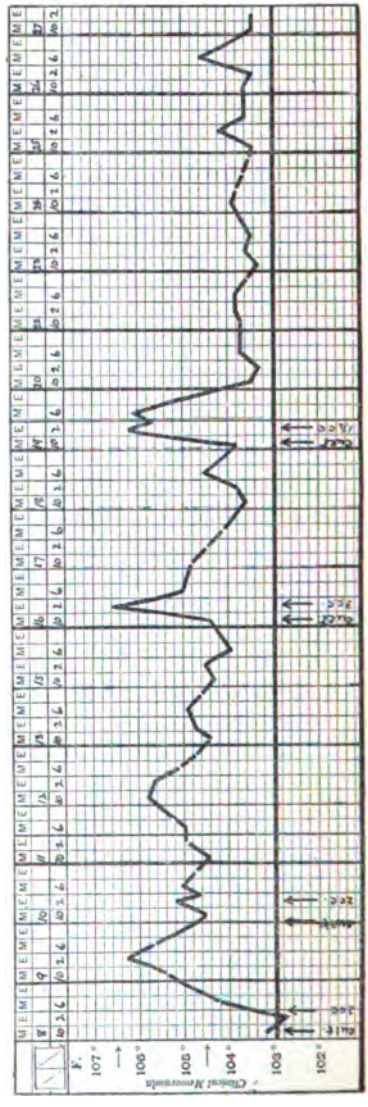
The animals, as is usual after toxic doses of typhoid bacilli, showed signs of poisoning, remaining quiet and refusing all food for some hours. The animals receiving protection with leucocyte extract, shortly after this treatment, always seemed much worse off than the control, and to the inexperienced would appear the most likely to die. This might possibly be due to a more rapid liberation of toxic substances by enhanced bacteriolytic processes, either brought about by a fuller complementing of immune bodies by the extract or to special digestive bodies of the leucocytic extract. That the poisoning in reality was fundamentally less severe than in the more normal appearing control is, however, evidenced by the rapid return of the treated animals to normal condition and weight, the weights following a perfectly logical order — untreated animal, animal treated late, animal treated early.

Another point of interest is the effect of the infecting doses on the temperatures of the animals. Leaving out of account the immediate effect of leucocytic extract on the temperature it is to be noted that following the later injections of typhoid bacilli the temperature of the untreated

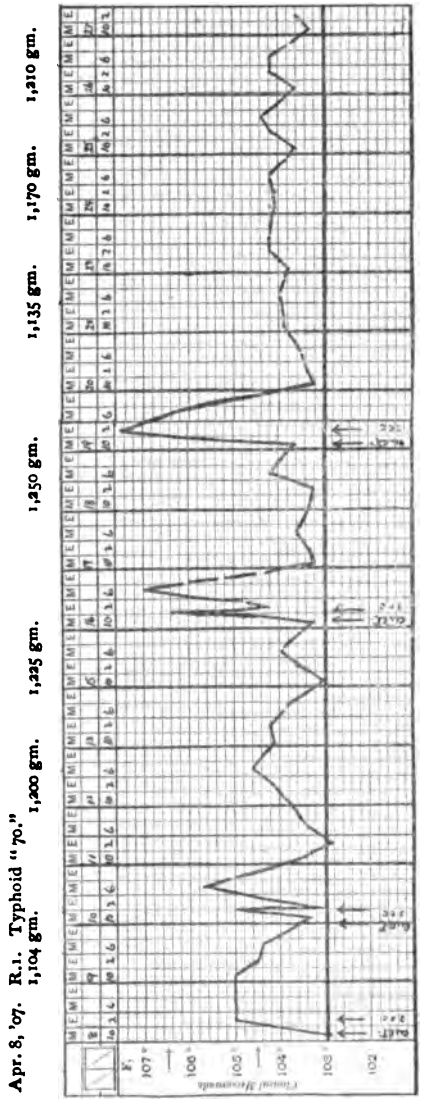
animal went up gradually and remained high, while the temperatures of the animal always receiving early treatment, at the later injections of bacilli, shot up abruptly and then returned rapidly to practically normal. There is a grading of these curves as there is of the weights — untreated animal, animal treated late, animal treated early. (See temperature charts.)



Typhoid. Exp. I. Temperature chart of untreated rabbit. Death. (103° C. is a fair normal temperature.)



Typhoid. Exp. I. Temperature chart of rabbit treated late. Animal lived.



Typhoid. Exp. I. Temperature chart of rabbit treated early. Animal lived.

No organisms were alive in the control, so that the animal died from poisoning and interference probably with metabolism and excretion due to cellular changes.

Experiment II. — April 17, 1907. This experiment will not be given in detail, as it largely repeats the foregoing one. The infecting dose was double the previous dose, and was repeated on the second day. The organism was culture "70" and the dose two-thirds of a twenty-four-hour agar culture. One animal was treated at the end of one hour, and one at the end of five hours. The dose of organisms was so large that its primary effect (after a very transient rise) was an abrupt lowering of the temperature followed by a rise shown at its height towards evening and the next morning.

The effect of the extract in the early treated animal was not a further lowering of the temperature, but an arrest and more abrupt rise than in the control and in the animal treated after five hours. Furthermore, both the control and the later treated animal had bad diarrhea within two hours, but the animal treated in one hour did not have diarrhea. The weights are again of interest and confirm our observations in Experiment I.

Control.	Treated late.	Treated early.
April 17. 1,030 grams.	1,020 grams.	980 grams.
" (Inoculation.)	(Inoculation.)	(Inoculation.)
" 18. 935 grams.	923 grams.	903 grams.
" (Inoculation.)	(Inoculation.)	(Inoculation.)
" 19. 907 grams.	880 grams.	922 grams.
" 22. 910 "	915 "	960 "
" 24. 890 "	900 "	940 "
" 26. 930 "	930 "	985 "
" 30. 880 "	945 "	1,015 "
May 1. 920 "	990 "	1,055 "
" 4. 840 "	965 "	1,010 "
" 7. 892 "	950 "	1,055 "

Here, slight fluctuations are noted, due no doubt to animals having been fed, at times, before noting temperature but not at others. The rule holds good, nevertheless — the animal receiving early treatment only lost weight transiently, rapidly regaining it, while the control lost weight more permanently, and the animal treated later held an intermediate position.

Another interesting observation was made in this experiment. The control and the animal receiving late treatment showed, on the day following the second injection of typhoid bacilli, a marked cyanosis of the ears and multiple petechial hemorrhages in the ears, suggesting the rose spots of typhoid fever. The animal treated early did not show these. The same appearances have been noted in animals receiving intravenous doses of meningococci, but not in connection with any other organism. This is a rather striking fact as both of these diseases in man are characterized by the appearance of such spots.

Experiment III. — May 5, 1908. As both of the earlier experiments (1907) had been performed with the same culture of B. typhosus, the present experiment was undertaken to see if the same result could be obtained when an organism of a different strain was used.

11 A.M. Three rabbits, weighing one thousand eighty, one thousand forty-four, and one thousand thirty grams respectively, were given an intravenous dose of one-third of a twenty-four-hour agar culture of typhoid " 12."

Two of the animals — one thousand eighty grams and one thousand thirty grams — showed an almost immediate drop in temperature of nearly three degrees. The one-thousand-forty-four-gram rabbit showed a slow but steady rise to 105° F., and was therefore presumably the most resistant animal and was kept as control. The one-thousand-thirty-gram rabbit received five cubic centimeters of extract at the end of one hour and the one-thousand-eighty-gram the same dose at the end of five hours. The control progressively lost weight and died on the nineteenth (*i.e.*, after fourteen

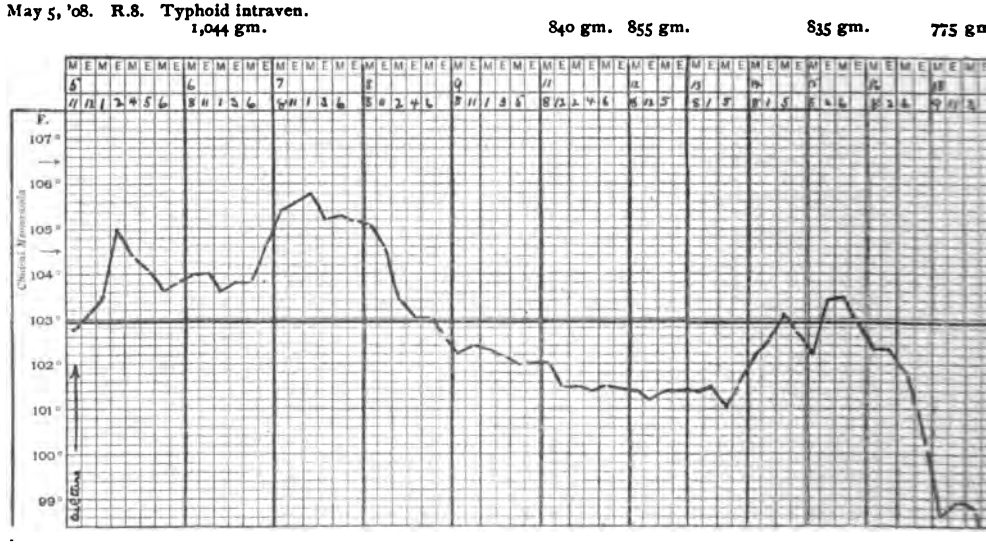
days), weighing only seven hundred and seventy-five grams, in spite of the fact that it rallied and ate voraciously even up to the eighteenth of May.

At autopsy, bloody fluid was found in the peritoneum containing pus cells and bacilli. There was an abscess around the left kidney. Cultures from the heart's blood were negative. Organisms, which proved to be typhoid bacilli, were recovered from the peritoneal fluid and the kidney abscess.

The treated animals lost weight transiently, the later treated one regaining weight more slowly than the one treated early. At the time of the death of the control the one-thousand-thirty-gram rabbit weighed one thousand one hundred grams, and the one-thousand-eighty-gram rabbit weighed one thousand ninety-three grams.

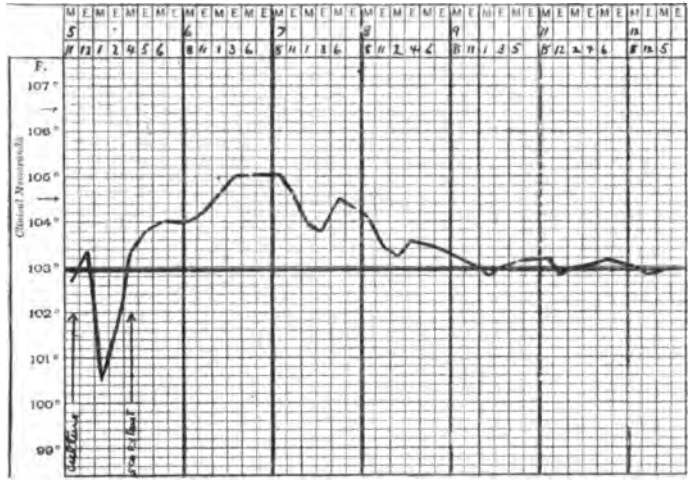
Two of the animals had hemorrhagic spots in the ears.

The charts in this experiment are very interesting and show distinctly the influence of the extract upon the course of the disease.



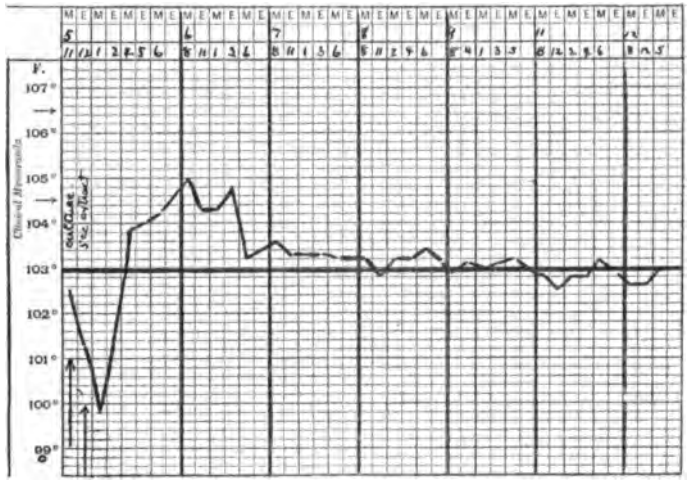
Typhoid. Exp. III. Temperature chart of untreated rabbit. Animal died in fourteen days.

May 5, '08. R.7. Typhoid intraven.
1,080 gm. 950 gm. 1,020 gm.



Typhoid. Exp. III. Temperature chart of rabbit treated late. Animal lived.

May 5, '08. R.9. Typhoid intraven.
1,030 gm. 1,010 gm. 1,060 gm.



Typhoid. Exp. III. Temperature chart of rabbit treated early. Note rapid return of temperature to normal. Animal lived.

These three experiments with leucocyte extract on typhoid infections in rabbits are sufficient for illustration, and the conclusion from them seems unavoidable, that leucocyte extracts have a markedly beneficial modifying action on the course of typhoid infections or poisonings in rabbits.

The same holds true of infections in guinea-pigs treated with rabbit leucocyte extracts, but apart from noting the fact here that subcutaneous injections, used curatively, are active in guinea-pigs, it does not seem of sufficient import to detail such experiments at this time.

Whether this action is to be attributed to products from polymorphonuclear or mononuclear cells can of course not be definitely stated, since all the exudates of course contain a certain percentage of mononuclear cells. It is not unlikely, however, the polymorphonuclear play an important part.

4. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON PNEUMOCOCCUS INFECTIONS IN RABBITS.

My first experiments with the pneumococcus were begun in April of 1907. Although these earliest experiments indicated that a modifying influence on pneumococcic infections, especially on temperature, was exerted by leucocyte extracts, they were unsatisfactory and were for the time abandoned.

These unsatisfactory results were due to the chance use of an extremely virulent organism and to the fact that small doses only of extract were employed at that time. Experiments with pneumococci were not resumed until early in 1908. In the meantime, leucocyte extracts had, however, been employed by us in pneumococcic infection in man with such encouraging results that the experiments about to be described were undertaken with much interest.

Experiment I. — Feb. 17, 1908. 11.15 A.M. Six rabbits, weighing one thousand four hundred and thirty, one thousand four hundred and twenty-five, one thousand four hundred, one thousand four hundred, one thousand three hundred and fifty-five, and one thousand three hundred and

twenty-five grams respectively, were given intravenously one cubic centimeter each of a twenty-four-hour ascitic broth culture of pneumococci "Ac."

The heaviest rabbits, one thousand four hundred and thirty grams and one thousand four hundred and twenty-five grams, were kept as controls. The two one-thousand-four-hundred-gram animals had been given, thirty minutes before receiving the pneumococcus, five cubic centimeters each, subcutaneously, of leucocyte extract. (This is the only instance in all our series of experiments that a prophylactic dose was given.) Notwithstanding this prophylactic treatment the temperatures of these animals, as well as that of all the others, rose consistently and steadily towards 105° F.

After the lapse of five hours the two remaining rabbits, one thousand three hundred and fifty-five grams and one thousand three hundred and twenty-five grams, were given subcutaneously five cubic centimeters of leucocyte extract (4 P.M.). At the expiration of two hours the one-thousand-three-hundred-and-fifty-five-gram animal showed a fall of temperature from 105.3° F. to 104° F., thus indicating a fairly rapid absorption of active substances. The one-thousand-three-hundred-and-twenty-five-gram rabbit's temperature did not fall.

No other treatment was given on this day.

February 18. The controls were still alive. The one-thousand-four-hundred-and-thirty-gram control's temperature reached 106° F. at 3 P.M., but fell to 105° by six o'clock and the animal was very sick. The temperature of the other control fell from 104.8° in the A.M. to 103° at 6 P.M., animal evidently dying.

All the treated animals seemed in fair condition with temperatures, however, ranging above 105° F. as a rule. All of them received treatments with the exception of one of the one-thousand-four-hundred-gram animals. These details are indicated on the charts.

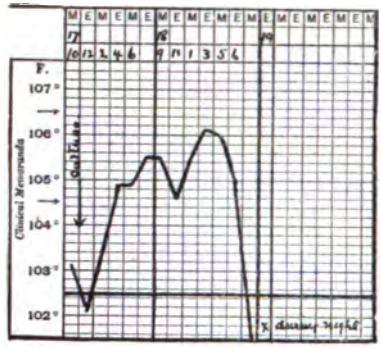
February 19. Both controls were found dead early in the morning (within thirty-six hours).

All the treated animals in fair condition, but temperatures high. No treatment given.

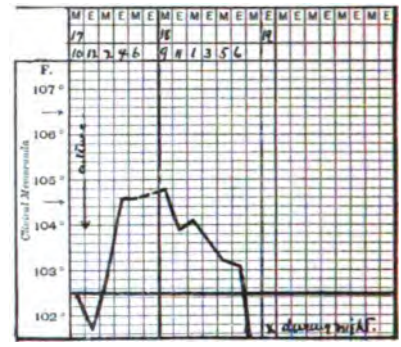
February 20 and 21. All the treated animals, with the exception of one of the one-thousand-four-hundred-gram animals, were doing very well, and in two the temperature fell to 104° during the twentieth, but ascended again on the twenty-first. The one-thousand-four-hundred-gram animal referred to as not doing well had from the first done badly, and had received two treatments on the eighteenth. On the twentieth its appearance was so bad that ten cubic centimeters of extract were given. The temperature dropped in an hour one degree and a half. In spite of this treatment it died during the night of the twenty-first (one hundred and eight hours). At autopsy there were evident signs of a double pleurisy, pericarditis, and peritonitis, but no pneumonia.

February 22. The temperatures of all the animals came down practically to normal, and continued from this time on practically flat. Animals in good condition, and eating well, from twenty-second on.

Feb. 17, '08. R.1. 1,430 gm. Pneumococcus.

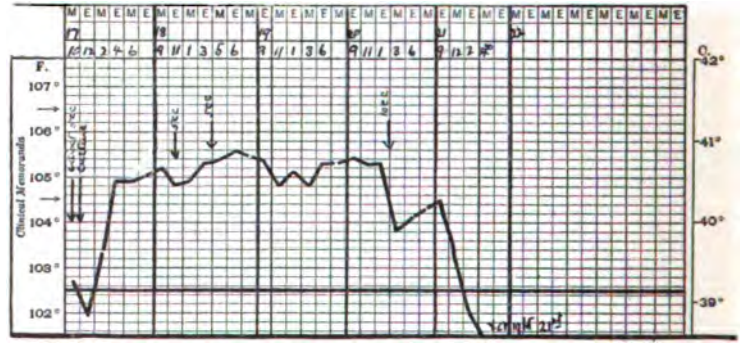


Feb. 17, '08. R.2. 1,425 gm. Pneumococcus.

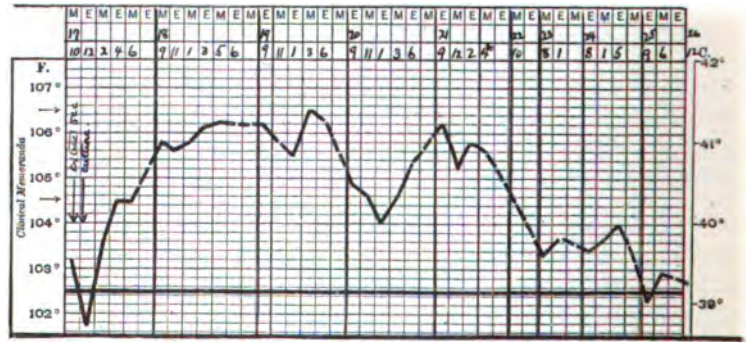


Pneumococcus. Exp. I. Temperature charts of untreated animals. Organisms given intravenously.

Feb. 17, '08. R.3. 1,400 gm. Pneumococcus.

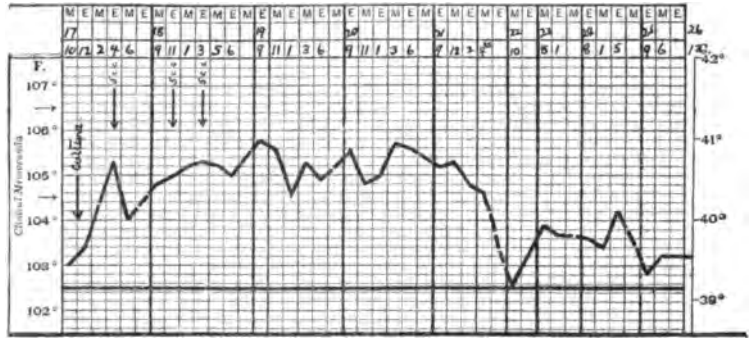


Feb. 17, '08. R.4. 1,400 gm. Pneumococcus.

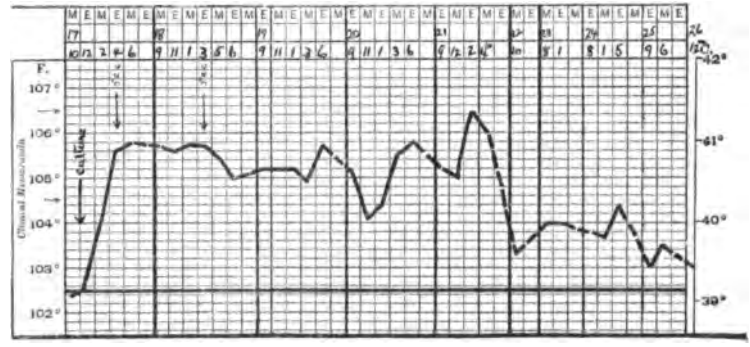


Pneumococcus. Exp. I. Temperature charts of rabbits receiving a prophylactic injection of extract.

Feb. 17, '08. R.5. 1,355 gm. Pneumococcus.



Feb. 17, '08. R.6. 1,325 gm. Pneumococcus.



Pneumococcus. Exp. I. Temperature charts of rabbits treated after five hours.

This experiment seemed to indicate a marked influence of leucocyte extract on pneumococcus septicemia in rabbits. The organism used was undoubtedly a virulent one, as indicated by the controls, both of which died in thirty-six hours. Seventy-five per cent of the treated rabbits recovered — two of them not having been given extract until the lapse of nearly five hours, while the other surviving one was only given a single prophylactic dose one-half hour before being infected, and had no further treatment. The one treated animal which died nevertheless survived the controls by over seventy hours (three days).

Experiment II. — Feb. 24, 1908. 11 A.M. Six rabbits, weighing one thousand two hundred and ten, one thousand one hundred and

seventy-five, one thousand one hundred and sixty, one thousand seventy, one thousand sixty, and one thousand fifty grams respectively, were each given intravenously two cubic centimeters of a twenty-four-hour ascitic broth culture of pneumococcus "Ac." It is to be noted in this experiment that all the rabbits were smaller than in Experiment I. and that the dose was doubled. Two animals were treated at the end of four hours and two after six hours.

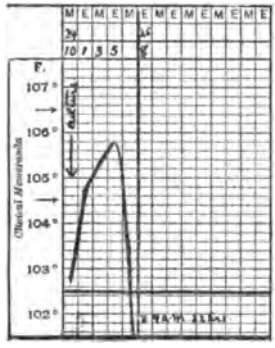
The controls died, one (one thousand two hundred and ten grams) in twenty-two hours, the other (one thousand one hundred and seventy-five grams) in twenty-nine and one-half hours.

One of the animals (one thousand fifty grams), treated in four hours, died in twenty-two hours, the other (one thousand seventy grams) at the end of seventy-one hours.

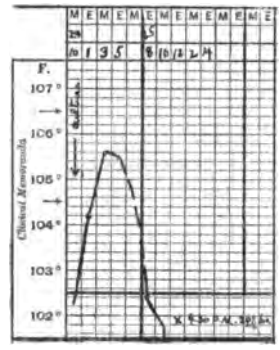
Of the two animals treated after six hours one survived, being normal on the seventh day, but the other one died (chloroformed) on the ninth day. The death of this animal was not directly due to pneumococcus infection, for the rabbit had contracted a common laboratory ailment which is often fatal, and known to us locally as "wet-mouth." This supposition was confirmed at autopsy, for there were no gross lesions of lungs, pleura, pericardium or peritoneum, and no fluid in the body cavities and no organisms by stain or by cultivation.

The test undertaken in this experiment was an extremely severe one, and again shows the influence of the extract. Both controls died, one in twenty-two hours, one in twenty-nine and one-half hours. Of the four treated animals, two died notwithstanding that they were treated — one died in twenty-two hours, the other one, however, only after three days — while the other two survived the infection, one recovering completely, the other one dying in nine days, but probably from an intercurrent disease, and with sterile blood and organs. It is also to be noted that all protective injections were made subcutaneously and, as indicated on the charts, markedly influenced the temperature in most instances. The doses of extract were usually five cubic centimeters. (See charts.)

Feb. 24, '08. R.7. Pneumococcus. 1,210 gm.

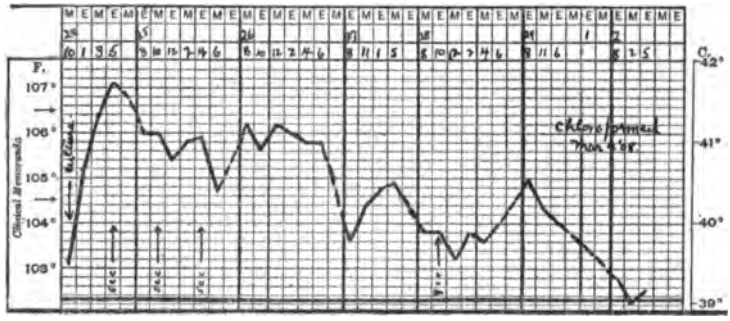


Feb. 24, '08. R.8. Pneumococcus. 1,175 gm.



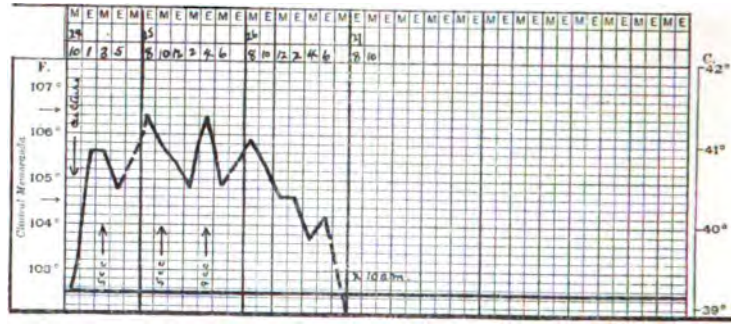
Pneumococcus. Exp. II. Temperature charts of the untreated rabbits.

Feb. 24, '08. R.9. 1160 gm. Pneumococcus. March.

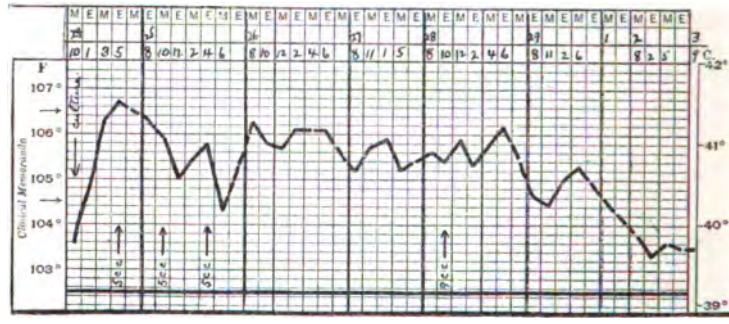


Pneumococcus. Exp. II. Temperature chart of treated animal.

Feb. 24, '08. R.10. 1,070 gm. Pneumococcus.



Feb. 24, '08. R.11. 1,060 gm. Pneumococcus. Mar.

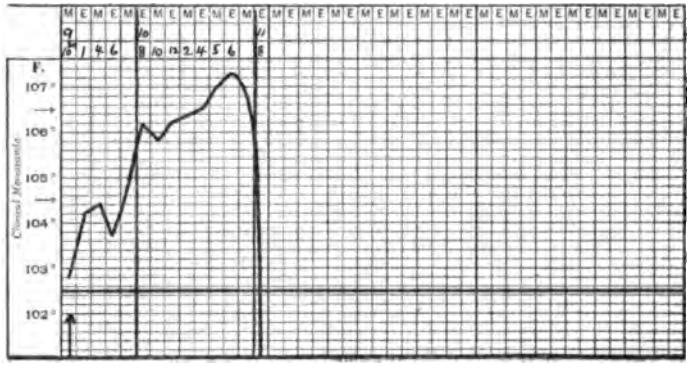


Pneumococcus. Exp. II. Temperature charts of the treated rabbits.

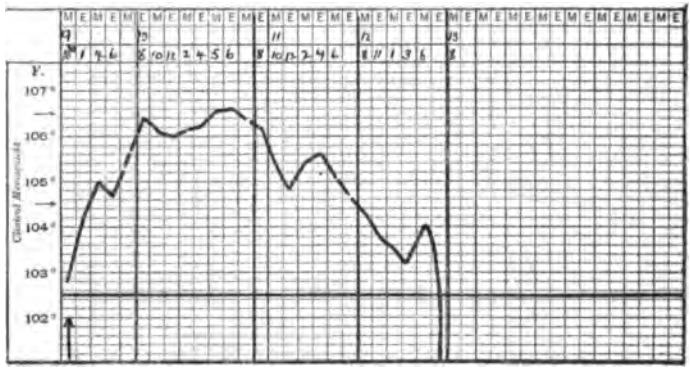
Experiment III. — March 9, 1908. 11 A.M. Six rabbits, weighing one thousand four hundred and forty, one thousand three hundred, one thousand two hundred and ninety, one thousand two hundred and seventy, one thousand two hundred and forty, and one thousand fifty grams respectively, were each given intravenously one cubic centimeter of a twenty-four-hour ascitic broth culture of pneumococcus "Ac." In this experiment we returned to the one cubic centimeter dose of culture and used animals slightly heavier than in Experiment II. All treatment was subcutaneously given, and no animal was treated before twenty-four hours after infection.

The controls, one thousand four hundred and forty grams and one thousand three hundred grams, died in forty-five and ninety-six hours respectively. All of the treated animals survived, only two of them, however, received more than one dose of five cubic centimeters of leucocyte extract. The doses and times of giving them are noted on the charts, where the effects of the extract on the temperature are also apparent. The temperatures of all were practically normal on the fifth to sixth day.

Mar. 9, '08. R.13. 1,440 gm. Pneumococcus.

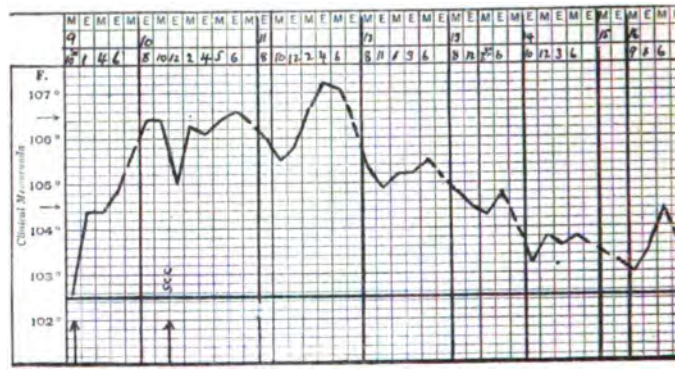


Mar. 9, '08. R.14. 1,300 gm. Pneumococcus.

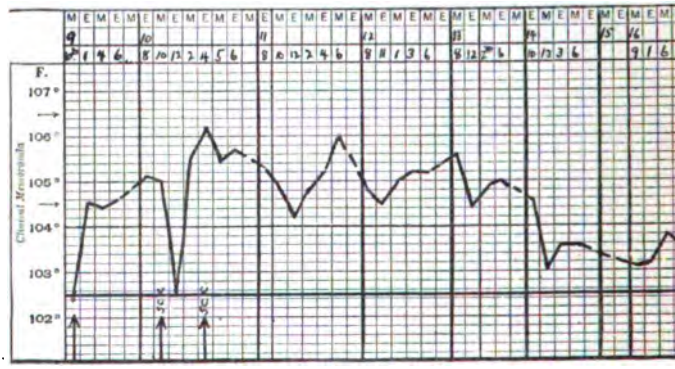


Pneumococcus. Exp. III. Temperature charts of the untreated rabbits.

Mar. 9, '08. R.15. 1,290 gm. Pneumococcus.

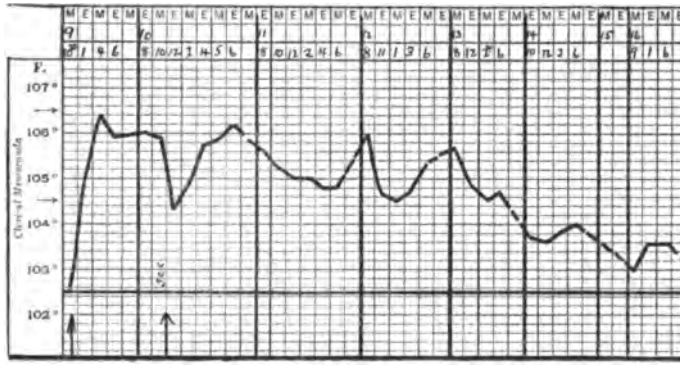


Mar. 9, '08. R.16. 1,270 gm. Pneumococcus.

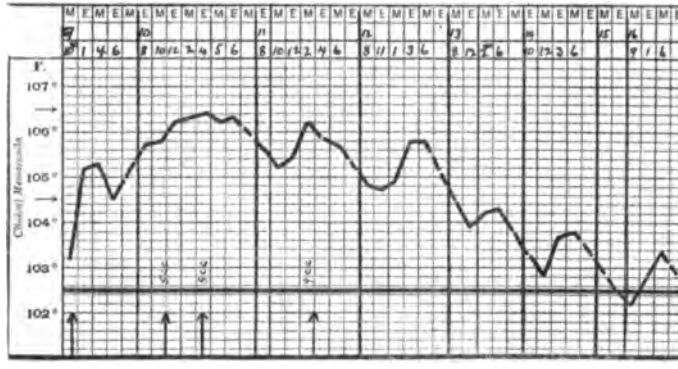


Pneumococcus. Exp. III. Temperature charts of rabbits treated twenty-four hours after intravenous inoculation.

Mar. 9, '08. R.17. 1,240 gm. Pneumococcus.



Mar. 9 '08. R 18. 1,050 gm. Pneumococcus.



Pneumococcus. Exp. III. Temperature charts of rabbits treated twenty-four hours after intravenous inoculation.

The effect of the extract is so self-evident in the experiment that no analytical remarks are necessary. Attention is especially, however, directed to the fact that the animals were saved from an infection fatal to one of the controls in forty-five hours, treatment not having been commenced until half of this time — twenty-four hours — had elapsed.

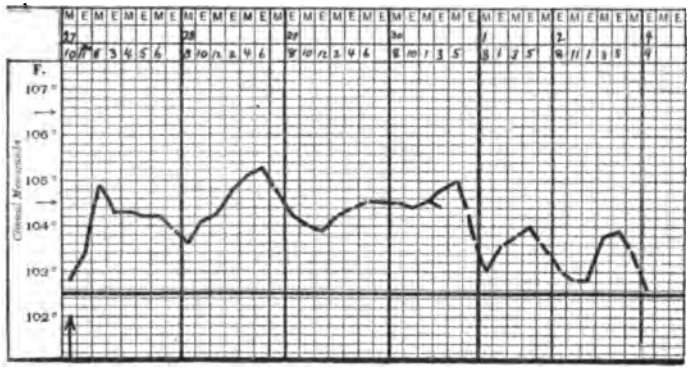
In order to demonstrate that such happenings were not peculiar to the given strain of organisms used, the following experiment is here given:

Experiment IV. — April 27, 1908. 10.30 A.M. Six rabbits, weighing one thousand three hundred and fifty, one thousand three hundred and fifty, one thousand three hundred and twenty-five, one thousand three hundred, one thousand two hundred and fifty, and one thousand two hundred and thirty grams respectively, were each given intravenously one cubic centimeter of a twenty-four-hour serum broth culture of pneumococcus "P." This organism had recently been isolated from a fatal case of pneumococcus meningitis. The organism heretofore used was from a pneumonic lung at autopsy.

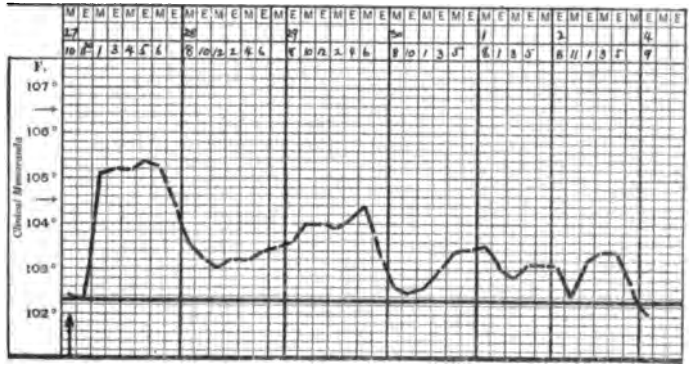
None of the animals died of the infection with this new organism, but the charts are interesting and illustrate beautifully the effect of leucocyte extract on the course of this more benign infection. It is worthy of note that the extract here used was over one month old (having been kept in the ice-chest) and was free from the usual red tinge, the exudate having been free from red cells.

In the charts one may observe the abrupt fall of the temperatures, veritable crises in some cases, following the absorption of the extract, which in each instance was given subcutaneously.

Apr. 27, '08. R.56. 1,350 gm. Pneumococcus "P." May.

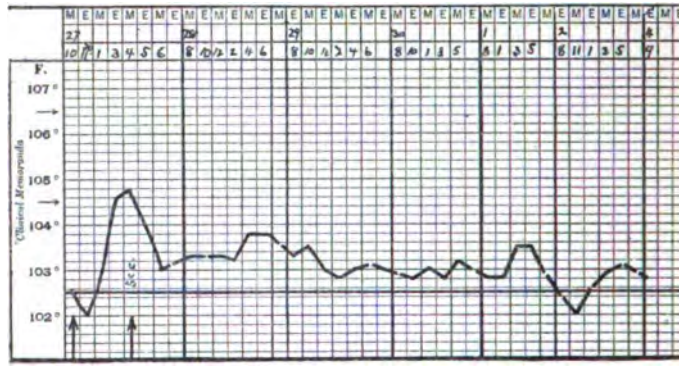


Apr. 27, '08. R.57. 1,350 gm. Pneumococcus "P." May.

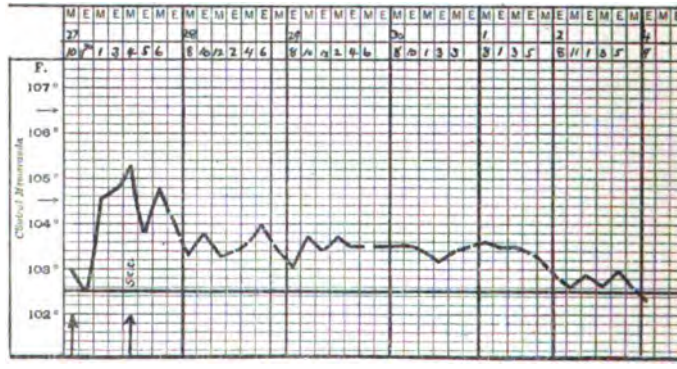


Pneumococcus. Exp. IV. Temperature charts of untreated rabbits surviving a mild infection.

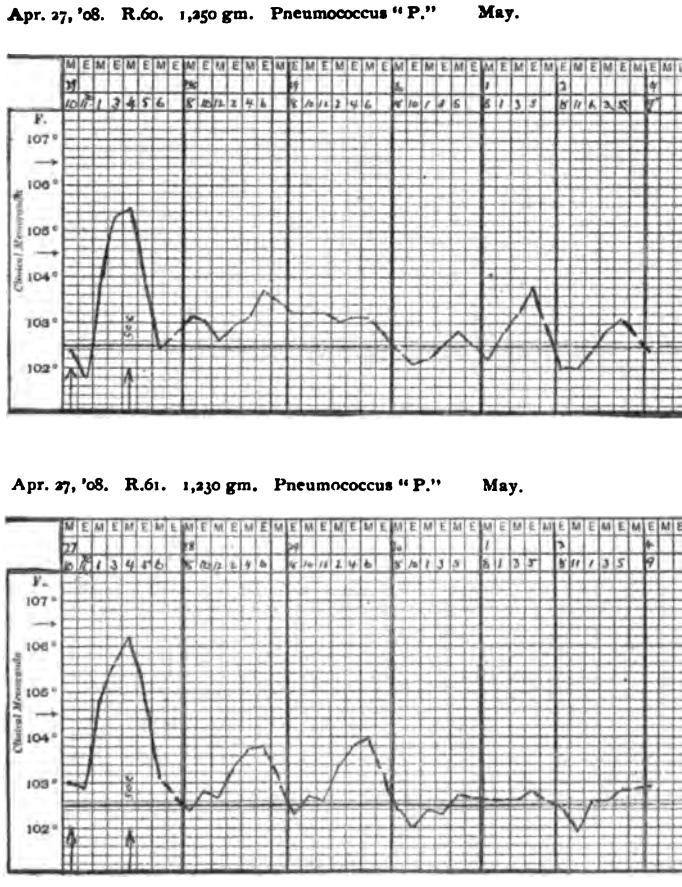
Apr. 27, '08. R.58. 1,325 gm. Pneumococcus "P." May.



Apr. 27, '08. R.59. 1,300 gm. Pneumococcus "P." May.



Pneumococcus. Exp. IV. Temperature charts of rabbits treated after six hours. Type of infection mild.



Pneumococcus. Exp. IV. Temperature charts of rabbits treated after six hours. Type of infection mild.

It may not be altogether illogical to suppose that there may be times during an infection when leucocytes, having emigrated from their normal environment and being under adverse or unusual conditions, such as probably exist in the exudate in the lung during pneumonia, may be disintegrated in numbers and by a rapid neutralization of poisons in their immediate vicinity or by the reabsorption of their products into the general circulation bring about the abrupt

terminations of infections known as crises; or at other times by their cyclical destruction give rise to the heretofore inexplicable swinging temperatures so common in certain septic conditions.

It seems unnecessary to further illustrate this phase of the subject although data are at hand.

The following experiments are given as illustrative of certain other points which seem to us to demand some mention in the present paper.

Experiment V. — March 26, 1908. 11.15 A.M. Seven rabbits, weighing one thousand three hundred and ninety, one thousand three hundred and sixty-five, one thousand three hundred and fifty, one thousand two hundred and ninety-five, one thousand two hundred and ninety-five, one thousand two hundred and seventy, and one thousand one hundred and sixty grams respectively, were each given intravenously one cubic centimeter of a twenty-four-hour serum broth culture of pneumococcus "Ac."

No rabbit was given treatment of any kind until 10.30 o'clock the following morning, after practically twenty-four hours. The following table shows the treatment given each animal and the result:

Date.	Animal.	Weight.	Culture Inoculated.	Hour of Treatment.		Result.	Treated with.
				24-hr.	48-hr.		
1908.							
March 26.	R. 19	1,390	Pn. "Ac" 1 cc. intravenously.	.....	.....	36 hrs.	
"	R. 20	1,365	Pn. "Ac" 1 cc. intravenously.	.....	.....	36 "	
"	R. 21	1,350	Pn. "Ac" 1 cc. intravenously.	5 cc.	.....	36 "	Received H_2O suspension of cell residue after two previous H_2O extractions. Made to volume just before inoculation.
"	R. 22	1,295	Pn. "Ac" 1 cc. intravenously.	5 "	5 cc.	84 "	Received clear supernatant fluid composing second H_2O extraction.
"	R. 23	1,295	Pn. "Ac" 1 cc. intravenously.	5 "	5 "	132 "	Received clear supernatant fluid composing first H_2O extraction.
"	R. 24	1,270	Pn. "Ac" 1 cc. intravenously.	5 "	5 "	Survived.	Received regular aqueous extract containing emulsified cells. Made from same extracts as preparations above.
"	R. 25	1,160	Pn. "Ac" 1 cc. intravenously.	5 "	.....	36 hrs.	Received same as R. 24.

The experiment was planned to determine if the cell residue played much part in saving the animals and whether our aqueous extracts were saturated. The cells were, therefore, subjected to two extractions with distilled water, and then rapidly emulsified in a third equal volume of distilled water and given to the animal in volumes equal to the injections of the other extracts.

From the table it is seen that the controls died in thirty-six hours, as did also the animal receiving the suspended cell detritus, this animal having shown no temperature depression worthy of note following the injection. The animal receiving the second aqueous extraction lived for eighty-four hours, and the one given the first aqueous extraction survived for one hundred and thirty-two hours. The one-thousand-two-hundred-and-seventy-gram animal which received the regular aqueous extract plus the cell

detritus (as usually given) survived, while the small one-thousand-one-hundred-and-sixty-gram (two hundred and thirty grams lighter than the control animal) died in thirty-six hours. The death of this animal does not entirely invalidate the experiment, for the culture was very active and the animal much under weight. Leaving this animal out of consideration, the grading of the effect of the different materials used for treatment is interesting. No apparent effect with the doubly washed cell detritus, a survival of eighty-four hours of the animal receiving the second aqueous extract, and of one hundred and thirty-two hours of the one receiving the first extract, while the animal receiving the regular mixture of cell detritus and extract lived. The temperature charts show extremely little difference between the "regular" extract and the clear supernatant fluid, and probably the determining amount is represented by the slight protective power shown by the second aqueous extract.

The conclusion is probably warranted that most of the protective and curative bodies are free in the aqueous fluid, the rapidity with which the substances are absorbed, as indicated by their influence on temperature, also supports this view.

Other experiments on the effect of extraction by various methods will be given in a separate paper.

Experiment VI. — March 31, 1908. In this experiment the effects of immune serum and extracts from the leucocytes of immune animals as compared with the extract from leucocytes of normal animals was undertaken. The plan and details of the experiment are shown in the table:

Date.	Animal.	Weight.	Culture.	Hour of Treatment.		Result.	Remarks.
				24-hr.	48-hr.		
1908.							
March 31.	R. 26	1,420	Pn. "Ac" 1 cc.	.....	.....	52 hrs.	Control.
"	R. 27	1,380	" " "	.....	.....	24 "	"
"	R. 28	1,360	" " "	2½ cc.	.....	36 "	Serum from immune Pn. 4.
"	R. 29	1,360	" " "	5 "	.....	36 "	Serum from immune Pn. 4.
"	R. 30	1,340	" " "	2½ "	.....	36 "	Serum from immune Pn. 6.
"	R. 31	1,340	" " "	5 "	5 cc.	60 "	Serum from immune Pn. 6.
"	R. 32	1,300	" " "	1 "	.....	36 "	Extract from immune Pn. 4.
"	R. 33	1,280	" " "	2½ "	.....	46 "	Extract from immune Pn. 4.
"	R. 34	1,275	" " "	1 "	5 cc.	Survived.	Extract from immune Pn. 6.
"	R. 35	1,275	" " "	2½ "	.....	36 hrs.	Extract from immune Pn. 6.
"	R. 36	1,225	" " "	2½ "	5 cc.	Survived.	Extract normal 24-III.
"	R. 37	1,205	" " "	5 "	.....	36 hrs.	" " "

The immune leucocytes were recovered from two rabbits immunized against pneumococci and the immune serum was from these same animals, Pn. 4 and Pn. 6, which were bled to death at the time of taking the leucocytes from the pleural cavities.

Record of immunization of Rabbit **Pn. 4**:

Feb. 4, 1908. One live agar culture of pneumococcus "R" subcutaneously.

" 20, " Five cubic centimeters of twenty-four-hour serum broth culture subcutaneously.

March 5, " Five cubic centimeters of twenty-four-hour serum broth culture subcutaneously.

" 18, " Aleuronat given.

" 19, " Bled to death and exudate obtained.

Record of immunization of Rabbit Pn. 6:

Feb. 8, 1908. Five cubic centimeters of a serum broth culture of pneumococcus "Ac" heated to 60° C. subcutaneously.

Feb.	19, 1908.	Five cubic centimeters of a serum broth culture of "Ac" heated to 60° C. intravenously.
March	6, "	Same dose repeated.
"	16, "	Aleuronat given.
"	17, "	Bled to death and exudate taken.

The animals for the test received intravenous inoculations of one cubic centimeter of a twenty-four-hour serum broth culture of pneumococcus "Ac." The serum tests were favored by being made on the heaviest animals. The immune leucocyte extracts were given to the medium weight and the regular extract to the lightest animals. Treatment at end of twenty-four hours. The controls died in fifty-two and twenty-four hours.

The animals receiving 2.5 cubic centimeters of immune sera, Pn. 4 and Pn. 6, died in thirty-six hours.

Of those receiving five cubic centimeters of the sera the one receiving Pn. 4 died in thirty-six hours, and the one receiving Pn. 6 died in sixty hours, having also been given five cubic centimeters more serum at the end of forty-eight hours.

Of those receiving the extract from immune animals, Pn. 4, the one treated with one cubic centimeter died in thirty-six hours, the one with 2.5 cubic centimeters died in forty-six hours.

The animal receiving one cubic centimeter of Pn. 6 immune extract and then five cubic centimeters more at the end of forty-eight hours survived.

The other animal treated with 2.5 cubic centimeters of Pn. 6 extract died in thirty-six hours, so that it was probably due much to the native resistance of the animal getting the one cubic centimeter that it lived to receive five cubic centimeters more protection at forty-eight hours and then survived.

In the case of the animals receiving normal extract the lightest one, receiving five cubic centimeters at twenty-four hours, died in thirty-six hours. The other one, given 2.5 cubic centimeters at twenty-four hours, lived to receive five cubic centimeters more at forty-eight hours and survived.

The only point cleared up by this experiment is the inefficiency of moderate (really large compared with the extract) doses of immune serum in combating pneumococcus infection as compared with leucocyte extracts.

Little is to be learned from the comparison of the "immune" leucocyte extracts and the normal extracts, especially when it is to be remembered that it is impossible to represent strength by exact quantities. In the case of "immune" extract Pn. 4, the corpuscles may not have been really in an immune state at the stage when the exudate was taken, and, at any rate, the primary doses were small, and the animals did not survive for the forty-eight-hour treatment. In the case of the Pn. 6 extract the result was about the same as that shown by the use of normal extract — fifty per cent of recoveries — *i.e.*, the animals surviving to receive treatment in forty-eight hours.

This and other experiments with leucocytes from immunized animals have made it clear to the writer that it is a matter that will require extensive experimentation to determine the time most suitable for withdrawing leucocytes from an animal after immunizing doses of organisms or bacterial extracts. It is possible that the augmentation of beneficial substances, if they do occur at all in leucocytes, is of a very transient nature.

Experiment VII. — May 19, 1908. This experiment was undertaken with two objects in view —

(*a.*) To determine whether living leucocytes taken perfectly fresh from one animal (simply being centrifugalized and emulsified in normal salt solution) and introduced subcutaneously or intraperitoneally into an infected animal, had any influence on the course of the systemic infection, and

(*b.*) What effect heating at 60° C. for one hour had on the curative influences of leucocyte extract.

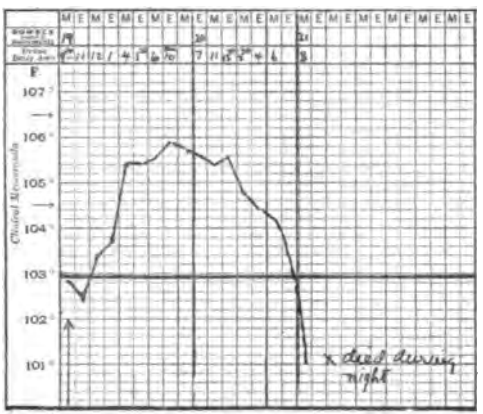
This experiment is also of interest as a different culture of pneumococcus from those employed in the former experiments was used. The animals received, intravenously, one

cubic centimeter of a twenty-four-hour broth culture of pneumococcus "$L_2 R_1$." Thus:

		Treatment.	Result.
R. 62.	1,360 grams.	.....	Died in 36 hours.
R. 63.	1,345 "	.....	" " 36 "
R. 64.	1,310 "	5 cc. cell emulsion subcutaneously.	" " 20 "
R. 65.	1,300 "	5 cc. cell emulsion intraperitoneally.	" " 20 "
R. 68.	1,260 "	5 cc. normal extract heated to 60°–63° C. subcutaneously.	" " 30 "
R. 69.	1,195 "	5 cc. normal extract subcutaneously.	" " 28 "
R. 70.	1,170 "	5 cc. normal extract heated to 60°–63° C. intraperitoneally.	" " 50 "
R. 73.	1,130 "	5 cc. normal extract intraperitoneally.	Survived.

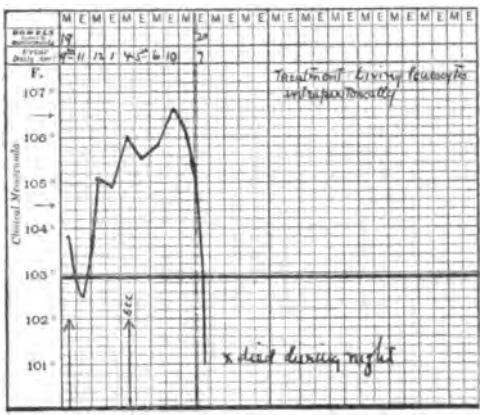
The infection was evidently a severe one. Only one animal of the series survived, the one receiving normal leucocyte extract intraperitoneally. In this animal the drop in temperature following injection of extract was remarkable — four degrees in less than one hour (from 104.7° to 100.4° F.). In the animal receiving heated extract intraperitoneally there was also a drop of two degrees, showing that the heating had not entirely destroyed its activity (see charts), although it was undoubtedly weakened. The same extract was, of course, used for the heated and unheated test.

May 19, '08. R.62. 1,360 gm. Pneumococcus "L2R1."



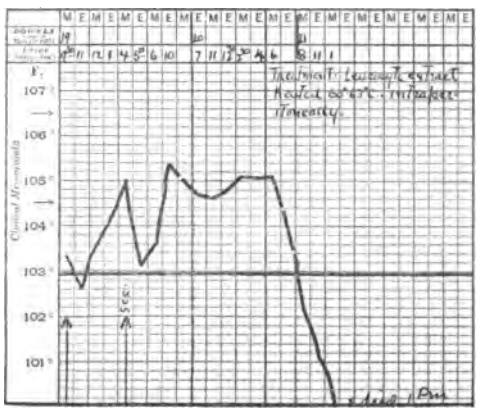
Pneumococcus. Exp. VII. Temperature chart of untreated control rabbit. Inoculation intravenously.

May 19, '08. R.65. 1,300 gm. Pneumococcus "L2R1."



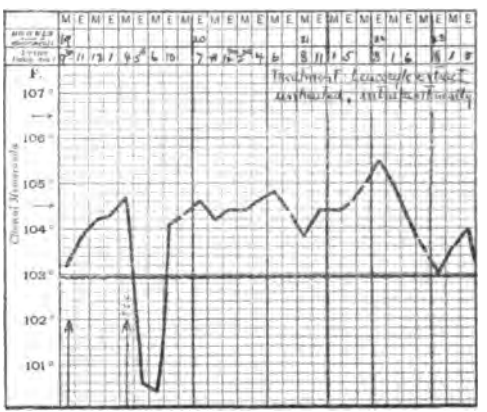
Pneumococcus. Exp. VII. Temperature chart of rabbit receiving living leucocytes intraperitoneally.

May 19, '08. R.70. 1,170 gm. Pneumococcus "L2R1."



Pneumococcus. Exp. VII. Temperature chart of rabbit receiving treatment, intraperitoneally, with leucocyte extract heated to 60°–63° C.

May 19, '08. R.71. 1,130 gm. Pneumococcus "L2R1."



Pneumococcus. Exp. VII. Temperature chart of rabbit receiving treatment, intraperitoneally, with regular unheated leucocyte extract. Animal survived.

The living leucocytes emulsified in normal salt had absolutely no effect, as administered, on the temperature or course of the disease — the animals dying even before the controls (see chart) — and this tends to support our supposition that even if they were active in absorbing toxins and giving up necessary substances to the plasma their action is too slow to be of avail unless they were introduced intravenously — a procedure certainly not to be thought of in the treatment of man — or were used in local infections such as occur in the pleural or peritoneal cavities or, possibly, in the subdural spaces.

Experiment VIII. — April 8, 1908. Up to the time of this experiment no definite determination of the degree of virulence of the culture "Ac" used in most of the tests had been made. No control had, however, survived a dose of one cubic centimeter of a twenty-four-hour serum broth culture given intravenously.

As is so well known, differences in weight of animals have so much influence on the outcome and course of infection that the exact virulence is hard to determine, and, further, in all experiments a certain percentage of deaths are undoubtedly due to preëxisting but not obvious conditions of the animals and should justly be allowed for in all series of tests. In other words, if all animals were in equally good and normal condition a much higher percentage of recoveries could certainly be counted on in the use of any beneficial therapeutic measure.

This present test was, therefore, made to get some idea of the approximate virulence of the organism used for most of our infections. The animals used were a little above the usual weight and the dose given intravenously was graded from .5 cubic centimeter to .01 cubic centimeter of a twenty-four-hour serum broth culture of "Ac." Thus:

Weight.	Dose.	Result.
1,525 grams.	1/2 cc.	Died in 7 days.
1,520 "	1/4 "	" " 7 "
1,480 "	1/10 "	" " 8 "
1,450 "	1/20 "	" " 4 1/2 "
1,300 "	1/40 "	Survived, normal on 8th day.
1,300 "	1/100 "	" " " 5th "

It was probable that our animals in previous tests had been saved from at least twenty times the fatal dose, and possibly more, as the culture had been cultivated solely on artificial media during the full time of experimentation and may have been more virulent at the beginning.

EXPERIMENTS ON PNEUMOCOCCIC INFECTIONS WITH EXTRACTS FROM LEUCOCYTES OF THE NORMAL DOG.

Only one experiment on this phase of our work will be given here.

On April 13, 1908, six rabbits, weighing one thousand six hundred and twenty, one thousand six hundred and ten, one thousand five hundred and sixty, one thousand five hundred and fifty, one thousand five hundred and fifty, one thousand five hundred and thirty grams respectively, were given 1.5 cubic centimeters of a twenty-four-hour serum broth culture of pneumococcus "Ac" intravenously. The two heaviest animals were, as usual, held as controls. The other animals were treated subcutaneously with a freshly prepared and strong aqueous extract of leucocytes from a healthy, normal dog. Two animals, one thousand five hundred and fifty grams and one thousand five hundred and thirty grams, were given five cubic centimeters after five hours, and a slight fall in temperature was noted in each instance. The remaining one-thousand-five-hundred-and-fifty-gram animal and the one-thousand-five-hundred-and-sixty-gram animal received

five cubic centimeters of extract after twenty-four hours with practically no effect on temperature. No further treatment was given.

Both of the control animals survived, possibly on account of their greater weight — only fifty grams, however, in one instance, and the heaviest control being only ninety grams heavier than the lightest treated — while all but one of the treated animals died. Of the two animals treated after five hours, one died in ninety-eight hours, the lighter one in forty-eight hours. Of the animals treated in twenty-four hours, the heaviest one died in thirty-six hours, the other one survived, one might almost say, in spite of the treatment. It is, in fact, difficult to judge of the value or none-value of dog leucocyte extracts from the experiment. It seems, however, that they had no favorable effect on the infection in rabbits as the control animals (only very little heavier), although distinctly sick, survived the dose with apparent ease.

On the other hand, from the experiment one might even be justified in concluding that their effect was harmful.

From the writer's experience in this and other attempts to determine the value of dog leucocytes in treating various infections in rabbits he feels justified in assuming that they are certainly by no means as efficient as those of rabbits. No experiments, however, have been tried on dogs with their own leucocyte extracts, and up to the present the writer has not felt justified in attempting their use in man.

SUMMARY OF RESULTS OF PNEUMOCOCCUS INFECTION EXPERIMENTS.

If, in the series of experiments on pneumococcus infections detailed in the foregoing pages, we consider the animals treated with the extract of leucocytes of normal rabbits, we find that in such animals an infection, surely fatal in untreated rabbits, becomes significantly modified in such treated animals even if this treatment be delayed many hours. Thus, out of eight control animals used in four experiments in which the infecting dose was the same, all died, averaging only forty-five hours of life after being infected. Of the animals treated

— some as late as twenty-four hours after infection — nine out of twelve survived the infection; three died with an average life of sixty hours after infection, two of them not having received treatment until the expiration of twenty-four hours.

When the infecting dose was double the one just mentioned, the controls (2) averaged only twenty-five hours, while one animal out of four of the treated survived, but the three dying averaged one hundred and one hours of life after being infected. These are not selected examples, but are records of events as they developed in our regular research tests, and have been fully confirmed by experiments undertaken in elucidation of other points, and are unmistakably indicative of the powerful beneficial action of such extracts on pneumococcus septicemia in rabbits.*

On the other hand, living leucocytes, introduced subcutaneously, or even peritoneally, have little or no effect on systemic infections.

5. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON STREPTOCOCCUS INFECTIONS IN RABBITS.

Many strains of streptococci, no matter what their source, whether from slight or severe infections in man, are primarily not very virulent for rabbits, even when administered in large amounts. This is one of the distinguishing marks of the streptococci as a class as compared with pneumococci, which organisms, as a rule, no matter what their source, whether recovered while leading a presumably harmless parasitic life in the mouth of man, or from a patient suffering from a severe pneumonia or fatal septicemia, are quite regularly fatal to rabbits when administered in fairly small amounts.

When streptococci are primarily virulent for rabbits or become so after repeated passages through these animals,

* The results of the treatment of pneumonia in man by such extracts are given in another article in this number of the Journal.

the character of the resulting infection is, however, seldom to be distinguished from that given rise to by pneumococci, and animal experiments are considered of minor import in distinguishing one of these organisms from the other.

The writer has maintained, since his first studies on the physiology of pneumococci and streptococci, that there were certain distinct differences in the fermentative abilities of these two organisms, which were sufficient for purposes of identification, *i.e.*, that pneumococci fermented inulin while streptococci did not. The fact that pneumococci may at times have not enough kinetic fermentative energy to permit of immediate identification by this method has not seemed worthy of discussion, for examples of suppression of function are too numerous for the ordinarily well-informed biologist to consider such a phenomenon greatly worthy of note. On the other hand, the actual assumption of fundamentally new functions, however, is so rare that the report of such occurrences, not based on the most careful studies or investigation, may well be accepted with extreme caution, especially when they contradict a long series of previous observations. The statement, therefore, that certain strains of Streptococcus pyogenes may ferment inulin is so contradictory of the writer's personal observation through years that he again asserts his belief that conclusions to this effect are more than likely based on insufficient data, and that the organisms thus described are much more likely aberrant types of the inulin fermenting species than members of the non-inulin fermenting species that have assumed this character. The question is here referred to on account of the absolute necessity, which became apparent while carrying on the following experiments, of knowing the species to which an organism belongs before attempting to draw conclusions from animal experiments which may be carried on with it, and because the experiments here detailed indicate certain marked differences between pneumococci and, at least, certain streptococci, which may aid us in solving the question of identity.

The experiments on the influence of leucocyte extracts on streptococcus infections have, therefore, a double interest for us, on the one hand the fundamental one, whether or not they change favorably the course of the disease, and on the other hand whether the result is exactly comparable to that found to obtain in pneumococcus infections.

The culture used in the experiments was isolated from the throat of a scarlet fever patient. It was at first not virulent for rabbits; four cubic centimeters of a twenty-four-hour serum broth culture gave rise to a high temperature, but was not fatal to an eight-hundred-and-eighty-gram rabbit. This rabbit having apparently recovered and its temperature being normal, on the third day, an intraperitoneal dose of five cubic centimeters was given and the animal chloroformed after twenty-four hours. Four and one-half cubic centimeters of a serum broth culture of the organism received from the peritoneum of this animal were given intravenously to a seven-hundred-gram rabbit, which died from it in eighteen hours with organisms in its blood.

Two cubic centimeters of a culture from the heart's blood of this animal were given to a third, which died in twenty hours, with organisms in the blood. The organism from this animal was then cultivated on serum broth and used for the tests. Inulin fermentation tests and stains for capsule gave negative results, and all characters confirmed our opinion that the organism was a true streptococcus.

Experiment I. — March 23, 1908. At 10.30 A.M. six rabbits, weighing one thousand one hundred, one thousand seventy-five, one thousand ten, nine hundred and ninety, nine hundred and sixty, and nine hundred and thirty grams, were given intravenously one cubic centimeter of a twenty-four-hour serum broth culture of the streptococcus.

The temperature of all rose steadily and consistently to 106° F. and tended to remain high unless interfered with by the injection of leucocyte extract, the maximum depression occurring after each injection of the extract at the end of about two hours. Two of the animals, the nine hundred and ninety grams and the nine hundred and thirty grams, received treatment after five hours. The temperature of the nine hundred and ninety grams did not fall within two hours (6 P.M.), but was at 104.2° the following morning. The temperature of the nine hundred and

thirty grams fell a degree and a half by 6 P.M. (two hours), but was at 105.8° F. the following morning.

The two remaining rabbits, one thousand ten grams and nine hundred and sixty grams, were given five cubic centimeters of extract subcutaneously at the end of twenty-four hours. The temperature of the one-thousand-ten-gram animal fell within two hours from 106.2° to 103°, but rose again to 106.6° by 4 P.M. The temperature of the nine-hundred-and-sixty-gram animal fell from 104.8° to 102.8° within two hours, and then rose steadily to 106°. The subsequent course of the disease and its treatment may best be followed on the charts. Attention is directed to the marked influence of the extract on the temperature.

The controls died, one in seventy hours (one thousand one hundred grams) and one in thirty-six hours (one thousand seventy-five grams). There were no marked lesions, but the spleen of the one dying in seventy hours was enlarged. Organisms were present in the blood of each and the picture was one of septicemia.

Of the animals treated in five hours, but with no subsequent treatment, one survived (nine hundred and thirty grams), and one (nine hundred and ninety grams) died in thirteen and one-half days.

During the course of the disease both of these animals developed involvement of the joints, especially the joints of the front paws; in the case of the animal which recovered, distinct abscesses formed at the joints of the front paws and at one joint of a hind leg.

At autopsy of the animal dying in thirteen and one-half days there was great emaciation but no gross internal lesions; organisms, however, were recovered from the heart's blood. There was still involvement of the joints at death.

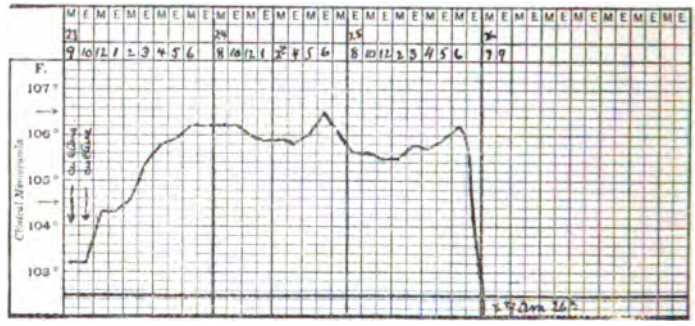
Both of the animals treated after twenty-four hours died, one (one thousand ten grams) in one hundred and eight hours, and one in five and one-half days. The latter animal was autopsied. There was bloody fluid in the peritoneum, the lungs were congested, and there was some fluid in the pleural cavities. The spleen was very large. Organisms were recovered from the blood.

The results are as follows:

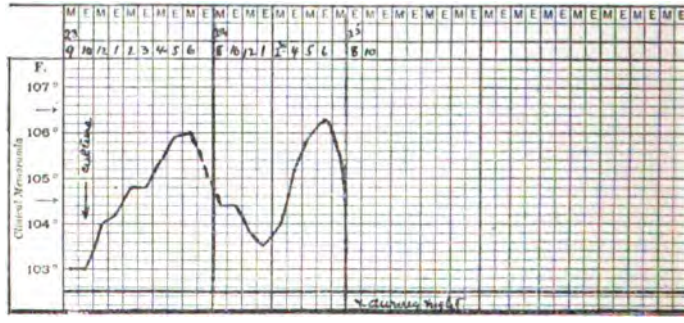
R. 1. 1,100 grams.	Died in 70 hours.	Controls.
R. 2. 1,075 "	" " 36 "	
R. 3. 1,010 "	" " 108 "	Treated, 24 hours.
R. 5. 960 "	" " 5½ days.	
R. 4. 990 "	" " 13½ "	Treated, 5 "
R. 6. 930 "	Survived.	

Such a result leaves little doubt of the beneficial action of the leucocyte extract on the streptococcus infection in rabbits, and this is seen not only in the prolongation of the life of the treated animals but also in the marked effect upon the temperature. (See charts.)

Mar. 23, '08. R.1, 1,100 gm. Streptococcus "104 R3."

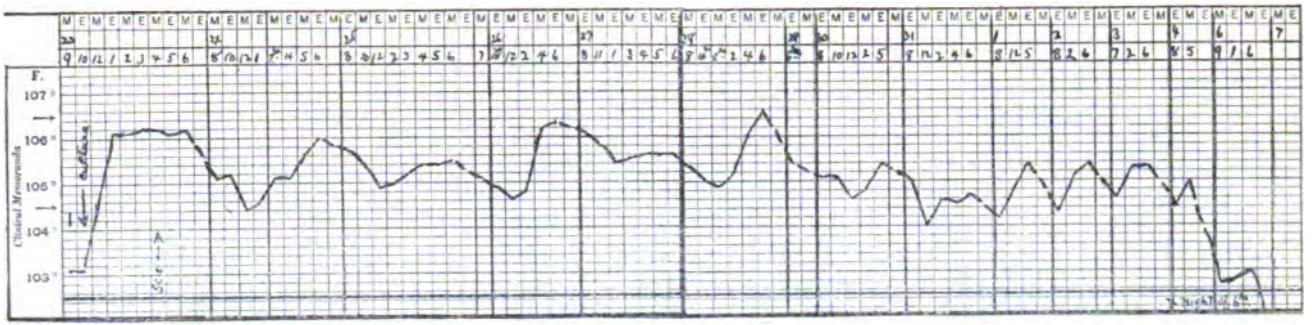


Mar. 23, '08. R.2. 1,075 gm. Streptococcus "104 R3."

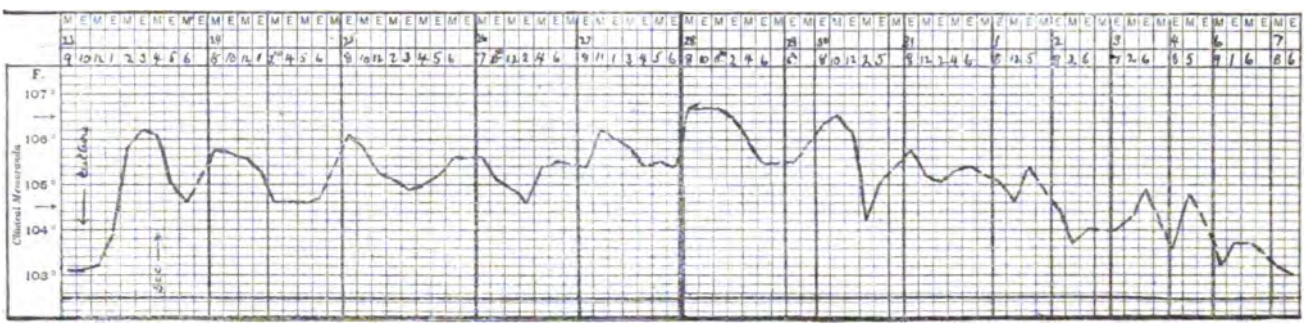


Streptococcus. Exp. I. **Temperature charts of untreated rabbits, receiving inoculation intravenously.**

Mar. 23, '08. R.4. Streptococcus " 104 R3." Apr.
990 gm. 915 gm.

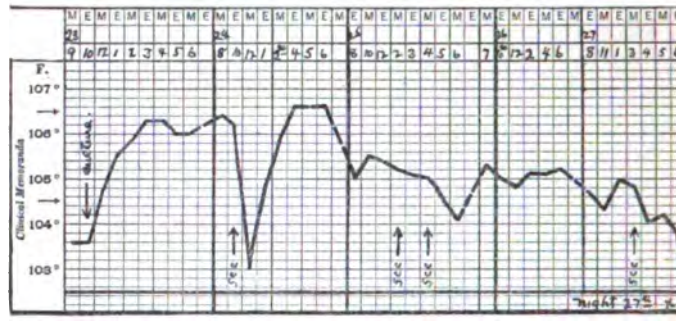


Mar. 23, '08. R.6. Streptococcus " 104 R3." Apr.
930 gm. 915 gm.

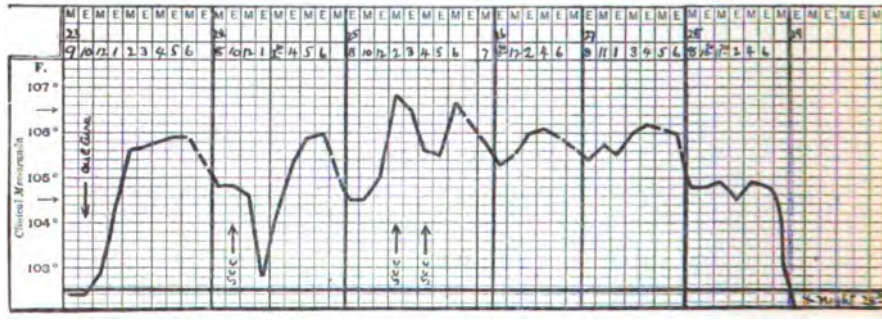


Streptococcus. Exp. I. Temperature charts of rabbits treated five hours after inoculation. R.6 survived, R.4 died.

Mar. 23, '08. R.3. 1,010 gm. Streptococcus " 104 R3."



Mar. 23, '08. R.5. 960 gm. Streptococcus " 104 R3."



Streptococcus. Exp. I. Temperature charts of rabbits treated twenty-four hours after inoculation. Note marked drop in temperature. Both animals eventually died.

Experiment II. — April 6, 1908. 11 A.M. Six fair-sized rabbits were given intravenously 1.5 cubic centimeters of a twenty-four-hour serum broth culture of the same streptococcus used in Experiment I. The results may best be seen by reference to the accompanying table:

R. 7. 1,455 grams.	Died in 30 hours.	Controls.
R. 8. 1,455 "	" " 45 "	
R. 9. 1,435 "	" " 9¼ days.	Treated in 6 hours; 5 cc. subcutaneously.
R. 10. 1,435 "	" " 60 hours.	
R. 11. 1,435 "	" " 7 days.	Treated in 6 hours; 10 cc. subcutaneously.
R. 12. 1,435 "	" " 36 hours.	

The results of this experiment confirm those of Experiment I. The dose of infecting organisms was evidently very severe, as was evidenced by a moderate primary rise in temperature followed by a distinct fall, in all cases, to normal or below. This was followed by rallying and rise of temperature to 105° or 106° F. The animals receiving treatment showed another fall in temperature by the end of two hours after injection of the leucocyte extract. The fact that the animals receiving ten cubic centimeters did not recover, or even outlive those receiving only five, may indicate that the primary poisoning was too severe in its effects to admit of recovery, or that in spite of the greater amount of extract that the animals received they lacked a sufficient amount of some protective element not furnished in the extract — suffered, for instance, from a paucity of or interrupted production of some interbody (amboceptor) necessary for the inhibition or destruction of the invading organisms. Further experiments may show that the deficient body can be furnished by immune serum.

In both of the animals surviving until the third day joint involvement manifested itself.

The regularity with which these articular or peri-articular involvements occurred with this special organism indicates that a difference may exist in the character of the infections

caused by streptococci and those caused by pneumococci. In our many observations on pneumococcus infections, both in treated and untreated animals, I have never observed this tendency to local manifestation. If further observations on other carefully identified strains of Streptococcus pyogenes show this to be a constant occurrence, it must certainly indicate a fundamental physiological difference and may aid us in definitely establishing the identity of certain of the organisms which have been reported as varying in cultural and fermentative characters from their type, and assuming characters typical of pneumococci.

Comparing these streptococcus experiments with those made with staphylococci we find that the streptococci although localizing at certain points — not unlikely points susceptible to injury from damp cages and constantly maintained cramped positions of sick animals — do not tend, so far as our experiments go, to give rise to the multiple abscesses which constitute the lesions of pyemia, so frequent a result of staphylococcus infection.

6. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON MENINGOCOCCUS INFECTIONS IN RABBITS.

The favorable impression, which the results of the writer's experiments on the effect of leucocyte extracts on staphylococcus infections and on the course of typhoid infections in animals had made upon him, led him as early as April, 1907, to attempt the treatment of another disease — acute cerebro-spinal meningitis — and this time in man, on what was largely theoretical grounds.

A boy of fifteen had been admitted to Roosevelt Hospital suffering from cerebro-spinal meningitis due to the meningococcus. On April twenty-third all hope of his recovery had been given up by the attending physician and house staff, and the writer requested permission to give him an injection. Only small primary subcutaneous doses were given, as this

was our first attempt to administer leucocyte extract in man. It was found that no bad effects followed its administration and that the extract was very rapidly absorbed. The history of this case is given in a following clinical paper, but our observations were so encouraging and the result of this treatment, on the profound intoxication from which the boy was suffering, was apparently so marked that we were encouraged, not only to treat other cases of the infection, but to thoroughly investigate the influence of extract on the course of this infection in animals.

After several tentative experiments which convinced us that leucocyte extracts influenced meningococcus infections, or rather poisoning, in animals, more thorough experiments were begun in the summer of 1907.

Rabbits suffer a marked intoxication, if not, indeed, in some instances, a true infection, when given sufficient quantities of almost any race of meningococcus intravenously. During the epidemic of cerebro-spinal meningitis occurring in New York several years ago the writer proved this to his satisfaction many times, so that for the purpose of the following experiments he had no hesitation in selecting rabbits as the test animals. The only care necessary was to have the organisms in proper condition to be administered intravenously. Agar cultures were used and, after being as thoroughly emulsified in salt solution as possible, they were always filtered through thin layers of absorbent cotton to remove the larger masses. Any chance of the organism masses serving as emboli was thus largely eliminated. The dose necessary for satisfactory results was often as large as four or five agar cultures.

Experiment I. — Aug. 9, 1907. At 11.15 A.M. three rabbits, weighing seven hundred and seventy-five, seven hundred and thirty, and seven hundred and twenty-five grams respectively, were given a full agar culture of meningococcus "James" intravenously. The temperature of the control, seven hundred and seventy-five grams, rose abruptly to 107.4° F. by two o'clock, and the animal died during the night.

The seven-hundred-and-thirty-gram rabbit was given two cubic centimeters of extract subcutaneously at 12.15 o'clock, *i.e.*, one hour after infection. At 4 P.M. its temperature reached its maximum — 105° — and

then fell to 104.5 F. by 5 P.M. The following morning the animal was in good condition and eating a little; temperature practically normal — 103° F. This animal survived.

The seven-hundred-and-twenty-five-gram rabbit was given two cubic centimeters of extract at 2.15 P.M., *i.e.*, after three hours. Its temperature reached its maximum at 3 P.M. — 106°, and fell to 104° by five o'clock, another two cubic centimeters of extract having been administered at 4.15 P.M. The temperature remained around 105° F. on the following day, and in spite of subsequent injections the animal died at the end of four days. The notes on this experiment indicate an intercurrent infection of "snuffles;" meningococci were, however, recovered from the heart's blood and probably should be looked upon as the cause of death. The undoubted presence of meningococci in the blood of the animal after four days is very unusual and indicates that possibly all the effects noted after such inoculation are not always to be referred to intoxication from endo-toxins alone.

We have shown to us in this experiment, as in the case of staphylococcus, typhoid, and pneumococcus infections, apparently at least, an influence on the course of the infection or poisoning by the leucocyte extract.

Experiment II. — Oct. 25, 1907. In this experiment four rabbits were each given intravenously four full agar cultures of meningococcus "Margie." The two treated rabbits received a dose of five cubic centimeters of extract subcutaneously about five minutes before being given the culture intravenously. This was not really a prophylactic experiment as little absorption could take place in so short a time.

The animals weighed one thousand four hundred and thirty-five, one thousand four hundred and twenty, one thousand three hundred and fifty-five, and one thousand three hundred and five grams and were inoculated at ten o'clock. The one-thousand-four-hundred-and-thirty-five-gram animal — one of the controls — had bad diarrhea by eleven o'clock, its temperature fell from the beginning, the animal dying with a temperature of 94° at 2 P.M., *i.e.*, in four hours. The other one-thousand-four-hundred-and-twenty-gram control had bad diarrhea within an hour and a falling temperature which reached 99.6° F. by 11.30 A.M., and then began to ascend. The following morning its temperature was 106° F., but on the next day the temperature fell rapidly and the animal died with a low temperature at the end of fifty-three hours.

Neither of the treated animals showed a fall of over one and a half degrees, and the temperature then ascended slowly to about 105° F. by evening. The animals did not have diarrhea. Both of these animals survived, although they were evidently poisoned by their infection and took several days to reach a normal condition. By November first they were

normal in every way. On the twenty-eighth, it is to be noted, they were each given five cubic centimeters of extract subcutaneously, although it was not apparent that this was necessary to save their lives.

This experiment shows, even better than Experiment I., the beneficial effect of treatment with leucocyte extract.

Experiment III. — Oct. 31, 1907. 12.30 P.M. Three animals, weighing one thousand three hundred and eighty, one thousand three hundred and seventy, and one thousand two hundred and fifty grams, were each given intravenously four full agar cultures of meningococcus "Margie." The control weighed one thousand three hundred and eighty grams. Its temperature rose steadily to 106° F. by 6 o'clock, and during the next day ranged higher, 107.2° at mid-day. The animal died during the second night — thirty-six hours.

The one-thousand-three-hundred-and-seventy-gram animal showed a drop of about one degree after infection and had diarrhea; as its temperature rose again during the afternoon to 106° it was decided to defer treatment until the day following. The animal's temperature was still high the next morning, and at 10 A.M. five cubic centimeters of extract were given, the temperature remitting about one-half degree and then ascending a little again. The animal was distinctly sick and somnolent. The day following (November 2) its temperature had fallen to 103.7° and from then on to normal, the animal recovering its spirits and appetite and remaining well.

The one-thousand-two-hundred-and-fifty-gram rabbit showed, after infection, an immediate rise of one degree in temperature. From then on the temperature fell so that, at the expiration of four hours, five cubic centimeters of extract were given subcutaneously. The animal was very ill; the temperature after the extract injection still fell, and on the following morning was at 99° F. at 8 o'clock, the animal being very ill and having had diarrhea during the night. Extract was given at nine o'clock and the temperature rose steadily to 104° F., where it remained during the day. The animal was much improved on November second, and from then on its temperature was practically normal and the animal remained well.

This experiment is a further confirmation of the impression gained from the preceding experiments that leucocyte extracts have a distinctly beneficial effect on meningococcus infections; the treatment of one animal having even been deferred to twenty-four hours in an infection proving fatal in thirty-six hours in a heavier control. The fact that the other animal with a continuously falling temperature, which is

usually indicative of profound poisoning and impending death, lived proves conclusively to the writer the marked contribution of the leucocyte extract to the result. The experiments were, in fact, so conclusive and so confirmative of results obtained in man that a simple repetition of them was considered unnecessary.

Other experiments were, however, undertaken with other objects in view, which further confirm the results already obtained.

The two following experiments may be given together, as the object of them was practically the same:

COMPARISON OF CURATIVE EFFECT OF IMMUNE SERUM, IMMUNE LEUCOCYTE EXTRACT AND NORMAL LEUCOCYTE EXTRACT ON MENINGOCOCCUS INFECTION IN RABBITS.

Experiments IV. and V. — November 14 and 20, 1907. Nineteen rabbits were used in these two experiments. Five of them served as controls and the infecting dose was the same in both experiments — three and one-half full agar cultures of meningococcus "Margie" given intravenously.

In Experiment IV. the treatment was commenced in five hours, in Experiment V. in twenty-one hours. The plan of Experiment V. required the use of so many animals that, two of the heaviest animals having died before the lapse of twenty-one hours, this was considered more than sufficient indication of the markedly lethal character of the dose, and the remainder were given treatment (Experiment of Nov. 20, 1907).

In the other experiment (IV., November 14) three animals served as controls, a light one dying in five hours (*i.e.*, before it was planned to commence treatment), one in twenty-one hours, and a large animal, nearly four hundred grams heavier than the next in weight of the series, died in thirteen days, so that there is little probability that any of the other animals would have survived had they not been treated.

The scheme and results of these two experiments may be easily seen in the appended table:

	Hour of Treatment.				Result.	Remarks.
	5-hr.	21-hr.	24-hr.	72-hr.		
Controls:						
Nov. 14. R. 11, 1,640.	.....	.....	.....	.....	13 days.	
" " R. 12, 1,275.	.....	.....	.....	.....	21 hours.	
" " R. 18, 1,085.	.....	.....	.....	.....	5 "	100% deaths.
" 20. R. 21, 1,375.	.....	.....	.....	.....	18 "	
" " R. 23, 1,285.	.....	.....	.....	.....	6 "	
Animals treated with normal extract:						
Nov. 14. R. 14, 1,240.	2½ cc.	.....	2½ cc.	.....	Survived.	
" " R. 16, 1,170.	5 cc.	.....	.....	.....	18 hours.	50% recoveries.
" 20. R. 24, 1,260.	.....	5 cc.	.....	.....	Survived.	
" " R. 27, 1,187.	.....	5 cc.	.....	.....	8½ days.	
Animals treated with immune extract:						
Nov. 14. R. 20, 1,040.	5 cc.	.....	.....	.....	Survived.	
" 20. R. 25, 1,255.	.....	5 cc.	.....	.....	Survived.	66⅔% recoveries.
" " R. 28, 1,165.	.....	5 cc.	.. ..	5 cc.	9½ days.	
Animals treated with immune serum:						
Nov. 14. R. 13, 1,255.	2½ cc.	.....	2½ cc.	.....	70 hours.	
" " R. 15, 1,220.	5 cc.	.....	.....	.....	Survived.	
" 20. R. 22, 1,320.	.....	5 cc.	.....	.....	36 hours.	20% recoveries.
" " R. 26, 1,190.	.....	5 cc.	.....	.....	70 hours.	
" " R. 29, 1,032.	.....	5 cc.	....	5 cc.	8½ days.	
Animals treated with immune serum and extract:						
Nov. 14. R. 17, 1,110.	2½ cc. extract. 2½ cc. serum.			.....	18 hours.	Immune serum and normal extract.
" " R. 19, 1,045.	2½ cc. extract. 2½ cc. serum.			.....	84 hours.	Immune serum and immune extract.

The rabbit furnishing the immune leucocyte extract was the same from which the immune serum was obtained —

Record of immunization of rabbit "9"

Sept.	25,	1907.	1	agar	culture	meningococcus	"Margie"	intravenously.
"	30,	"	1	"	"	"	"	"
Oct.	5,	"	2	"	"	"	"	"
"	11,	"	3	"	"	"	"	"
"	21,	"	5	"	"	"	"	"
Nov.	6,	"	Aleuronat given.					
"	7,	"	Bled to death and exudate taken,					

so that the result of this comparison is very interesting. The test was an unusually severe one in both experiments. No control, with the exception of the very heavy one of November fourteenth, which should not be taken into account, lived over twenty-one hours. In the animals receiving normal leucocyte extract we have fifty per cent of recoveries in each experiment; and one out of the two animals which died lived for nearly eight and one-half days. In the case of the animals receiving immune leucocyte extract we have the only animal so treated after five hours living, and fifty per cent of those treated after twenty-one hours, thus giving sixty-six and one-third per cent recoveries, while the only animal of this series which died survived nine and one-half days.

Of the animals receiving immune serum fifty per cent of those treated in five hours died, while one hundred per cent of those treated after twenty-one hours died, although one survived eight and one-half days after having received further treatment at the end of seventy-two hours. The percentage of recoveries was only twenty per cent. The amounts of serum used must certainly be considered large and the animal furnishing it was fairly highly immunized.

The most interesting point in the experiment is the apparent, if not real, superiority of immune leucocyte extract over normal. This of course may possibly be simply a matter of concentration, for as before remarked there is no way at present of standardizing these extracts, but results obtained by use of the immune extracts in man make us feel that in the case of meningococcus immune extracts, at least, this difference is a real one.

No conclusion can be drawn from the experiments in which

immune serum and extracts were given to the same animal. The administration of the serum and extract was simultaneous, but into different places in the animals, so as to avoid any possible diversion of complement, the serum undoubtedly being absorbed more slowly than the extract.

Not enough of these tests were made to determine whether the administration of immune serum with the extract was in any way beneficial, but that it was not markedly so is evident from the comparatively early death of the two animals —the amounts given, however, were small and the animals very ill.

Some of the animals in the experiments showed hemorrhagic spots in the ears similar to those noted in rabbits suffering from typhoid infections.

SUMMARY OF EXPERIMENTS WITH MENINGOCOCCUS INFECTIONS.

If we analyze briefly the results of our experiments on rabbits infected with meningococcus we find the following:

In every experiment the controls died.

The total number of control animals used in the experiments, in which the treated animals received normal leucocyte or immune leucocyte extract, was nine. In one of the experiments one of the animals was greatly over weight and should not have been used. The animal died, however, in thirteen days. Leaving this animal out of account, we have eight controls averaging one thousand two hundred and fifty-four grams, with an average life of twenty hours after infection.

Of the treated animals there were thirteen. Nine of these recovered and four died, over seventy per cent of recoveries.

The average weight of the nine animals which recovered was one thousand two hundred grams, and of the four which died one thousand sixty-two grams, with an average life after infection of 5.7 days.

The majority of the animals did not receive treatment until the expiration of five hours after inoculation, and a number

of them not until twenty-one to twenty-four hours, some of the controls having at times died before these animals were treated with leucocyte extract. Severer tests could hardly be devised, and when results of such tests are compared with those obtained with the use of serum, they point strongly to the value of leucocyte extracts in the treatment of this infection.

GENERAL SUMMARY AND DISCUSSION.

In the earlier sections of this paper certain facts and hypotheses of immunity were discussed, and reasons were advanced in support of the idea that leucocytes play a dominant part in the protection of the animal economy; a part, which in many infections, especially those in which poisoning is supposed to depend upon endotoxins, necessitates the direct intervention of the leucocytes themselves between the invading microörganisms and their poisons and the more highly specialized cells of the animal.

It was assumed that there were two classes of bodies active in protection and immunity, one class, such as the antitoxins, the bacteriolysins (amboceptor), agglutinins, etc., which are readily overproduced and given off or secreted by the cells producing them, and are thus present free in the blood stream and equally available for the protection of all cells of the body; and another class of bodies serving directly for the protection of the individual cells possessing them, and only indirectly, through the intervention of these cells, for the protection of the other cells of the animal economy, but not as a customary thing given off into the blood stream during the life of the cells possessing them.

From this assumption it was argued that these substances — digestive, poison-neutralizing and complementary — might possibly be liberated from leucocytes by methods of extraction, and introduced in this free condition into infected animals or man, and that they might thus act not only in protecting the flagging leucocytes, and permitting them to recuperate, but also as a shield for the more specialized cells.

This hypothesis was put to test in experiments with the inciting organisms of some of our most prevalent infections, and the results have apparently justified our expectations.

Animals suffering from severe septicemias and poisonings, following intravenous injections of such organisms as staphylococci, streptococci, pneumococci, typhoid bacilli, and meningococci,* have shown the beneficial effect of treatments with extracts of leucocytes, and have, in many instances, survived infections fatal to the control animals in thirty-six hours, even when treatment has been delayed as late as twenty-four hours.

The action of the extracted substances is evidenced in many instances by a marked fall in temperature, and by a conservation or rapid return to normal of the animal's weight.

Animals receiving treatment with the extracts often appear, however, for a time, much sicker than the controls. This is especially true of typhoid and meningococcus infected rabbits and, to a certain extent, even of pneumococcus infected animals.

What the exact action of the extracted substance is, is, of course, at present largely a matter of conjecture. The fact that treated animals in some instances appear more intoxicated than the untreated may indicate an enhanced bacteriolytic action and liberation of endotoxins, thus suggesting the presence in the extracts of complementing bodies, or of digestive bodies peculiar to the leucocytes. These bodies may, of course, be present and play an active part, but the strongest impression given to one carefully following the experiments and noting the immediate effect on temperature and the conservation and quick return to normal weight of the treated animals is that the principal substance at work is one active in neutralizing poisons, and thus able to relieve the animal economy and give the phagocyting cells an

* The report of our investigations on tuberculosis and dysentery and cholera infections in animals will soon be ready for publication. It may be said here, however, that infections due to the various strains of the dysentery bacilli and to the Spirillum of Asiatic cholera are markedly influenced by leucocyte extracts.

opportunity to carry on their work of ingesting the micro-organisms and thus permanently rendering them harmless.

Freshly obtained living leucocytes, when introduced into an infected animal, even intraperitoneally, are practically without effect on systemic infections. The lives of the animals are not lengthened and these intact leucocytes seem to have no influence on the temperatures.

Intravenous injections of living leucocytes have not been tried, since the results of such a procedure are of purely academic interest, being entirely outside the realm of possibility in the treatment of human infections. The use of living leucocytes in the treatment of local infections, such as those of the pleura or peritoneum, or even subdural infections, is of course possible, but of limited application, both theoretically and practically, and their beneficial action would probably be due to the simple regeneration of the phagocyte army, or to extracts which, unsuspectedly, accompanied the supposedly intact leucocytes introduced.

CONCLUSIONS.

Extracts of leucocytes from normal rabbits have a distinct modifying and curative action when given subcutaneously or intraperitoneally to rabbits, even on systemic infections which are rapidly fatal in untreated rabbits.

Rabbit leucocyte extracts are also active in guinea-pigs, saving them from fatal infections.

Extracts of leucocytes from immunized animals seem, in some instances at least, to possess even greater curative powers than extracts from leucocytes of normal animals.

The action of the leucocyte extract may be due to the enhancement of the bacteriolytic action of the animal's plasma by the introduction of complement or to the action of digestive substances usually not liberated from the leucocytes; but is most likely chiefly due to poison-neutralizing or destroying bodies, which act on the endotoxins, *i.e.*, endo-antitoxins or antiendotoxins, and thus relieve the leucocytes of the animal from fatal poisoning and protect the higher cells of the animal so that their functions are not deranged.

If these protective bodies are, as we suppose, poison-neutralizing in their action, it does not seem improper to refer to them as endoantitoxins, since they are apparently antibodies, which are not normally given up to the plasma by the cells possessing them, and in this peculiarity correspond to the bodies against which, it seems, they are chiefly directed, and which being fixed constituents of the bacterial cell are, therefore, referred to as endotoxins. It would seem, then, more logical to refer to these nondiffusible bodies of the animal cell as endoantitoxins than as antiendotoxins.

It does not seem unlikely, then, that extracts of leucocytes (polymorphonuclear and mononuclear), and possibly of the blood-forming organs, furnish us with means of combating infections incited by those microörganisms generally looked upon as giving rise to endotoxin poisonings, and which have steadily refused to yield to the action of immune sera alone.

[It is not only a pleasure to the writer, but a duty, to mention the invaluable services of his constant and faithful assistant in this work, James May, through whose careful observation of the animals and skill in the preparation of the extracts this series of experiments was largely made possible.]

II.

ON THE PRECIPITATION OF BACTERIAL EXTRACTS BY EXTRACTS OF LEUCOCYTES.*

PHILIP HANSON HISS, JR., M.D., AND HANS ZINSSER, M.D.

INTRODUCTORY. — The results obtained by one of us (preceding paper) in mitigating and often curing infections in animals by subcutaneous injections of aqueous extracts of rabbit leucocytes naturally led to speculation as to the mode of action of the injected leucocyte substances.

The close observation of treated subjects, both animal and human, together with the results obtained by experiments to be separately recorded, pointed rather to a neutralizing power on the part of the leucocyte extracts toward the bacterial poisons — probably the endotoxins — than to any marked or immediate bactericidal, bacteriolytic, opsonic, or antitoxic action in the sense of that of the diphtheria or tetanus antitoxins.

All the evidence collected at the time this work was begun, while by no means conclusive, seemed, therefore, to indicate that the protective action of leucocyte extracts might lie in their endotoxin-neutralizing or coagulating properties.

With this as a premise, it did not seem improbable that the leucocyte extracts when brought into contact with bacterial extracts under proper conditions might give rise to precipitates not unlike those observed when homologous immune sera are mixed with similar bacterial extracts.

It appears from the work of Kraus and Levaditi (C. R. Acad. Sc., cxxxviii, 1904; Ref. Bull. Ann. Past., 1904, p. 498) that the specific precipitins themselves may possibly originate in the leucocytes. These considerations gave considerable additional encouragement to the proposed scheme of experimentation.

* Received for publication Sept. 8, 1908.

Preparation of extracts. — The leucocyte extracts employed for the experiments were obtained in the same manner as those used for therapeutic purposes, in fact, were, in most cases, from the same stock supply.

Aleuronat exudates from the pleural cavities of rabbits were centrifugalized, the supernating fluid decanted, and the cell residue extracted in sterile distilled water for periods ranging from five or six hours to several weeks. The extractions are probably fairly complete in a short time when distilled water is used. After a few hours at 37.5° C. the extracts were preserved in the ice-box until used.

Just prior to using, the leucocyte remains were separated by centrifugalization at high speed for from fifteen to thirty minutes, the yellowish supernatant fluid being removed with sterile pipettes. This clear aqueous extract was used for the experiments.

The bacterial extracts employed were obtained by the extraction of twenty-four to forty-eight hour agar slant cultures in sterile distilled water or, in some cases, in physiological salt solution (.85 per cent). Extraction of the bacteria was usually continued for two or three days at 37.5° C., followed by three or four days at room temperature. The bacterial bodies were then removed by filtration under suction through Berkefeldt candles.

EXPERIMENTS. — Mixtures of the various substances were made in small sterile agglutination tubes in quantities as indicated in the tables:

Experiment I. — Aqueous extract of typhoid bacilli, aqueous leucocyte extract:

(*a*)	Bacterial extract	.5 cc.	+	leucocyte extract	.5 cc.	=	+	ppt.
(*b*)	"	" .5 "	+	"	" .5 "	=	+	"
(*c*)	"	" .5 "	+	distilled water	.5 "	=	O.	
(*d*)	Leucocyte	" .5 "	+	"	" .5 "	=	O.	

The mixtures were incubated for one hour, then kept at room temperature. After twenty-four hours (*a*) and (*b*) showed slight flocculent precipitate. (*c*) and (*d*) were clear.

Experiment II. — Aqueous extract of diphtheria bacilli, aqueous leucocyte extract:

(*a*) Bacterial extract .5 cc. + leucocyte extract .5 cc. = ppt. slight.
(*b*) " " .5 " + " " .5 " = " "
(*c*) " " .5 " + distilled water .5 " = O.
(*d*) " " .5 " + " " .5 " = O.

Twelve hours at room temperature brings no precipitate; twelve hours incubation after this brings a barely visible precipitate in (*a*) and (*b*), which intensifies slightly after seventy-two hours, but, even then, remains slight. (*c*) and (*d*) remain clear.

Experiment III. — Aqueous extract of cholera vibrio, aqueous leucocyte extract:

(*a*) Bacterial extract .5 cc. + leucocyte extract .5 cc. = ppt.
(*b*) " " .5 " + " " .5 " = "
(*c*) " " .5 " + distilled water .5 " = O.
(*d*) " " .5 " + " " .5 " = very slight ppt.

After forty-eight hours a marked precipitate appeared in (*a*) and (*b*) flocculent and slightly yellowish. (*c*) is clear but (*d*) showed a very slight precipitate about one-quarter in volume of that in (*a*) or in (*b*), and of a whitish, finely granular appearance.

Experiment IV. — Aqueous extract of cholera vibrio, aqueous leucocyte extract:

(*a*) Bacterial extract .5 cc. + leucocyte extract .5 cc.
(*b*) " " .5 " + " " .5 "
(*c*) Leucocyte " .5 " + distilled water .5 "

After twenty-four hours at room temperature (*a*) and (*b*) show heavy yellowish flocculent precipitates. (*c*) shows a slight white granular precipitate.

Experiment V. — Aqueous extract of cholera vibrio, aqueous leucocyte extract:

(*a*) Bacterial extract 1 cc. + leucocyte extract 1 cc.
(*b*) " " 1 " + distilled water 1 "
(*c*) Leucocyte " 1 " + " " 1 "

After eighteen hours at room temperature (*a*) shows a marked flocculent yellowish precipitate, (*b*) is clear, and (*c*) shows a fine granular white precipitate about one-quarter in volume of that in (*a*).

Experiment VI. — Aqueous extract of meningococcus, aqueous leucocyte extract:

(*a*) Bacterial extract 1 cc. + leucocyte extract 1 cc.
(*b*) " " 1 " + distilled water 1 "
(*c*) Leucocyte " 1 " + " " 1 "

After twenty-four hours (*a*) and (*c*) show precipitates, the one in (*a*) slightly greater and more flocculent than the one in (*c*). (*b*) is clear.

Experiment VII. — Aqueous extract of streptococcus, aqueous leucocyte extract:

(*a*) Bacterial extract .5 cc + leucocyte extract .5 cc.
(*b*) " " .5 " + distilled water .5 "
(*c*) " " .5 " + " " .5 "

After twenty-four hours at room temperature (*a*) shows a slight yellowish flocculent ppt. (*b*) is clear and (*c*) shows a very slight white granular ppt. The difference between (*a*) and (*c*) is well marked, easily observed, and beyond question.

In the preceding experiments, simple mixtures of equal quantities of aqueous extracts of the bacteria and the leucocytes were made. In all of these tests the precipitate formed after the mixture of leucocyte and bacterial extracts was easily distinguishable, both by its greater quantity and its yellowish flocculent nature, from that taking place when water alone was added to either one of the two extracts.

It will be noticed that in the experiments recorded above, leucocyte extract plus bacterial extract was controlled by leucocyte extract plus sterile distilled water.

The precipitates in the extract mixtures, tested by the various proteid reactions, showed a positive but faint xanthoproteic reaction and an extremely faint, hardly noticeable Biuret reaction. When washed with salt solution and the washings salted out with Magnesium sulphate, small traces of globulin could be detected.

It seemed not impossible that the bacterial extracts might contain salts which, in acid mixtures (the leucocyte extract having an average titer of about .3 per cent acidity to NaOH, — the bacterial extracts varying from .1 per cent to

.05 per cent acidity), might have a tendency to cause proteid precipitation. The precipitates formed in the experiments might then be shown to be merely an expression of proteid insolubility under the given conditions. Qualitative tests of the bacterial filtrates with $AgNo_3$ with HNO_3 and ammonium molybdate, and with HCl and $BaCl_2$ showed these to contain very faint traces of chlorides, phosphates and in sulphates respectively. Since, in slightly acid or neutral environment, globulins are held in solution by dilute sodium chloride, it was consequently determined to extract both leucocytes and bacteria with .85 per cent salt solution and with these extracts to parallel the experiments previously done. In the various mixtures the amount of salt present would then be sufficient in each case to preclude precipitation due merely to insolubility of the globulin.

The following experiments were tried:

Experiment VIII. — Salt solution extract of staphylococcus pyogenes aureus, salt solution leucocyte extract:

(*a*)	Bacterial extract	.5 cc.	+	leucocyte extract	.5 cc.
(*b*)	" "	.5 "	+	" "	.5 "
(*c*)	" "	.5 "	+	salt solution .85%	.5 "
(*d*)	Leucocyte "	.5 "	+	" " .85%	.5 "

After twenty-four hours (*a*) and (*b*) show heavy flocculent precipitates. (*c*) shows none. (*d*) shows a very slight granular precipitate.

Experiment IX. — This experiment was a complete repetition of Experiment VIII., except for a doubling of the quantities used. (*a*) and (*b*) again show flocculent yellowish precipitates. (*c*) is clear, and (*d*) shows a fine granular precipitate about one-eighth in quantity of that in (*a*) or in (*b*).

Experiment X. — Salt solution extract of meningococcus, salt solution leucocyte extract:

(*a*)	Bacterial extract	.5 cc.	+	leucocyte extract	.5 cc.
(*b*)	" "	.5 "	+	" "	.5 "
(*c*)	" "	.5 "	+	salt solution .85%	.5 "
(*d*)	Leucocyte "	.5 "	+	" " .85%	.5 "

After twelve hours at room temperature (*a*) and (*b*) show heavy yellowish

flocculent precipitates. (*c*) is clear. (*d*) shows a fine granular white precipitate about one-fifth in quantity of that in (*a*) or in (*b*).

Experiment XI. — This experiment was an exact repetition of Experiment X., except for a doubling of the quantities used. The results were exactly similar to those of Experiment X.

The foregoing four experiments showed, then, that extraction with salt solution gives exactly the same results as those obtained in experiments done with aqueous extracts. They show, therefore, that the precipitates formed, when bacterial extract is added to leucocyte extract, do not depend merely upon globulin insolubility under the conditions of the experiment.

The experiments which follow were carried out primarily with the intention of determining the influence of dilution of the leucocyte extracts upon the formation of precipitates. While giving little information in this respect because of unavoidable differences in the various leucocyte extracts as produced with the technic in use at present, the experiments tended, in a general way, to confirm those done before. Variations in leucocyte extracts manufactured at different times from different rabbits became evident in various ways. Some of them, possibly because of too high a concentration with the extracted substances, gave slight precipitates on standing, without any additions. This phenomenon, at first confusing, could usually be obviated by dilution of the extract in question with distilled water. Just what the cause of such spontaneous precipitation is, cannot at present be stated.

At other times, in aqueous leucocyte extract-bacterial extract mixtures which, at first, gave no precipitates whatever, such precipitates could be brought out by the addition of a few drops of 1.5 per cent NaCl solution, the similarly treated controls remaining clear. This phenomenon cannot be explained at the present time but is extremely suggestive because of its similarity to the observation of Bordet, who

found the presence of NaCl necessary for the occurrence of agglutination reactions. Whatever the explanation of this may be, however, the tables given for the following experiments will show that, in all cases, where bacterial extract and leucocytic extract were mixed, either with or without the addition of small quantities of salt solution, the precipitate which was formed was quantitatively and qualitatively different from the slight granular white precipitate formed in the control tubes.

Experiment XII. — Aqueous extract of meningococcus, aqueous leucocyte extract:

(*a*)	Bacterial extract	1	cc.	+	leucocyte extract	1	cc.
(*b*)	"	"	1	"	+	"	"	.5	"
(*c*)	"	"	1	"	+	"	"	.2	"
(*d*)	"	"	1	"	+	"	"	.1	"
(*e*)	Bacterial	"	1	"					
(*f*)	Leucocyte	"	1	"					

After twenty-four hours at room temperature none of the tubes show any precipitate. To (*a*), (*b*), and (*f*) are added a few drops of 1.5 per per cent salt solution.

After further twenty-four hours at room temperature (*a*) shows a heavy flocculent precipitate. (*b*) shows a moderate precipitate similar to that of (*a*). (*f*) shows nothing until further twenty-four hours have elapsed when a slight granular precipitate appears.

Experiment XIII. — Aqueous extract of staphylococcus pyogenes aureus, aqueous leucocyte extract:

(*a*)	Bacterial extract	1	cc.	+	leucocyte extract	1	cc.
(*b*)	"	"	1	"	+	"	"	.5	"
(*c*)	"	"	1	"	+	"	"	.2	"
(*d*)	"	"	1	"	+	"	"	.1	"
(*e*)	"	"	1	"					
(*f*)	Leucocyte	"	1	"					

After twenty-four hours at room temperature (*a*) shows a very slight precipitate, all the other tubes remain clear. Salt solution 1.5 per cent is added in quantities of two drops to (*a*), (*b*), (*d*), and (*f*). After further twenty-four hours (*a*), (*b*), and (*d*) show marked yellowish flocculent precipitates, less in quantity in (*d*) than in the other two. (*f*) shows no precipitate.

Experiment XIV. — Aqueous extract of meningococcus, aqueous leucocyte extract:

(*a*)	Bacterial extract	.5	cc.	+	leucocyte extract	.5	cc.						
(*b*)	" "	.5	"	+	" "	.5	"						
(*c*)	" "	.5	"	+	" "	.5	"	+	1.5%	salt sol.	.1	cc.	
(*d*)	" "	.5	"	+	" "	.5	"	+	1.5%	" "	.1	"	
(*e*)	Leucocyte "	.5	"	+	distilled water	.5	"						
(*f*)	" "	.5	"	+	" "	.5	"	+	1.5%	" "	.1	"	
(*g*)	Bacterial "	.5	"	+	" "	.5	"	+	1.5%	" "	.1	"	

In this experiment it was attempted to determine whether any difference could be detected between tubes to which salt had been added at the beginning and those to which no salt had been added. Also, whether salt solution added in exactly the same quantity to a mixture of leucocyte extract and distilled water would give rise to similar precipitates. The leucocyte extract used for this experiment did not belong to the same lot as that used in Experiments XII. and XIII. and, unlike the latter, did not show spontaneous precipitation. In the experiments recorded above (*a*) and (*b*), (*c*) and (*d*) showed heavy yellowish flocculent precipitates which came out within twenty-four hours, whereas the controls (*e*), (*f*), and (*g*) showed, in the same time, very slight finely granular white precipitates not one-fifth in volume of those shown in the first four tubes.

Experiment XV. — Aqueous extract of staphylococcus pyogenes aureus, aqueous leucocyte extract:

(*a*)	Bacterial extract	.5	cc.	+	leucocyte extract	.5	cc.						
(*b*)	" "	.5	"	+	" "	.5	"	+	1.5%	salt sol.	.1	cc.	
(*c*)	Distilled water	.5	"	+	" "	.5	"						
(*d*)	" "	.5	"	+	" "	.5	"	+	1.5%	" "	.1	"	
(*e*)	Bacterial extract	.5	"	+	distilled water	.5	"	+	1.5%	" "	.1	"	

After twenty-four hours at room temperature (*a*) and (*b*) showed heavy yellowish coarse precipitates. (*c*) and (*d*) showed fine granular white precipitates, not one-fourth in volume of those in (*a*) or (*b*). (*e*) remained entirely clear.

Experiment XVI. — Aqueous extract of meningococcus, aqueous leucocyte extract:

(*a*) Leucocyte extract 1 cc. + bacterial extract 1 cc. + 1.5% salt. sol. .2 cc.
(*b*) " " 1 " + distilled water 1 "
(*c*) " " 1 " + " " 1 " + 1.5% " " .2 "

After twenty-four hours at room temperature (*a*) shows a heavy, coarsely flocculent precipitate. (*b*) and (*c*) show fine granular precipitates about one-fifth in volume of that in (*a*).

In the preceding four experiments the addition of small quantities of salt solution does not seem to have influenced the reaction in any way.

In all of the foregoing experiments a slight granular sediment was noted in the controls. While this has been quantitatively and qualitatively easily distinguishable from the precipitates taking place in the extract mixtures, it was deemed advisable to make an attempt to eliminate all precipitation in the controls by diluting the leucocyte extract. With this in view the following experiment was done:

Experiment XVII. — Aqueous extract of meningococcus, aqueous leucocyte extract:

I. (*a*) Leucocyte extract .5 cc.
(*b*) " " .5 " + distilled water .5 cc.
(*c*) " " .25 " + " " .75 "
(*a*) " " .1 " + " " .9 "

After twenty-four hours at room temperature (*a*) shows a very slight granular sediment. (*b*), (*c*), and (*d*) are absolutely clear.

II. (*a*) Leucocyte extract .5 cc. + bacterial extract .5 cc.
(*b*) " " .25 " + " " .5 " dist. water .25 cc.
(*c*) " " .1 " + " " .5 " " " .4 "

After twenty-four hours (*a*) shows heavy flocculent precipitate far heavier than any of those found in set I. (*b*) shows a similar precipitate but very much less in amount. (*c*) is clear.

Experiment XVIII. — Aqueous extract of staphylococcus pyogenes aureus, aqueous leucocyte extract:

(*a*) Leucocyte extract .5 cc. + bacterial extract .5 cc.
(*b*) " " .25 " + " " .5 " dist. water .25 cc.
(*c*) " " .1 " + " " .5 " " " .4 "

After twenty-four hours (*a*) shows very heavy yellowish precipitate. (*b*) and (*c*) are clear.

The observations in the preceding two experiments seem to show that spontaneous precipitation of leucocyte extract can be eliminated by dilution with distilled water, and further tend to show that dilution of the leucocyte extract before mixing with bacterial extract leads to diminution of the precipitate formed.

It was noticed in the course of these experiments that spontaneous precipitation in the leucocyte extract could often be avoided without dilution by the addition of small quantities of sodium chloride. One-tenth of a cubic centimeter of a 1.5 per cent salt solution added to one cubic centimeter of leucocyte extract in most cases prevented the formation of any spontaneous precipitates. Thus, in the following:

Experiment XIX. — Aqueous extract of meningococcus, aqueous leucocyte extract:

(*a*) Leucocyte extract 1 cc. (after twenty-four hours) slight ppt.
(*b*) " " 1 " + .1 cc. 1.5% salt sol. no ppt.
(*c*) " " 1 " + .1 " 1.5% " " " "

In the preceding, the salt solution added to tubes (*b*) and (*c*) has held in solution the substances precipitated in (*a*). To (*b*) was then added .5 cubic centimeter aqueous extract of meningococcus. To (*c*) was added .5 cubic centimeter distilled water. After twenty-four hours (*b*) shows heavy flocculent precipitate. (*c*) is clear.

Experiment XX. — Aqueous extract of meningococcus, aqueous leucocyte extract:

(*a*) Bacterial extract 1 cc. + leucocyte extract 1 cc. + .85% salt sol. .5 cc.
(*b*) " " .5 " + " " 1 " + .85% " " .5 "
(*c*) " " 1 " + distilled water 1 " + .85% " " .5 "
(*d*) " " 1 " + " " .5 " + .85% " " .5 "

After twelve hours at room temperature (*a*) and (*b*) show moderate flocculent precipitates. (*c*) and (*d*) show extremely slight granular ones.

Experiment XXI. — Aqueous extract of cholera vibrio, aqueous leucocyte extract.

(*a*) Leucocyte extract .5 cc. + .85% salt sol. .2 cc.
(*b*) " " .3 " + .85% " " .5 "

Three tubes of each of the preceding mixtures were made, and after standing twenty-four hours none of them showed any precipitate. At the end of this time, to one tube of each set was added .5 cubic centimeter of an aqueous extract of the cholera vibrio. To another tube of each set was added .5 cubic centimeter of distilled water. The third tube of each set was left for further observation without any additions. After further twelve hours at room temperature the tubes to which the extract of cholera vibrio have been added showed heavy flocculent precipitates. The tubes to which pure distilled water had been added showed no precipitates and the control tubes to which nothing had been added continued to remain clear.

SUMMARY AND CONCLUSIONS.

In summing up the experiments recorded in the preceding pages, it is but fair to state that other experiments were done beside those recorded, and some of these did not give such uniform results as those given in the tables. Spontaneous precipitation of the leucocyte extract was often so heavy that differences between experiment and control were hard to recognize. Whatever irregularities occurred seem to depend upon differences between the various lots of leucocyte extract. The scant knowledge which we possess at the present time, regarding the physical and chemical composition of these substances, makes it impossible to prepare the extracts so that they are completely uniform in composition and strength.

In spite, however, of irregularities and many experimental difficulties, it seems to us from our observations that there can be no reasonable doubt as to the formation of a distinct precipitate when leucocyte extract and bacterial extract are mixed, a precipitate which is quantitatively and qualitatively easily differentiated from that occasionally formed in the control tubes. We believe, therefore, that the results obtained probably justify the following conclusions:

I. That distinct precipitates are formed when aqueous or saline leucocyte extracts are added to aqueous or saline extracts of bacteria.

II. That these precipitates are not merely indications of the insolubility of proteid or other substances, due to adventitious circumstances under the given experimental conditions, but are reactions not unlike other immune reactions,

and are due to a combination of leucocytic with bacterial substances.

III. That the precipitates formed while varying in quantity with different species of bacteria, are probably not to be regarded as specific, and the differences in the quantities of precipitates may possibly be indications of the more complete liberation of bacterial cell contents in the case of some organisms than in that of others.

1 2 3 4 5 6

Photograph showing the quantitative differences between the precipitates occurring in the leucocyte extract-bacterial extract mixtures and those appearing in the controls.

Tubes 1, 2, and 3, aqueous extract of meningococcus plus aqueous leucocyte extract.

Tubes 4, 5, and 6, distilled water plus leucocyte extract.

III.

OBSERVATIONS ON THE MECHANISM OF PROTECTION BY LEUCOCYTE EXTRACTS.*

HANS ZINSSER, M.D., AND PHILIP HANSON HISS, JR., M.D.

The extensive animal experiments reported by Hiss in a previous paper have demonstrated that aqueous extracts of rabbit leucocytes exert a considerable curative influence upon animals infected with various microöganisms.

The purpose of the experiments which form the subject of the present paper was to ascertain, if possible, whether the manner of action of the leucocytic substances could be determined by a controlled study of the phenomena occurring after intraperitoneal infection in the presence of leucocyte extracts.

Considerable and careful work has been done upon the bactericidal value of leucocytes and their extracts by Petterson (Centralblatt f. Bakt., 1905, I., xxxix, 423 and 613). Working with various strains of proteus, Petterson comes to the conclusion that leucocytes and leucocyte extracts possess distinct bactericidal properties for these microorganisms.

In regard to the cholera vibrio and typhoid bacillus, however, he agrees with Schattenfroh (Arch. f. Hyg., xxxi, 27) in denying the bactericidal action of leucocytes and leucocyte extracts.

The conclusions reached by Petterson in his very instructive paper are embodied in an ingenious summary in which he asserts that the destruction of bacteria in the animal organism, immunity, in other words, may depend upon two entirely separate factors. On the one hand, upon the lytic substances of the serum, on the other hand, upon the bactericidal substances contained in the leucocytes. As an example of the former type of immunity he cites the marked

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lysis of cholera spirilla in immune guinea-pig serum. As an example of the latter, the natural and artificial immunity against anthrax of the dog and cat, animals which may be immune against this microörganism without showing the slightest trace of lytic power in their serum.

I.

It was thought by the writers that the phenomena, clinical and experimental, observed during their work with leucocyte extracts gave little indication of immediate bactericidal power possessed by these substances, but pointed rather to a marked power on the part of the leucocyte extract to reduce the purely toxemic manifestations in infected subjects.

The following studies were made with the purpose of gaining further light upon this question:

EXPERIMENTS UPON INTRAPERITONEAL INFECTION WITH VIBRIO CHOLERÆ ASIATICÆ.

Experiment I. — Two guinea-pigs were used.

(*a*.) Weight, five hundred grams. Received intraperitoneally three cubic centimeters of an emulsion of four loopfuls of an agar slant culture emulsified in broth, plus four cubic centimeters of plain broth. A volume of seven cubic centimeters.

(*b*.) Weight, five hundred grams. Received three cubic centimeters of same cholera emulsion plus four cubic centimeters of aqueous extract of rabbit leucocytes = seven cubic centimeters.

The exudate was taken from the peritoneal cavity and examined morphologically at intervals as follows:

(*a*.)	(*b*.)
10 minutes. Exudate shows few lymphocytes and few free vibrios.	10 minutes. Same as (*a*).
30 minutes. Lymphocytes and a few polynuclear leucocytes. Slight increase in free bacteria. No phagocytosis.	30 minutes. Distinctly more leucocytes than in (*a*) — especially larger proportion of polynuclears. No phagocytosis.
1 hour. General increase of leucocytes and of free bacteria. Slight phagocytosis. No evidence of bacteriolysis.	1 hour. Differs from (*a*) only in that there are distinctly more leucocytes. Bacteria and phagocytosis same as in (*a*).

2½ hours. Extracellular vibrios much more numerous. No marked increase in leucocytes. Slight phagocytosis.	2½ hours. Distinctly more leucocytes than in (*a*). Otherwise the same.
3½ hours. Very little change from 2½ hours except for increase in number of vibrios. No distinct swelling or degeneration of bacteria. Phagocytosis slightly increased.	3½ hours. Differs from (*a*) only in larger numbers of leucocytes present.
6 hours. Enormous increase in polynuclear leucocytes. Enormous increase in free bacteria. Vibrios look normal. Phagocytosis slightly increased.	6 hours. Almost exactly like (*a*). No distinct difference.
20 hours. Exudate a solid mass of leucocytes. Vibrios in large numbers; many appear swollen and degenerated. Guinea-pig decidedly sick.	20 hours. Almost no leucocytes present. Few vibrios and a few cocci from secondary infection present in exudate.
(*a*) dies after thirty hours.	(*b*) alive and well four days after inoculation.

Experiment II. — Two guinea-pigs were used.

(*a*.) Weight, about four hundred grams. Received intraperitoneally one cubic centimeter of an emulsion of cholera vibrios in salt solution (five loopfuls of a twenty-four agar slant, emulsified in five cubic centimeters of salt solution) plus two cubic centimeters normal salt solution. A volume of three cubic centimeters.

(*b*.) Weight, about four hundred grams. Received intraperitoneally one cubic centimeter of same cholera emulsion plus two cubic centimeters aqueous extract of rabbit leucocytes.

Exudate observed morphologically at intervals as below:

(*a*.)	(*b*.)
1 hour. A few lymphocytes present and a few free spirilla.	1 hour. Same as (*a*).
3 hours. Increase in leucocytes and free bacteria. No phagocytosis.	3 hours. Distinctly more leucocytes, especially polynuclear, than are found in (*a*).
6 hours. Leucocytes steadily increasing; vibrios also increased but less so than leucocytes. More phagocytosis; many of the vibrios appear vacuolated and degenerated.	6 hours. Differs very little from (*a*).

Agar plates poured with one drop of exudate each, from (*a*) and from (*b*) show thick growth in both. No noticeable difference in numbers of colonies.

7½ hours. Leucocytes numerically increasing and appear degenerated. Vibrios increasing less rapidly and show more degeneration forms. Moderate phagocytosis.	7½ hours. Does not differ from (*a*).
24 hours. Enormous leucocytosis. But few intact vibrios can be found.	24 hours. Same as (*a*).
(*a*) dies on third day after inoculation.	(*b*) dies on the fourth day after inoculation surviving (*a*) by twenty-four hours.

The exudates after death show very few vibrios intact; many degeneration forms are present and enormous numbers of leucocytes, many in a state of degeneration.

Experiment III. — The plan of this experiment was exactly like that of Experiment II. Again there was a slightly earlier appearance of leucocytes in the exudate of the guinea-pig which had received the leucocyte extract. This difference was soon equalized, however, and the exudates, taken six and more hours after inoculation, showed practically no differences between (*a*) and (*b*). Agar plates were poured after six hours with a drop of exudate each from (*a*) and (*b*). These showed no difference between the two in the numbers of colonies developed. Guinea-pig (*a*) died forty-eight hours after inoculation; (*b*) died twelve hours later than (*a*).

EXPERIMENTS UPON INTRAPERITONEAL INFECTION WITH STAPHYLOCOCCUS PYOGENES AUREUS.

The staphylococcus used for these experiments was unusually virulent.

Experiment I. — Two guinea-pigs were used.

(*a*.) Weight, six hundred grams. Received intraperitoneally three cubic centimeters of an emulsion of staphylococcus in broth plus three cubic centimeters of broth, a volume of six cubic centimeters.

(*b*.) Weight, six hundred and ten grams. Received three cubic centimeters of the same emulsion plus three cubic centimeters of leucocyte extract. Exudate observed at regular intervals as below:

(*a*.)	(*b*.)
1½ hours. Very few leucocytes present, consisting chiefly of lymphocytes. Few free bacteria present.	1½ hours. Leucocytes more plentiful than in (*a*), especially the polynuclears. Otherwise same as (*a*.).
4 hours. Slight increase in leucocytes. Many leucocytes vacuolated and degenerated. Free bacteria are more plentiful. There is marked phagocytosis.	4 hours. Marked increase in leucocytes. There are incomparably more than in (*a*). Free bacteria are increasing rapidly. Marked phagocytosis.

5 hours. General increase in leucocytes and in free bacteria. Phagocytosis is increasing.	5 hours. There is still a marked excess of leucocytosis over that in (*a*). Free bacteria and phagocytosis are rapidly increasing.
8 hours. The leucocytes have not increased in number. They appear degenerated and are crammed full of cocci.	8 hours. The exudate now resembles that of (*a*) in almost every particular.

Both animals died within twenty hours after inoculation.

Experiment II. — The plan of this experiment duplicated exactly Experiment I. Throughout, however, there was no distinct difference between the exudates of (*a*) and (*b*). Both animals died within thirty-six hours after inoculation.

Experiment III. — Two guinea-pigs were used.

A staphylococcus emulsion was made by emulsifying five loopfuls of a fresh agar culture in five cubic centimeters of normal salt solution.

(*a*.) Weight, five hundred grams. Received one cubic centimeter of the staphylococcus emulsion plus three cubic centimeters of salt solution intraperitoneally, a volume of four cubic centimeters.

(*b*.) Weight, four hundred and eighty grams. Received one cubic centimeter of the same staphylococcus emulsion plus three cubic centimeters of leucocyte extract. Exudate was observed at regular intervals as below:

(*a*.)	(*b*.)
1½ hours. Exudate contains a few lymphocytes and free bacteria.	1½ hours. There are distinctly more polynuclear leucocytes than in (*a*).
3 hours. Leucocytes and free bacteria have markedly increased. Phagocytosis is active.	3 hours. Does not differ from (*a*).
5 hours. There is marked numerical increase in the leucocytes and in the bacteria. Phagocytosis is still increasing.	5 hours. Does not differ from (*a*).
7 hours. About the same as after five hours except for a marked increase in free bacteria. There is enormous phagocytosis.	7 hours. Sudden and marked increase in the leucocytes has taken place. These now far exceed the number in (*a*). At the same time there seems to have been a marked increase in the numbers of bacteria, which also exceed those found in (*a*).
(*a*) died in three days.	(*b*) died in four and one-half days.

EXPERIMENTS UPON INTRAPERITONEAL INFECTION WITH VIRULENT PNEUMOCOCCI.

The pneumococcus for the experiments was a laboratory strain, designated as "L 2.," from the collection of Dr. Wadsworth, extremely virulent and a good inulin fermenter.

Experiment I. — Two guinea-pigs were used.

(*a*.) Weight, five hundred grams. Received intraperitoneally three cubic centimeters of (*a*) twenty-four-hour broth culture of pneumococcus plus three cubic centimeters of sterile salt solution, a volume of six cubic centimeters. Temperature before inoculation, 100.2° F.

(*b*) Weight, four hundred and fifty grams. Received intraperitoneally three cubic centimeters of same pneumococcus culture plus three cubic centimeters of aqueous extract of rabbit leucocytes. Temperature taken before inoculation, 100.8° F.

Temperature taken at intervals as below, exudate examined at same intervals.

(*a*.)	(*b*.)
3 hours. Temperature, 101° F. The exudate shows numerous polynuclear leucocytes and many mononuclear cells. Few bacteria present.	3 hours. Temperature, 102.2° F. The exudate differs from (*a*) only in that there are about one-fifth as many leucocytes.
6 hours. Temperature, 100° F. Leucocytes are more numerous and consist now chiefly of polynuclears. Leucocytes show much degeneration and vacuolization. Isolated leucocytes contain one or two cocci. The extracellular are more numerous.	6 hours. Temperature, 102° F. The exudate differs from (*a*) only in that there are fewer leucocytes. The bacteria are in about the same number as in (*a*). No positive evidence of bacteriolysis.
9 hours. Temperature, 102.2° F. The leucocytes have increased in number. There is a slight increase in phagocytosis but this is still very little. Extracellular organisms have increased.	9 hours. Temperature, 102.2° F. Does not differ from (*a*).
20 hours. Temperature, 97.8° F. Guinea-pig is decidedly sick, does not eat, fur ruffled. The exudate consists chiefly of extracellular bacteria and of large numbers of polynuclear leucocytes many of which are vacuolated and degenerated.	20 hours. Temperature, 101.6° F. Guinea-pig eating and apparently well. The exudate is hard to obtain and, when obtained, shows many polynuclear leucocytes, a few lymphocytes and large endothelial cells. Very few cocci present.
(*a*) grows gradually sicker. Dies during the night between thirty-six and forty-eight hours after inoculation.	(*b*) is alive and well on May 12, four days after inoculation; remained alive.
Autopsy shows fibrinous exudate in peritoneum. Bacteria in heart's blood.	

Experiment II. — Two guinea-pigs were used.

(*a*.) Weight, four hundred and seventy-five grams. Received intraperitoneally two cubic centimeters of a twenty-four-hour broth culture of pneumococci plus two cubic centimeters of sterile broth, a volume of four cubic centimeters. Temperature before inoculation, 99°.

(*b*.) Weight, four hundred and twenty-five grams. Received intraperitoneally two cubic centimeters of the same pneumococcus culture plus two cubic centimeters of an aqueous extract of rabbit leucocytes (leucocyte extract was two months old). Temperature before inoculation, 98.8° F.

(*a*.)	(*b*.)
1 hour. Temperature, 99° F. Exudate shows a few leucocytes, chiefly lymphocytes. Free bacteria in moderate numbers.	1 hour. Temperature, 98.8° F. Exudate does not differ from that of (*a*).
3 hours. Temperature, 97.2° F. There is a general increase both in leucocytes and in bacteria. No phagocytosis. Relatively more polynuclear leucocytes than before. There is no granulation or swelling of bacteria.	3 hours. Temperature, 97° F. Exudate differs but slightly from (*a*). Bacteria seemed clumped about leucocytes but there is no phagocytosis.
6 hours. Temperature, 97.2° F. There is slight increase in bacteria, otherwise no change.	6 hours. Temperature, 97.2° F. Exudate same as (*a*).
9 hours. Temperature, 100.2° F. Polynuclear leucocytes have increased in number. No phagocytosis.	9 hours. Temperature, 102° F. There has been an enormous increase in the leucocytes which are at least four times as numerous as in (*a*).
20 hours. Guinea-pig has died over night. Exudate in peritoneum shows a few leucocytes and many bacteria. Bacteria are found in heart's blood.	20 hours. Temperature, 98° F. Guinea-pig appears sick. Exudate shows enormous numbers of bacteria and many leucocytes.

The surviving pig (*b*) was given another dose of leucocyte extract (two cubic centimeters) at this time intraperitoneally. Exudate was taken one, two, and three hours after this injection, but showed no marked changes. Bacteria steadily increased in numbers; leucocytes were present in large clumped masses. There was no phagocytosis and no evidence of bacteriolysis. Guinea-pig (*b*) died twenty-four hours after (*a*).

Experiment III. — Two guinea-pigs were used.

(*a*.) Weight, four hundred and fifty grams. Received intraperitoneally 1.5 cubic centimeters of an emulsion of a twenty-four-hour agar slant culture of pneumococcus plus two cubic centimeters of sterile salt solution, a volume of 3.5 cubic centimeters. Temperature before inoculation, 98.8°.

(*b*.) Weight, four hundred and sixty grams. Received 1.5 cubic centimeters of the same pneumococcus culture plus two cubic centimeters of

aqueous leucocyte extract. The leucocyte extract used was two months old. Temperature before inoculation, 98° F. Temperature taken and exudate observed at intervals as below:

(*a*.)	(*b*.)
2 hours. Temperature, 99.8° F. The exudate shows sparse leucocytosis. There are moderate numbers of free bacteria. Very slight phagocytosis.	2 hours. Temperature, 101° F. The exudate shows decidedly more leucocytes than (*a*). Slight phagocytosis. Free bacteria numerically equal to those in (*a*).
3 hours. Temperature, 103° F. Exudate shows a marked increase in leucocytes and in free bacteria. No shadow forms or swelling of bacteria observed.	3 hours. Temperature, 103.6° F. The leucocytes are much more numerous than in (*a*); otherwise exudate shows no difference from (*a*).
6 hours. Temperature, 102.4° F. The leucocytes in the exudate are not increasing and are about as numerous as they were after three hours. Free bacteria present in large numbers.	6 hours. Temperature, 103.6° F. Exudate shows a tremendous increase in leucocytes. There is slightly more phagocytosis. Free bacteria are about the same as in (*a*).
20 hours. Temperature, 102.2° F. No exudate can be obtained.	20 hours. Temperature, 103.4° F. No exudate can be obtained.
26 hours. Temperature, 102° F. Exudate shows but few leucocytes, but enormous numbers of bacteria.	26 hours. Temperature, 103.6° F. Exudate shows enormous numbers of leucocytes, but moderate numbers of bacteria.
48 hours. Temperature, 98° F. Guinea-pig is dying. No exudate can be obtained.	48 hours. Temperature, 101.2° F. Guinea-pig seems lively and well. Exudate shows enormous numbers of leucocytes with a relative increase in the number of lymphocytes and large endothelial cells.
50 hours. Guinea-pig lying on bottom of cage breathing spasmodically. Is killed by chloroform. Autopsy shows intestines matted together with fibrinous exudate. Free exudate shows but few leucocytes and contains tremendous numbers of bacteria. Bacteria are plentiful in heart's blood.	50 hours. Guinea-pig is still alive and remains in fairly good condition, but gradually grows sicker. Dies on May 19, seven days after inoculation. No autopsy.

Experiment IV. — Two guinea-pigs used.

(*a*.) Weight, four hundred and sixty-five grams. Received intraperitoneally two cubic centimeters of an emulsion of a twenty-four-hour agar culture of pneumococcus in salt solution plus two cubic centimeters of sterile salt solution, a volume of four cubic centimeters. Temperature before inoculation, 101° F.

(*b.*) Weight, four hundred and twenty grams. Received two cubic centimeters of the same pneumococcus emulsion plus two cubic centimeters of an aqueous extract of rabbit leucocytes. Temperature before inoculation, 101.6° F. Temperatures taken and exudate taken as follows:

(*a.*)	(*b.*)
1 hour. Temperature, 99.2° F. Exudate shows few leucocytes, chiefly lymphocytes. Many free bacteria; no phagocytosis.	1 hour. Temperature, 101.8° F. Exudate shows moderate numbers of polynuclear leucocytes and lymphocytes. Many free bacteria; no phagocytosis.
2½ hours. Temperature, 101.4° F. The leucocytes in the exudate have increased only slightly. The free bacteria are numerous. There is slight phagocytosis. There is no swelling or morphological evidence of lysis.	2½ hours. Temperature, 102.8° F. The leucocytes in the exudate are decidedly more numerous than in (*a*). Free bacteria about the same as in (*a*). There is very slight phagocytosis.
5 hours. Temperature, 101° F. General increase of leucocytes in exudate and of free bacteria.	5 hours. Temperature, 102.6° F. There are decidedly more polynuclear leucocytes than in (*a*); otherwise there is no difference.
10 hours. Temperature, 102.2° F. Exudate shows many leucocytes and free bacteria. Very slight phagocytosis. There is marked clumping of the leucocytes.	10 hours. Temperature, 103.6° F. The exudate shows practically no difference from that of (*a*).
20 hours. Temperature, 99.8° F. Guinea-pig appears decidedly sick. Does not eat. The exudate shows fewer leucocytes, but enormous numbers of free bacteria.	20 hours. Temperature, 101.8° F. Appears well, runs about and eats. No exudate can be obtained.
Guinea-pig died twenty-four hours after inoculation. Autopsy showed few leucocytes, but enormous numbers of bacteria in peritoneal cavity. Bacteria found in heart's blood.	Guinea-pig remained alive until three days after inoculation, when it died, thus outliving pig (*a*) by two days. Autopsy showed a thick exudate in peritoneal cavity which contained large numbers of leucocytes and endothelial cells. Bacteria present in heart's blood.

The preceding experiments having shown the plan of experimentation, those following, simply confirmatory repetitions, will be summarized.

Experiment V. — Two guinea-pigs were used.

(*a.*) Weight, three hundred and fifty grams. Received intraperitoneally 1.5 cubic centimeters of an emulsion of one agar slant of

pneumococcus in salt solution plus two cubic centimeters of sterile salt solution. Temperature before inoculation, 99.8° F.

(*b*.) Weight, three hundred and sixty grams. Received intraperitoneally 1.5 cubic centimeters of the same pneumococcus emulsion plus two cubic centimeters of aqueous leucocyte extract.

The peritoneal exudate was taken one and one-half hours, four, seven, twelve, and twenty-four hours after inoculation. Comparative study of the exudates of (*a*) and (*b*) show practically no difference in appearance or in numbers of bacteria. The leucocytes in the exudate of the untreated guinea-pig were less rapid in appearance and fewer in number throughout. Shadow forms and swelling of bacteria was scarce in both, showing no difference in this respect between the two. Streak plates on agar made with a drop of exudate from (*a*) and (*b*) each, seven hours after inoculation, showed confluent growths along the central streaks, with isolated colonies along the periphery. There is no difference in the extent of growth in the plates planted from (*a*) and from (*b*). The growth of bacteria in the exudates seems to have been equally rapid in both pigs.

Temperatures:

1 hour 30 minutes:	(*a*) 98.8° F.,	(*b*) 102.6° F.
4 hours:	(*a*) 100° F.,	(*b*) 103.6° F.
7 "	(*a*) 100° F.,	(*b*) 102.6° F.
12 "	(*a*) 101° F.,	(*b*) 103.6° F.
24 "	(*a*) 97° F.,	(*b*) 97.8° F.

The untreated pig died after twenty-four hours. The guinea-pig which had received leucocyte extract remained alive until four days after inoculation. No autopsies done.

Experiment VI. — Two guinea-pigs used.

(*a*.) Weight, three hundred and eighty grams. Received intraperitoneally 1.5 cubic centimeters of an emulsion of pneumococcus in salt solution plus two cubic centimeters of sterile salt solution, a volume of 3.5 cubic centimeters. Temperature before inoculation, 99° F.

(*b*.) Weight, three hundred and fifty grams. Received 1.5 cubic centimeters of same pneumococcus emulsion plus two cubic centimeters of aqueous extract of rabbit leucocytes. Temperature before inoculation, 99° F.

Temperatures taken and exudates studied four, ten, and twenty hours after inoculation. Throughout, there was no difference in speed of multiplication or in appearance of bacteria. Again the polynuclear leucocytes were more rapid in appearance and far more plentiful in the exudate of the animal receiving the leucocyte extract than in the other. This difference in amount of leucocytic reaction was almost equalized after twenty hours, however, and evident only during the first hours after inoculation.

Temperatures:

4 hours:	(*a*) 100.8° F.,	(*b*) 101.8° F.
10 "	(*a*) 102.4° F.,	(*b*) 103.6° F.
20 "	(*a*) 98° F.,	(*b*) 98.4° F.
30 "	(*a*) 95.4° F.,	(*b*) 96.8° F.

(*a*) died about thirty-six hours after inoculation.
(*b*) died about forty hours after inoculation.

Experiment VII. — Five guinea-pigs were used.

(*a.*) Weighed three hundred and ninety grams. Received intraperitoneally 1.5 cubic centimeters pneumococcus emulsion in salt solution plus one cubic centimeter aqueous extract of rabbit leucocytes plus two cubic centimeters salt solution, a volume of 4.2 cubic centimeters. Temperature before inoculation, 100.4° F.

(*b.*) Weighed five hundred and fifty grams. Received intraperitoneally 1.5 cubic centimeters pneumococcus emulsion plus two cubic centimeters leucocyte extract plus one cubic centimeter salt solution. Temperature before inoculation, 99.8° F.

(*c.*) Weighed four hundred and ninety-five grams. Received intraperitoneally 1.5 cubic centimeters pneumococcus emulsion plus three cubic centimeters of leucocyte extract. Temperature before inoculation, 100° F.

(*d.*) Weighed six hundred and sixty grams. Received intraperitoneally 1.5 cubic centimeters pneumococcus emulsion plus three cubic centimeters salt solution. Temperature before inoculation, 100° F.

(*e.*) Weighed five hundred and five grams. Received intraperitoneally 1.5 cubic centimeters pneumococcus emulsion plus three cubic centimeters salt solution. Temperature before inoculation, 99.6° F.

Attention is called in this experiment to the markedly greater weights of the controls (*d*) and (*e*). The animals were inoculated in the evening and the temperatures taken and exudates studied at irregular intervals as follows:

Temperatures:

4 Hours.	6 Hours.	15 Hours.
(*a*) 99° F.	(*a*) 98.8° F.	(*a*) 97° F.
(*b*) 100.6° F.	(*b*) 99° F.	(*b*) 99° F.
(*c*) 102° F.	(*c*) 101.2° F.	(*c*) 101.6° F.
(*d*) 101.6° F.	(*d*) 100.6° F.	(*d*) 100° F.
(*e*) 102° F.	(*e*) 100.4° F.	(*e*) 94.2° F.

First exudate taken fifteen hours after inoculation.

(*a.*) Exudate shows enormous numbers of free bacteria, few leucocytes, no phagocytosis.

(*b.*) Leucocytes are more numerous than in (*a*) but appear degenerated.

(*c.*) The exudate shows leucocytes in enormous numbers, at least ten times as many as in (*a*) or (*b.*) Bacteria are numerous, but clumped

about leucocytes. There are no shadow forms and apparently no degenerated bacteria.

(*d*) and (*e*) show exudates most similar to that of (*a*) containing many free bacteria and markedly fewer leucocytes than are contained in the exudate of (*c*).

In spite of the much larger size of (*d*) and (*e*) these guinea-pigs were the first to die. (*e*) died eighteen hours after inoculation.

(*a*) died twenty-one hours after inoculation, (*b*) thirty hours after inoculation, and (*c*) twenty-six hours after inoculation.

In this experiment the animals died in almost the exact order of the amounts of leucocyte extract which had been injected. The characters of the exudates will be discussed in the final summary.

Experiment VIII. — Three guinea-pigs were used.

(*a*.) Weighed six hundred and sixty-five grams. Received intraperitoneally .5 cubic centimeter of a pneumococcus emulsion in salt solution plus two cubic centimeters of leucocyte extract plus two cubic centimeters of salt solution, a volume of 4.5 cubic centimeters Temperature before inoculation, 99° F.

(*b*.) Weighed six hundred and sixty-five grams. Received .5 cubic centimeter of the same pneumococcus emulsion plus four cubic centimeters of leucocyte extract. Temperature before inoculation, 98.8° F.

(*c*.) Weighed seven hundred grams. Received .5 cubic centimeter of the pneumococcus emulsion plus four cubic centimeters of salt solution. Temperature before inoculation, 99.2° F.

After two and one-half hours: (*a*) temperature, 102.4° F.
(*b*) " 100° F.
(*c*) " 101.6° F.

The exudates of all three of the pigs at this time showed active reactions as to leucocytes, but an equally active increase in free bacteria.

After five hours: (*a*) temperature, 101.4° F.
(*b*) " 102° F.
(*c*) " 99° F.

The intraperitoneal exudates after five hours show an equal increase of bacteria in all three.

(*c*) shows markedly fewer leucocytes than (*a*) or (*b*).

There is nothing in any of the three which could morphologically be interpreted as bacteriolysis. There is slight phagocytosis about equal in all three. A drop of exudate from each smeared over the surface of agar plates after five hours shows, after forty-eight hours' cultivation, the same amount of growth in (*a*) and (*c*), (*b*) being contaminated cannot be considered.

After twenty hours: (*a*) temperature, 100.6° F.
(*b*) " 99.2° F.
(*c*) " 97° F.

The exudates show a moderate increase of bacteria in (*b*), more rapid increases in (*a*) and (*c*). The exudates of (*a*) and (*b*), however, contain many more leucocytes than in (*c*) in which the exudate seems composed almost entirely of bacteria.

(*c*) rapidly grew sicker and died at 12.33 P.M., twenty-four hours after inoculation.

(*a*) and (*b*) died during the night following between thirty and forty hours after inoculation, thus outliving the control by six and sixteen hours.

A careful consideration of the evidence presented in the preceding experiments gives, it seems to the writers, definite information which will be considered in detail in the summary which follows.

It seemed desirable, however, at this stage of the work with leucocyte extracts, to obtain some information as to the influence of these extracts upon phagocytosis in vitro and for this purpose the following experiments were carried out.

II.

ON THE INFLUENCE OF AQUEOUS EXTRACTS OF RABBIT LEUCOCYTES UPON PHAGOCYTOSIS OF PNEUMOCOCCUS IN VITRO.

The reasons for choosing pneumococcus for these experiments were, first, because the leucocyte extracts had been previously found to exert a more or less profound influence upon the course of pneumococcus infections, and, second, because the well-known low index of phagocytosis toward virulent pneumococci would favor the detection of even slight differences produced by the leucocyte extract.

The technic of Wright was employed. The experiments were as follows:

Experiment I. — (*a*.) NaCl emulsion of pneumococci (twenty-four-hour agar slant), NaCl solution, dog corpuscles, equal parts.

(*b.*) NaCl emulsion of pneumococci, aqueous extract of leucocyte, dog corpuscles, equal parts.

These were left at 37.5° C. for thirty minutes. The results were as follows:

(*a.*) 100 cells counted, no phagocytosis.
(*b.*) 100 cells counted, no phagocytosis.

Experiment II. — (*a.*) Pneumococcus (twenty-four-hour broth cultures), NaCl solution, dog corpuscles, equal parts.

(*b.*) Pneumococcus (twenty-four-hour broth culture), aqueous extract of leucocytes, dog corpuscles, equal parts.

These were left at 37.5° C. for thirty minutes. The results were as follows:

(*a*) 110 cells counted, no phagocytosis.
(*b.*) 100 cells counted, phagocytic index .55

Experiment III. — (*a.*) Pneumococci (NaCl solution emulsion), NaCl solution, dog corpuscles, equal parts.

(*b.*) Pneumococci (NaCl solution emulsion), aqueous extract of leucocytes, dog corpuscles, equal parts.

These were left at 37.5° C. for thirty minutes. The results were as follows:

200 cells counted in each case, no phagocytosis evident in either (*a*) or (*b*).

Experiment IV. — (*a.*) Pneumococci (broth culture), NaCl solution, dog corpuscles, equal parts.

(*b.*) Pneumococcus broth culture, aqueous extract of leucocytes, dog corpuscles, equal parts.

These were left at 37.5° C. for thirty minutes and two hundred cells counted, with the following results:

(*a.*) Phagocytic index .05.
(*b.*) Phagocytic index .02.

Experiment V. — In this experiment the pneumococcus emulsion in salt solution was made somewhat thicker than before.

(*a.*) Pneumococcus emulsion, NaCl solution, washed human leucocytes, equal parts.

(*b.*) Pneumococcus emulsion, aqueous extract of leucocytes, washed human leucocytes, equal parts.

These mixtures were made in duplicate and left at 37.5° C. for thirty minutes, and two hundred cells were counted with the following results:

(*a.*) 1 phagocytic index .83.
2 phagocytic index .1.
(*b.*) 1 phagocytic index .4.
2 phagocytic index .0.

Experiment VI. — Same emulsion as in Experiment V. and the same corpuscles; incubation for only twenty minutes at 37.5° C.

(*a.*) Pneumococci, NaCl solution, human corpuscles, equal parts.

(*b.*) Pneumococci, aqueous extract of leucocytes, human corpuscles, equal parts.

These mixtures were in duplicate and two hundred cells were counted with the following results:

(*a.*) 1 phagocytic index .0.
2 phagocytic index .0.
(*b.*) 1 phagocytic index .4.
2 phagocytic index .0.

Experiment VII. — In this experiment a twenty-four-hour broth culture, concentrated by centrifugalization, was used. The mixtures were as follows:

(*a.*) Pneumococci, aqueous extract of leucocytes, equal parts.

(*b.*) Pneumococci, NaCl solution, equal parts.

These mixtures were placed at 37.5° C. for twenty minutes. After this they were mixed with equal parts of freshly prepared human leucocytes and again placed at 37.5° C. for thirty-five minutes. Several preparations were made of each of the above mixtures and in each of the specimens prepared one hundred cells were counted. The results were as follows:

(*a.*) 1 phagocytic index .1.
2 phagocytic index .26.
3 phagocytic index .02.
4 phagocytic index .08.
(*b.*) 1 phagocytic index .12.
2 phagocytic index .3.
3 phagocytic index .0.

From the foregoing seven experiments it appears logical to conclude that the leucocyte extract has no practical effect upon the phagocytosis of pneumococci in vitro, at least when serum is not also used in the mixtures. Whether there would be shown a difference between experiments with serum alone and serum plus leucocyte extract is a point which we have not endeavored to settle in the present series of experiments. The fact, however, that there is no apparent augmentation of phagocytosis shown in the experiments carried on in the peritoneal cavity would rather argue against any particular increase in phagocytosis in mixtures to which leucocyte extract had been added.

SUMMARY AND DISCUSSION.

The experiments recorded in the first section of this paper were carried out with the purpose of ascertaining whether the protective action of leucocyte extracts could be explained by any visible influence they exerted upon the phenomena occurring after intraperitoneal infection. The technic of all such experiments, however, is subject to so much difficulty that only infinite repetition can completely guard against error. Some of the results obtained, nevertheless, have been so regular in their recurrence that the writers believe them to be of no inconsiderable value in helping to explain the manner of action of the leucocytic substances.

Apart from these results, which will be summarized shortly, this work has confirmed the actual protective value of the leucocyte extracts, which, especially in the pneumococcus experiments, was regular and almost directly in proportion to the amounts of extract used. (See pneumococcus Experiment VII.).

The failure to protect in the case of the staphylococcus experiments is believed by the writers to depend upon the extreme virulence and the too heavy dosage of the particular culture used.

A critical survey of the individual experiments shows, in spite of the distinct protective action exerted by the leucocyte extracts in the cholera and pneumococcus infections, a surprising similarity between the exudates of the animals receiving leucocyte extracts and those of the controls. In no case was there any reliable evidence that the bacteria were either immediately destroyed or greatly hindered in their development by the presence of the leucocytic substances. In no case did the presence of the leucocyte extract seem to immediately increase the degree of phagocytosis taking place intraperitoneally.

In one respect, however, the exudates of the animals receiving leucocyte extracts showed a difference from those of the controls, a difference noticeable to a more or less marked extent in all the experiments. The treated animals

showed, almost without exception, a more rapid and extensive accumulation of polymorphonuclear leucocytes in the peritoneal exudate. This feature was noticeable, however, only during the early hours after inoculation, the conditions in the peritoneal cavities becoming more equalized in regard to leucocytes after twelve or more hours. This observation, depending upon the rather unreliable evidence of numerical comparisons in smears of uncertain thickness is, nevertheless, mentioned because of the regularity with which the phenomenon recurred. The obvious inference from this fact is that the leucocyte extracts may have neutralized some bacterial substance of negative chemotactic activity. Whether such neutralization is aimed at toxins, endotoxins or "aggressins" in the sense of Bail, must be left undecided until further work has been done.

Another feature of the experiments in which extract and organisms were given simultaneously, regular in occurrence, and plainly evident, was the marked contrasts in the degree of prostration between the treated animal and the control, throughout all stages of the infection. Two animals in which six or more hours after inoculation there was hardly any difference in the appearance of the exudates would differ strikingly in apparent well being, the one which had received the leucocyte extract usually eating, running about and apparently well, the other crouching in a corner of its cage with ruffled fur and showing marked weakness. To this was added in the pneumococcus cases the evidence of the temperature, always lower in the untreated than in the treated animal. A low temperature immediately following the inoculation of an animal with a particularly large and powerful dose of any infectious agent is, of course, a recognized indication of a high degree of toxemia, while any rise in temperature at an early stage of such an infection seems to warrant the assumption of a more perfectly active protective mechanism.

Attention should be called, furthermore, to the apparently exact parallelism between the height of temperature and the leucocytosis in the exudate.

The conclusions which these observations seem, to the writers, to justify may be summarized as follows:

I. Aqueous extracts of rabbit leucocytes, intraperitoneally injected, exert marked protective influence upon guinea-pigs intraperitoneally infected with Vibrio choleræ Asiaticæ and Pneumococcus.

II. Observation of the intraperitoneal phenomena, after injection of bacteria and leucocyte extracts, tends to show that the leucocytic substances exert but slight, if any, bactericidal action, and do not, of themselves, inhibit to any considerable extent the development of the bacteria used for the experiments.

III. Similar observation shows that the leucocyte extracts do not, to any marked degree, directly increase intraperitoneal phagocytosis.

IV. The more rapid appearance and greater numbers of polynuclear leucocytes in the exudates of animals receiving leucocyte extract, seems to indicate a possible neutralization by such extract of negatively chemotactic bacterial products.

V. In the absence of bactericidal, bacteriolytic or phagocytosis-stimulating effects referable to the leucocytic extracts, the favorable influence which these substances were repeatedly shown to exert upon the temperature and general condition of the animals and upon the ultimate outcome of the infections is probably dependent upon a faculty of neutralization possessed by the leucocyte extract toward certain toxic bacterial products.

VI. The experiments recorded in Section II. indicate that leucocyte extracts do not influence in vitro phagocytosis of Pneumococcus, at least in the absence of serum.

IV.

THE CURATIVE INFLUENCE OF EXTRACTS OF LEUCOCYTES UPON INFECTIONS IN MAN.

PHILIP HANSON HISS, JR., M.D., AND HANS ZINSSER, M.D.

I. A REPORT OF TWENTY-FOUR CASES OF EPIDEMIC MENINGITIS TREATED WITH LEUCOCYTE EXTRACT.

Parallel with the work done upon the influence of extracts of rabbit leucocytes upon various infections in animals, a series of observations have been made during the past two years upon the value of such extracts in the treatment of human disease.

Cases of meningococcus, pneumococcus, typhoid, streptococcus, staphylococcus, and gonococcus infections have been treated, but among these the two first alone have been treated in sufficient number to warrant even a preliminary publication.

The cases treated were, with one exception, patients at various of the public hospitals of New York, and the work was made possible only by the courtesy and coöperation of the attending physicians and house staffs at these institutions.

The extracts used were, throughout, aqueous extractions of rabbit leucocytes, invariably administered subcutaneously. In no case, it may be stated, was any harmful action on the part of these substances observed. Local reactions were invariably slight and lasted at most for a few hours.

The number of cases of epidemic cerebro-spinal meningitis treated by us is twenty-four.

The results of this treatment are best shown in the brief records of the cases themselves.

With the exception of the first case to be described, the cases are divided into two groups. Those which recovered are given in one group, those which died in another. The cases are arranged according to hospital, not in direct order of treatment.

The following history is given first, because the patient,

an Italian boy, was the first person to whom leucocyte extract was given:

CASE I. — Splendidi, male, fifteen years, laborer. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Two days before admission the boy had severe headache, tried to work but had to give up on account of the violent pain. Vomited once.

Day before admission became unconscious and delirious; was very violent and noisy.

April 22, 1907. — Admitted to Roosevelt Hospital. Spinal puncture. Meningococci morphologically and by cultivation.

April 23. — When seen patient was noisy and delirious. Opiates had had no quieting effect. Temperature had fallen, following lumbar puncture, from 104° at admission to 100.4° on the twenty-second, but had returned to 103 on the twenty-third. Two cubic centimeters of leucocyte extract were given subcutaneously. Opiates discontinued.

April 24. — Patient quieter, temperature, 101°. Five cubic centimeters extract given subcutaneously.

April 25. — Temperature reached 99° at 4 A.M. Patient quiet and rational. Asks for water and is able to take light food. Spinal fluid is markedly less turbid, meningococci apparently decreasing in numbers.

From this time on the patient for a time steadily improves, takes food; retraction of head disappears and he even attempts to get out of bed by himself. Is clear mentally and converses rationally with his friends.

His condition was so good that he was supposed by us to be convalescing, and no further treatment was given until May 13, when he was so much worse again — complaining of severe pain in head — that ten cubic centimeters of extract were given and the dose repeated on May 14. In spite of this treatment, which was followed by a transient fall in temperature, there was an abrupt rise of temperature on the fifteenth, and on the seventeenth the boy died.

At autopsy there was seen some exudate over the pons and medulla. The ventricles were dilated and contained about two ounces of turbid fluid. The cord was covered with a thick exudate. Other organs normal, spleen somewhat enlarged.

Note: First injection given third day of disease.
Total quantity given, twenty-eight cubic centimeters.
Patient died about twenty-seventh day.

This boy was in an extremely grave condition from the beginning, seemingly moribund at the time treatment was begun and had been given up by the physicians in charge As he was the first patient to whom leucocyte extract was given, the small amounts administered are explained. Notwithstanding these small doses, rapid improvement was

shown and pointed strongly to a favorable influence of the extract on the toxic symptoms at least. The improvement in the patient coincident with the administration of the extract, if not due to it, was so marked that treatment was discontinued until the patient showed marked signs of a relapse, and even then we hesitated — about fifteen days having elapsed since treatment — for fear of possible anaphylaxis. Finally, when the patient was in a grave condition, treatment was again given — no signs of anaphylaxis appearing — but the disease had made too much headway to be again controlled.

Notwithstanding this outcome, certain factors appeared to us to argue so strongly for a beneficial effect of the leucocyte extract that we did not hesitate to apply the treatment to other cases of the disease.

RECORD OF CASES WHICH RECOVERED.

CASE II. — J. W., male, five years. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Past history and family history negative.

Present illness: On day before admission the child on getting up in morning vomited and wanted to lie down. Mother thought him unable to walk. Vomiting continued, complained of headache. On day of admission had convulsions and became irrational.

April 30, 1907. — Admitted to Roosevelt Hospital. Temperature, 103; pulse, 120; respiration, 32. Pupils large, neck stiff, Kernig's sign present. Meningococcus in spinal fluid.

Leucocyte extract was given on the day of admission and at irregular intervals thereafter in ten-cubic-centimeter doses. No marked temperature reaction after individual injections, but there was gradual improvement to final recovery.

Note: First injection given second day of disease.

Total quantity given, one hundred and forty-five cubic centimeters.

Duration of disease about seventy days.

This boy, who was unconscious at the time of admission to the hospital, was rational the morning following the first injection of leucocyte extract. No opiates were given and the boy was never very restless or irritable. The retraction and stiffness of the neck rapidly disappeared and the child

although weak remained fairly well nourished and in good mental condition throughout his rather protracted illness. His temperature was practically normal, with a few slight rises, from June 20.

CASE III. — G. D. W., child, six years. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Present illness: On day before admission, April 25, 1907, sharp pains in abdomen, general convulsion. Temperature, 102°; pulse, 160; respiration, 20. Leucocytes, thirty thousand; polynuclears, 92.5 per cent. Spinal fluid was obtained but no meningococci were found. One day after admission leucocyte extract was injected subcutaneously twice, six cubic centimeters each time. Temperature dropped from 105° to 102°. A third injection of seven cubic centimeters was given on the following day. Temperature dropped to normal. Rapid convalescence.

Note: First injection third day of disease.

Total quantity given, nineteen cubic centimeters.

In spite of the fact that meningococci were not found in the spinal fluid, this case was looked upon by the attending physician as clinically one of epidemic spinal meningitis. The mental condition of the patient cleared immediately subsequent to the first injection. Restlessness and irritability were allayed in spite of discontinuance of opiates. Temperature dropped immediately to normal and all physical signs of meningitis rapidly disappeared.

CASE IV. — I. T., female, eight years. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Past history negative.

Present illness: On day of admission, vomiting, headache, and fever, in afternoon semi-conscious.

May 9, 1907. — Admitted to Roosevelt Hospital. Temperature, 100.5°; pulse, 132; respiration, 26. Strabismus, Kernig's sign present. Petechial eruption over the whole body. Neck rigid and painful. Meningococci present in spinal fluid. Leucocytes, twenty-five thousand. Typical severe meningitis.

May 10. — Leucocyte extract, ten cubic centimeters, was given in the morning. A further ten cubic centimeters in the afternoon. Temperature drops gradually, but increases again. Further injections were given at intervals of one or two days until May 29, then at intervals of about one week. Temperature gradually falls, symptoms clear and child is discharged cured July 10. Temperature remains normal from June 19.

Note: First injection given second day of disease.
Total quantity given, one hundred and thirty cubic centimeters.
Duration of disease, sixty days.

In this patient there was a marked effect upon the temperature after the injections, and after each injection apparently some benefit to the general condition. The child was quite irritable for a time and although wandering mentally at intervals maintained in general a normal mental condition. The physical signs of meningitis cleared up fairly rapidly but the convalescence was a long one. There were no sequelæ.

CASE V. — I. R., female, colored, twenty-three years, housework. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Family history and past history irrelevant.

Present illness: Nine days before admission, violent chill, headache, and vomiting. Stiff neck and throbbing in head.

May 29, 1907. — Admitted to Roosevelt Hospital. Temperature, 104.8°; pulse, 104; respiration, 28. Neck very stiff, spinal fluid turbid, and meningococci present. Leucocytes, thirty-nine thousand. Temperature of the septic type, rising to about 101° in P.M.

June 6. — On this day ten cubic centimeters of leucocyte extract were given. Thereafter daily injections until June 12, temperature then comes down flat. Gradual clearing up of symptoms. Discharged cured July 26.

Note: First injection given seventeenth day of disease.
Total quantity given, ninety cubic centimeters.

In the case of this patient injections of leucocyte extract seem markedly to have affected the general condition and especially the ability to take food. There was no very marked immediate effect noted on the temperature. There were no sequelæ.

CASE VI. — Sweeney, female, six years. St. Luke's Hospital. Service of Dr. Collins.

Past history and family history negative.

Present illness: On day before admission to hospital fretful and cried when handled. Vomited on the same afternoon. On morning of admission to the hospital there was stiff neck, and the child vomited several times. Irritability much increased.

May 5, 1907. — Admitted to St. Luke's Hospital at 1.30 P.M. Temperature, 101°; pulse, 72; respiration, 30. Child is very irritable and not

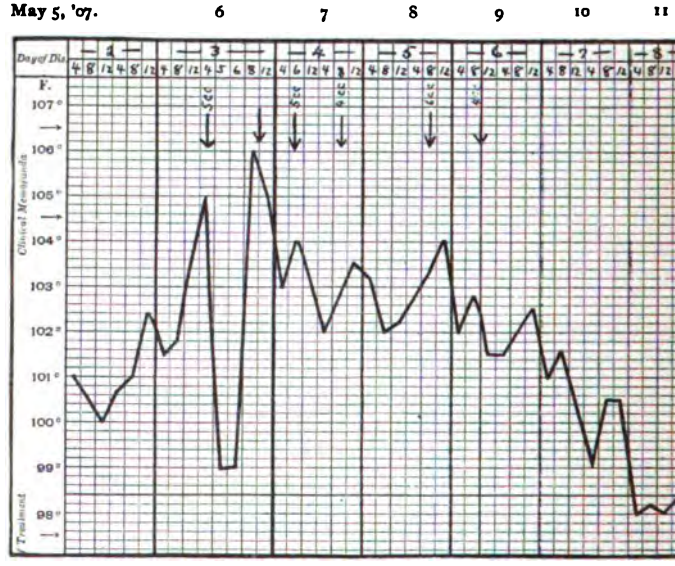
entirely rational. Neck rigid and retracted. Pulse occasionally drops a beat. Abdomen retracted. Kernig's sign marked. Knee jerks not obtained. Child lies in slight opisthotonos. Lumbar puncture forty cubic centimeters of fluid. Meningococci obtained in pure culture.

May 6. — First dose of leucocyte extract given on this day followed by an immediate drop in temperature (see chart). In this patient the drops in temperature following injections of the extract are marked. Six injections in all were given subcutaneously and were followed by a steady decline of temperature and clearing up of all symptoms. Temperature was normal on May 12.

Note: First dose given third day of disease.

Total quantity given, thirty- five cubic centimeters.

The immediate effect of the leucocyte extract, apart from its effect on the temperature, which is well illustrated in the chart, was in allaying irritability and improving the child's mental condition.



May 5. — Spinal fluid contains meningococci.
May 6. — Blood taken just before first injection of serum. Leucocytes, 36,000. Polynuclears, 84.25%. Lymphocytes, 15.75%.
May 7. — Leucocytes, 24,600. Polynuclears, 74%. Lymphocytes, 25¾%. Eosin, ¼%.
May 8. — Leucocytes, 19,200. Polynuclears, 66%. Lymphocytes, 33%. Eosin, 1%.
May 9. — Leucocytes, 15,900. Polynuclears, 70%. Lymphocytes, 30%.
May 11. — Leucocytes, 14,600. Polynuclears, 60%. Lymphocytes, 40%.
May 12. — Leucocytes, 12,300. Polynuclears, 71¼%. Lymphocytes, 27¼%. Eosin, 1%.

Acute meningitis. Sweeney. Note marked drop in temperature following treatment. Duration of fever four and one-half days after first treatment.

CASE VII. — S. S., female, nine years. New York Hospital. Service of Dr. S. W. Lambert.

Family history and past history irrelevant.

Present illness: A day and a half before admission there was sudden vomiting, chills, fever, headache, stiff neck, delirium.

July 4, 1907. — Admitted to New York Hospital. Temperature, 103.4°; pulse, 160; respiration, 32. Pupils dilated, nystagnus, Kernig's sign well developed, knee jerks absent. Leucocytes, thirty thousand six hundred; polynuclears, eighty-eight per cent. Meningococci present in the spinal fluid. Ten cubic centimeters of leucocyte extract was given on July 4, the day of admission. Thereafter, injections were given each day, or at intervals of one or two days. The temperature was markedly affected by each injection, but continued irregular until August 6, when it fell to normal. Mental condition had cleared up very soon after the first few injections but the stiffness and other symptoms had disappeared more gradually. Child was discharged cured. There were no sequelæ.

Note: First injection given third day of disease.

Total quantity given, one hundred and twenty cubic centimeters.

CASE VIII. — J. B., female, eleven years. New York Hospital. Service of Dr. S. W. Lambert.

Family history and past history negative and irrelevant.

Present illness: Two days before admission had headache and vomited three or four times. No convulsions. On the following day there was delirium and pain in back of the head.

May 15, 1907. — Admitted to New York Hospital. Temperature, 101°; pulse, 100; respiration, 20. Neck stiff and painful, knee jerks absent, Kernig's sign present. Leucocytes, twenty-nine thousand; polynuclears, ninety-one per cent. Meningococci present in spinal fluid.

Until June 3 child in very poor condition. Temperature up and down, ranging between 99° and 104°.

June 4. — Ten cubic centimeters of leucocyte extract given subcutaneously, temperature falls to 99.2° by next morning, child in good condition.

June 5. — Another injection of ten cubic centimeters given, temperature falls to normal.

June 6. — Temperature remains normal.

June 7. — Ten cubic centimeters of extract given.

Temperature continues to remain normal; the child gradually improves and is discharged cured, with no sequelæ.

Note: First injection given twentieth day of disease.

Total quantity given, thirty cubic centimeters.

In this case there was prolonged illness with a gradual change for the worse for almost three weeks, which apparently was brought to abrupt improvement by two injections of leucocyte extract.

CASE IX. — F. M., female, seven months. New York Hospital. Service of Dr. S. W. Lambert.

Family history and past history irrelevant.

Present illness: For ten weeks before admission, fever and cough. Two days before admission child vomited and had convulsions. Since then, convulsions and constant twitching of head and extremities.

May 4, 1907. — Admitted to New York Hospital. Temperature, 103.6°; pulse, 126; respiration, 40. Strabismus, nystagmus. Kernig's sign well developed, neck rigid and retracted. Spinal fluid turbid and contains fifty-three per cent polynuclear leucocytes. Meningococci present in spinal fluid. After admission child's temperature gradually falls and remains normal after May 11. The child's general condition, however, is one of gradual emaciation and constant vomiting. Almost all foods taken are vomited. Stiffness of neck and extremities persist.

June 18. — On this day the first injection of ten cubic centimeters of leucocyte extract was given. Thereafter injections of five cubic centimeters or ten cubic centimeters were given daily until June 21, and at intervals of one or two days until June 29. General condition improves steadily, child takes food, and gains weight. Discharged from hospital cured, with no sequelæ.

Note: First injection given forty-seventh day of disease.
Total quantity given, fifty cubic centimeters.

In this case the injections appeared to have a marked effect in controlling the vomiting and restlessness and permitting of the retention of food, which led to the gradual restoration of the child to its normal condition.

CASE X. — E. R., male, five years. Presbyterian Hospital. Service of Dr. David Bovaird.

Family history and past history irrelevant.

Present illness: Eight days before admission patient began to vomit and complained of pain in his head. Vomiting continued for three days and patient became irrational. There were herpes, retraction of the neck, and fever.

July 8, 1907. — Admitted to Presbyterian Hospital. Temperature, 100.4°; pulse, 140; respiration, 30. Herpes on cheeks, neck stiff, Kernig's sign marked. Knee jerks absent. Meningococci in spinal fluid.

Child continues to run irregular temperature and loses ground, gradually emaciating until August 10. During this time the child has taken nourishment poorly, and has been in a continuous state of stupor with general stiffness increasing. Condition apparently hopeless.

August 10. — First injection, eight cubic centimeters of leucocyte extract given subcutaneously.

Injections given thereafter ten cubic centimeters at a time each day

until August 15, then at intervals of two or three days. The child's temperature gradually descends to normal, he begins to take nourishment more freely, his mental condition clears up, and his rigidity relaxes. Because of his extremely poor condition and the long duration of the disease the child has to be kept in the hospital, but steadily improves and walks before leaving the hospital on December 22.

Note: First injection given forty-first day of disease.

Total quantity given, one hundred and eighteen cubic centimeters.

This case was one of exceptional interest to us. The boy was one of two children of the same age then in the hospital suffering from meningitis. The course of the disease in each case was remarkably similar. One of them, however, was considered to be on a fair road to convalescence, and was not treated by us. The other one was considered by Dr. Bovaird, the attending physician, to be steadily growing worse with little chance of eventual recovery. It was decided, therefore, to treat this case, and compare the conditions of the treated and untreated children day by day to determine, if possible, the value of the leucocyte extract treatment. The result of this treatment has been detailed in the preceding history. The untreated boy, who was thought at the time our treatment was commenced to stand a fair chance of recovery, gradually but steadily lost ground and eventually died, in spite of the fact that a few treatments were given him towards the end, but with no beneficial results on account of the grave anatomical lesions which had supervened.

CASE XI. — J. C., male, four years. Presbyterian Hospital. Service of Dr. Bovaird.

Family history and past history irrelevant.

Present illness: For one week before admission feverish and restless at night. Four days ago fell down hitting head. That night vomited. Three days ago great pain in head and retraction of neck.

Aug. 14, 1907. — Admitted to Presbyterian Hospital. Temperature, 102°; pulse, 124; respiration, 20. Neck rigid, flexion of head painful. Kernig's sign on right side. Urticaria on thighs and legs. Lumbar puncture bring slight amount of slightly turbid fluid. No meningococci found. Leucocytes ranging from thirteen thousand seven hundred to eighteen thousand three hundred; polynuclears, eighty per cent.

August 16. — Ten cubic centimeters of leucocyte extract given subcutaneously.

August 17. — Temperature falls to normal and remains normal until the twenty-first when ten cubic centimeters of extract was given. Symptoms clear up immediately. Patient is discharged cured.

Note: First injection given fifth day of disease.
Total quantity given, twenty cubic centimeters.

This case was considered by the physicians in charge to be acute cerebro-spinal meningitis and has, therefore, although no meningococci were demonstrated in the spinal fluid, been included in our series. Symptoms cleared up coincident with the administration of the extract.

CASE XII. — Martin Lowry, nine years. Bellevue Hospital. Service of Dr. Frank Meara.

Family history and past history, negative.

Present illness: On midnight of March 16, projectile vomiting and abdominal pain. Twitching of the extremities, delirious and restless. Does not seem to notice surroundings. Incontinence of urine and feces.

March 17, 1907. — Admitted to Bellevue Hospital. In coma. Ptosis of right eyelid and flattening of right side of face. Patellar reflexes very active, Kernig's sign present. Petechial spots. Pupils unequal, react quickly. Internal strabismus of right eye.

Under symptomatic treatment and spinal puncture, from this time on, child continued having a septic temperature reaching occasionally 103° F., sinking to 98° F. The condition of the child during this time does not vary much from the condition at time of admission except for gradual improvement in mental condition and improvement in the taking of food. By April 9, child has considerably improved, is rational and comfortable. Temperature drops to normal. Temperature remains low and child continues to improve until May 6. Spinal puncture during this time brings 1.5 ounces of turbid fluid.

May 6. — There is a sharp rise in temperature to 102.6°. At the same time child begins to vomit, to complain of pain in head and neck. Is fretful and very weak. Retains little nourishment. Spinal puncture yields sixteen ounces of turbid fluid.

May 8. — Leucocyte extract given, ten cubic centimeters. The following notes taken by the house physician in charge of child and on record in hospital chart are given literally:

May 9. — Since injection of "serum," child is quieter and temperature has fallen to normal. Passed quiet night and did not vomit. Received further five cubic centimeters leucocyte extract.

May 10. — Spent quiet day yesterday, no headache, no vomiting. Took milk in small quantities. This morning is restless, irritable, and

vomited after 8 A.M. feeding. Is hungry and thirsty. There is some hyperesthesia.

May 12. — Had bad day on the eleventh, vomited several times in the morning and almost continually in afternoon, although given nothing but cracked ice. Vomitus at first yellow, then green and then typically coffee-ground. Nutrient enema not retained. At 6 P.M. received ten cubic centimeters of "serum" (leucocyte extract) and after this the temperature fell, child slept greater part of night and retained nutrient enema eight hours after injection. Not vomiting after early evening.

May 13. — Child quiet and weak all day. Retained milk in small quantities, and to-day seems much better and brighter. Less hyperesthesia. At 5 P.M. received ten cubic centimeters of "serum" (leucocyte extract).

May 14. — Injection of ten cubic centimeters of leucocyte extract.

May 15. — After injection of "serum" (leucocyte extract) on fourteenth, temperature remained sub-normal. Noted an increase of appetite, absence of headache and vomiting, and general improvement. Leucocyte extract, ten cubic centimeters on May 18 and May 20.

May 21. — Since last note, after two days without "serum" (leucocyte extract) child began to vomit (on seventeenth), first complaining of headache. Hyperesthesia and headache demanded morphine; after a bad night remained in stupor for the next twenty-four hours, refusing all nourishment but taking small pieces of cracked ice. On nineteenth became incontinent of urine for first time. Yesterday refused milk but last night retained nutrient enema.

May 22. — Leucocyte extract, ten cubic centimeters. To-day child is hungry. Cries for milk and does not vomit.

May 23. — No hyperesthesia. Retains nourishment which he craves. Kernig's sign marked. General condition improved.

After May 22 the general condition of the child continued to improve, nourishment is taken well, stiffness relaxes and gradual but complete convalescence is established. Child discharged cured July 16, 1907.

Note: First injection given fifty-third day of disease.
Total quantity given, seventy-five cubic centimeters.

In the case of this patient the leucocyte extract seems to have exerted marked influence upon the temperature, reducing this to normal and even sub-normal on several occasions. The most striking effect of the leucocyte extract, however, was noticed in its apparently complete control of the vomiting. The child unable to retain even cracked ice was enabled after a single injection of leucocyte extract to take the fluid diet advised for it. On two occasions when leucocyte extract had been omitted the vomiting

returned and continued until another dose was given. After a few treatments all signs of poisoning as evidenced by these gastric disturbances disappeared, and the boy gradually but steadily regained weight and returned to his normal condition.

CASE XIII. — B. Z., female, twelve years. Bellevue Hospital. Service of Dr. Norrie.

Past history irrelevant. Slight deafness for several months.

Present illness: On day of admission to hospital suddenly taken ill with a headache, stiff neck, and Kernig's sign. During this day vomited several times.

May 25, 1907. — Admitted to Bellevue Hospital. Temperature, 104.8°; pulse, 140; respiration, 32 Child extremely ill. Meningococci found in spinal fluid. Treatment by baths, symptomatic medication and spinal puncture. Temperature gradually comes down.

July 1. — Temperature is now fluctuating between 98° and 101°. The child appears to be going into chronic stage. It is poorly nourished and emaciating. Its mental condition is listless and somnolent.

July 3. — Ten cubic centimeters of leucocyte extract given subcutaneously.

July 4. — Ten cubic centimeters of leucocyte extract again given.

Thereafter leucocyte extract is injected at intervals of three or four days. Child's general condition improves, temperature comes down gradually.

July 20. — Temperature normal. Child begins to eat. From this time child steadily improving and gains weight.

August 14. — Child in fair condition and ready to go home. Original deafness persists, slightly worse if anything. Otherwise no sequelæ.

Note: First injection given thirty-ninth day of disease.

Total quantity given, one hundred and ten cubic centimeters.

In this case the symptoms at the time treatment was begun were chiefly those of the chronic stage. The effect of the leucocyte extract was noticeable chiefly in its beneficial influence upon the mental condition of the patient and her nutrition.

CASE XIV. — Arthur McCreve, male, twenty-six years, policeman. Bellevue Hospital. Service of Dr. Draper.

Past history irrelevant.

Present illness: Three weeks before admission began to have pains in various parts of body and felt weak. For one week, vomiting after meals and diarrhea. Patient noticed rash on body for one week.

March 6, 1908. — Admitted to Bellevue Hospital. Temperature, 104.8°; pulse, 100; respiration, 30. From this date until the twenty-third of March a positive diagnosis was not made, the temperature running an irregular, swinging course.

March 23. — Neck became rigid and by lumbar puncture about one drachm of cloudy fluid under moderate pressure was obtained. Meningococci were present in the smears and were cultivated from the fluid. Kernig's sign positive. Incontinence of urine and feces. Tremor. Patient's temperature at this time fluctuated between 104° and 101° F. daily. Leucocytes, twenty-five thousand nine hundred; polynuclears, 86.5 per cent.

April 2. — Spinal puncture gave three cubic centimeters of clear yellow fluid. Thirty cubic centimeters of Flexner's anti-meningitis serum was injected. Two hours after injection of serum patient's hands and lips became cyanosed, and he seemed very weak. Towards night, however, his mental condition seemed improved but patient still irrational.

April 3. — Spinal fluid shows almost no polynuclear leucocytes and no diplococci. Many mononuclear cells present. As there was no microscopic evidence of epidemic meningitis the discontinuance of the serum treatment was advised. It was suggested that the case might be one of tuberculosis.

April 19. — Lumbar puncture gave five cubic centimeters of cloudy fluid which was reported to be sterile. Patient's temperature continued fluctuating as before, between 104° and 97° F. There was great emaciation, stiffness of the extremities with contractures, and some flatness of the left side of the face. This general condition continued until May 11.

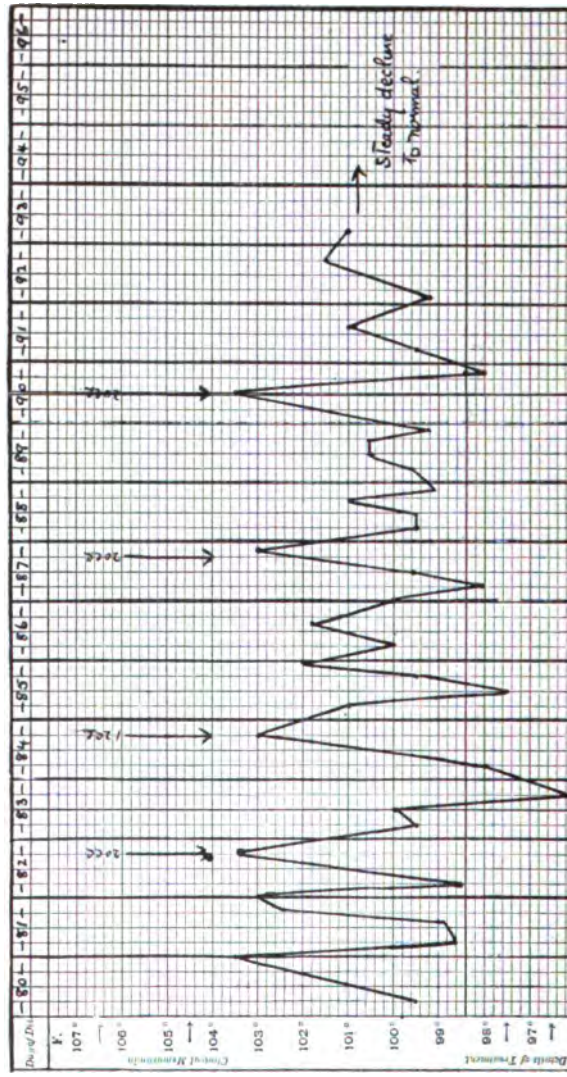
May 11. — On this day twenty cubic centimeters of leucocyte extract were given subcutaneously. Following this the patient sweated profusely and his temperature dropped to 96.2°. On the following day the temperature did not rise, the first time since the beginning of the disease that the temperature has not risen in the afternoon.

May 14. — Another injection of twelve cubic centimeters of extract was given. The next day temperature does not go above 102°.

May 16. — Twenty cubic centimeters of extract given. For the two following days temperature did not go above 101.2°. Patient seems generally improved.

Further injections were given at intervals of three or four days until June 8. During this interval temperature gradually fell, its highest point at any time being 102.4°. By June 1 the temperature was normal and remained so. Patient's appetite continuously improved and stiffness relaxed. By July the patient gaining weight and returning to normal, practically cured except for emaciation.

Note: First injection given eighty-second day of disease.
Total quantity given, one hundred and fifty cubic centimeters.



Chronic meningitis. McCreve. Note marked remissions following administration of the extract. Temperature remained normal after June 1. First treatment given May 11.

CASE XV. — H. L., male, six years. Private case. Dr. Leopold Stieglitz. Dr. Wm. P. Northrup, consultant.

The history of this case, of which the following is an abstract, was very kindly furnished to us by Dr. Stieglitz:

Previous history irrelevant.

Present illness: On Sept. 16, 1907, the child had tonsilitis, but apparently convalesced of this by September 18.

September 20. — On this day child began to have headache, and his temperature went to 101° F. On the following day there was photophobia, restlessness, petechial eruption, retraction of head, and irregular respiration and pulse.

September 22. — Temperature 103.6°, child hard to arouse, pronounced rigidity of the neck, child cries when touched. Great restlessness. *Cri cerebrale.*

September 23. — Temperature 102.8°, stupor more pronounced, more hypersensitiveness, and retraction of head very pronounced. Coma. Kernig's sign present. On lumbar puncture about an ounce of cloudy fluid was obtained. Meningococci present. Five cubic centimeters of leucocyte extract were injected. There was a gradual drop in temperature to 100.6° within twenty-four hours. Four or five hours after the injection the child became quieter and the twitching of the face and limbs much diminished. "The injection seemed to have soothing effect. It is also easier to arouse the child sufficiently to feed it."

September 24. — Distinct improvement in general condition, child much quieter. Seven cubic centimeters of leucocyte extract from an immune rabbit administered. "Very pronounced improvement in general condition, temperature dropping from 101.2° to 99.6° in course of twenty-four hours. Child very much quieter, takes nourishment more freely. Can be aroused. . . . Retraction of head still pronounced, rigidity distinctly diminished."

September 25. — Improvement more pronounced, child entirely rational, takes nourishment freely.

September 27. — Temperature rises to 102.2°. General condition same as before. A third injection of ten cubic centimeters of immune leucocyte extract given. During the night the temperature dropped to 100.4°, and the child slept quietly.

Steady and rapid improvement from this time on. All rigidity gradually disappearing, so that by October 2 the child can touch his chest with his chin.

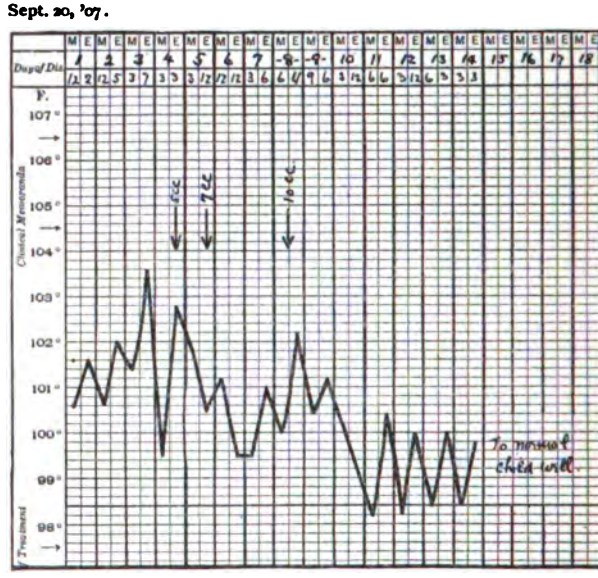
October 8. — Allowed to sit up and convalescent.

Note: First injection given fourth day of disease.

Total quantity given, twenty-two cubic centimeters.

It is worthy of note in this case that only one spinal puncture was made, and that for the purpose of diagnosis. The response to the injections was apparently marked and

unmistakable. It is not impossible that the rapidity of the reaction to the injections may have been due to the fact that the leucocyte extract employed was taken from immune rabbits.



Acute meningitis. Private case. H.L. Note fall of temperature following treatment. Only one lumbar puncture for diagnosis, day of first injection.

RECORD OF CASES WITH FATAL OUTCOME.

CASE XVI. — A. T., male, five years. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Past history irrelevant.

May 7, 1907. — Admitted to Roosevelt Hospital. Temperature, 100.2°; pulse, 84; respiration, 24. Meningococci present in the spinal fluid. Neck stiff, Kernig's sign present. Leucocytes, twenty-one thousand six hundred.

May 8. — Seven cubic centimeters of leucocyte extract given subcutaneously on the night of admission and ten cubic centimeters on May 8. Following the administration of these doses the child showed a marked improvement in all symptoms and was perfectly rational.

On May 13 five cubic centimeters more of extract were given, and from this time on injections of from five cubic centimeters to ten cubic centimeters were given at irregular intervals until May 25, and then at much

longer intervals until the end of the disease. Temperature during the last three weeks of the disease ran a swinging course, often sinking below normal, never exceeding 102° and rarely going above 101°.

Until towards the end the mental condition of the child was fairly normal, but during the last few weeks he passed into a more or less stuporous condition which was maintained until the end. The course of the disease being one of gradual emaciation, the child dying on July 30 after an illness of eighty-seven days.

Note: First injection given third day of disease.
Total quantity given, two hundred and ninety cubic centimeters.

CASE XVII. — Cephas, male, adult. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Past history not obtained.

Present illness: no history obtained.

May 21, 1907. — Admitted to Roosevelt Hospital. Temperature, 103.4°; pulse, 92; respiration, 40. Patient not rational and no history could be obtained. Lumbar puncture yields turbid fluid which contains meningococci. Patient has stiff neck, marked Kernig's sign. Presents typical clinical picture of severe epidemic cerebro-spinal meningitis.

On day of admission leucocyte extract, ten cubic centimeters, injected subcutaneously. This was followed by marked remission of the temperature. Patient slightly less noisy and restless.

May 22. — Leucocyte extract, ten cubic centimeters, injected. Again slight temporary improvement in general condition and temperature.

Leucocyte extract, ten cubic centimeters, injected May 23 and May 24, without markedly affecting condition of patient.

Patient dies May 27, 1907. Day of disease not known because no history could be obtained.

Note: First injection on day of admission (day of disease not known).
Total quantity given, forty cubic centimeters.

This case was one of extreme severity. The effect of the leucocyte extract, while apparently lowering the temperature, had no striking influence on the course of the disease.

CASE XVIII. — P. K., male, adult, laborer. Roosevelt Hospital. Service of Dr. W. H. Thomson.

Past history not obtained.

Present illness: No record of the present illness of the patient could be obtained. The patient was found unconscious in a lodging-house by the ambulance surgeon and brought to the hospital.

May 30, 1907. — Admitted to Roosevelt Hospital. Temperature, 101°; pulse, ?; respiration, 30. At time of admission patient was irrational. The extremities were covered with petechial spots. Lumbar puncture

gave turbid fluid which contained meningococci. The neck was stiff. Kernig's sign was present. Leucocytes were twenty-six thousand.

May 31. — Ten cubic centimeters of leucocyte extract were injected subcutaneously without marked effect upon the temperature or upon the general condition.

June 2. — Patient still unconscious and restless. Lumbar puncture again yields eight cubic centimeters of turbid fluid.

June 4. — Twenty cubic centimeters leucocyte extract injected. No marked effect upon patient follows.

Injections of leucocyte extract were given daily from June 4 until June 10. Patient continued irrational, temperature gradually rising and presenting larger excursions, swinging from 99° to 104° almost daily. Spinal puncture on June 11 yielded six cubic centimeters of slightly turbid fluid.

There was practically no change in condition of patient throughout June and the first week of July in spite of periodical injections of leucocyte extract. Patient never regained consciousness.

July 7. — Patient dies suddenly.

Note: First injection on second day after admission to hospital. Day of disease unknown.

Total quantity given, one hundred and ninety cubic centimeters.

Duration of disease thirty-eight days.

In this case, which was of marked severity from the very beginning, no definite beneficial effect of the leucocyte extract injections could be noted.

CASE XIX. — M. T., female, four years. Roosevelt Hospital. Service of Dr. Jackson.

Past history irrelevant.

Present illness: One week before admission child caught a cold, had a cough with scanty expectoration and slight hemoptysis. On day before admission to hospital child became much sicker, vomited, had a general convulsion, and became irrational.

June 7, 1907. — Admitted to Roosevelt Hospital. Temperature, 102.4°; pulse, 120; respiration, 40. On admission the neck was stiff. Kernig's sign was present. Physical examination was otherwise negative. Lumbar puncture yielded eight cubic centimeters of turbid fluid in which meningococci were found. On afternoon of day of admission leucocyte extract was injected, subcutaneously, ten cubic centimeters. Temperature dropped to 100° and remained low until afternoon of following day, when again rises to 104°.

Leucocyte extract given in quantities of ten cubic centimeters daily until June 15. During this time the temperature swings irregularly between 99° and 103°; is of distinctly septic type.

After June 15 leucocyte extract given at irregular intervals in quantities of ten cubic centimeters. The temperature gradually becomes lower,

reaching normal by June 30 and remaining so thereafter. Signs of toxemia disappear, but child rapidly passes into a stage of chronic meningitis. During this stage injections of leucocyte extract seem to have but little effect upon the condition. Child grows gradually weaker and dies Aug. 8, 1907.

Note: First injection second day of disease.
Total quantity given, two hundred and thirty cubic centimeters
Duration of disease sixty-two days.

CASE XX. — J. C., male, forty years. St. Luke's Hospital. Service of Dr. Theodore C. Janeway.

Past history not obtained.

Present illness: For one week the patient had been complaining of headache. He kept on working in spite of this until the day of admission, when he came to the hospital because of the great severity of the pain.

July 16, 1907. — Admitted to St. Luke's Hospital. Temperature, 103°; pulse, 80; respiration, ? On admission patient had slight rigidity of the neck and slight Kernig's sign, but seemed rational. Lumbar puncture yield turbid fluid in which meningococci are found. Leucocytes, twenty-four thousand two hundred; polynuclears, ninety per cent. On day of admission patient received ten cubic centimeters of leucocyte extract subcutaneously injected. Following this, temperature dropped to 100°, but gradually rises again on following day.

July 17. — Patient's condition practically unchanged except that his mental condition is no longer clear. Leucocyte extract, eighteen cubic centimeters, injected.

July 18. — The injection of leucocyte extract given on July 17 seems to have had little, if any, effect. Temperature continues to rise and general condition of the patient is not apparently improved. Lumbar puncture yields sixty-five cubic centimeters of turbid fluid in which meningococci are again found in large numbers. Leucocyte extract injected, twenty cubic centimeters.

From this day on injections of leucocyte extract in quantities from ten to twenty cubic centimeters are injected daily without noticeable effect upon temperature or general condition. Patient's temperature continues to rise, reaching 106° on July 26. At the same time the patient goes into profound coma from which he does not recover. Patient died on July 29.

Note: First injection given on seventh day of disease.
Total quantity given, one hundred and twenty cubic centimeters.
Duration of disease twenty days.

This case was one of extreme severity from the very beginning. The effect of the leucocyte extract was noticeable only after the first two injections and then was slight. Later injections seemed to have had no effect.

CASE XXI. — A. C., male, twenty-five years, porter. New York Hospital. Service of Dr. S. W. Lambert.

Past history and family history irrelevant.

Present illness: Twenty-four hours before admission to the Hospital the patient became suddenly ill, complaining of headache, general pains, and vomited.

June 29, 1907. — Admitted to New York Hospital. Temperature, 99.2°; pulse, 92; respiration, 22. There was a petechial eruption all over the body. The patient had rigidity of the neck and Kernig's sign. There was marked stupor. Lumbar puncture yield turbid fluid in which a moderate number of meningococci were found. Leucocytes, twenty-one thousand three hundred; polynuclears, ninety-three per cent. On day of admission ten cubic centimeters of leucocyte extract were injected. The temperature which had risen to 101° before the injection dropped to 100°, but there was no other change in the condition of the patient.

June 30. — The stupor has become more profound, approaching coma. Leucocyte extract, ten cubic centimeters, injected.

From this time on leucocyte extract was injected daily in quantities of ten cubic centimeters without apparently benefiting the condition of the patient. Patient died July 10.

Note: First injection given second day of disease.
Total quantity given, one hundred cubic centimeters.
Duration of disease thirteen days.

CASE XXII. — W. D., male, seven months. Bellevue Hospital. Service of Dr. Meara.

Family history irrelevant.

Past history: One week ago child was discharged from North Brother's Island where it had been for two weeks suffering from measles.

Present illness: Since coming home from North Brother's Island baby has been listless, has not cared for food and vomited after every feeding. During this time it has lost weight.

Oct. 2, 1907. — Admitted to Bellevue Hospital. Temperature, 101.4°. On admission child had a stiff neck and Kernig's sign. There was a yellow purulent discharge from the left ear. Lumbar puncture yielded turbid fluid in which meningococci were found.

October 5. — The retraction of the head is marked. Leucocyte extract, five cubic centimeters, injected subcutaneously.

October 6. — Temperature dropped after yesterday's injection of leucocyte extract to normal but general condition of child did not improve.

October 7. — Leucocyte extract, six cubic centimeters, injected. Temperature remains normal but general condition of child is worse. The neck is less rigid, but Kernig's sign is more marked. The child is extremely weak.

After October 8 the temperature gradually rises and in spite of two further injections of leucocyte extract of seven cubic centimeters and eight

cubic centimeters respectively, the condition of the child steadily grows worse. Child died Oct. 14, 1907.

Note: First injection given fifth day of disease.
Total quantity given, twenty-five cubic centimeters.
Duration of disease not certain, probably seventeen days.

It is but fair to note in this case that the infant was extremely weak and that the meningeal condition was complicated by a preëxisting gastro-enteritis.

CASES OF UNCERTAIN OUTCOME.

CASE XXIII. — K. C., female, three years. Bellevue Hospital. Service of Dr. Norrie.

Family history irrelevant.

Past history: The child had been in Bellevue Hospital during May and again during June of this year suffering from influenza and acute bronchitis.

Present illness: After the last discharge on June 19, 1907, child did not seem to thrive. Four days before admission to hospital complained of headache, vomited, and was slightly irrational.

July 22, 1907. — Admitted to Bellevue Hospital. Temperature, 105°; pulse, 112; respiration, 36. Child has a stiff neck, Kernig's sign, and is very restless. Vomits so that it has to be fed by gavage.

July 24. — General condition of the child is unchanged. Lumbar puncture yields turbid fluid in which meningococci are found in pure culture.

July 25. — Leucocyte extract given, ten cubic centimeters. After injection, child for a short time does not seem as well as before and has several convulsions. Lumbar puncture at this time shows the spinal fluid to be so thick that it will hardly run through the needle.

Leucocyte extract, ten cubic centimeters, was now given daily for a long time. During this time there is a gradual decline in the temperature, which nevertheless remains of a septic type. The most marked effect of the leucocyte extract injection seems to be evident in a diminution of the vomiting and of the restlessness. The child gradually passed into the chronic stage. After August 15, leucocyte extract injections are given at longer intervals and seem to control the vomiting which becomes more severe whenever injections are omitted. The child seems to be gradually improving when taken out of the hospital against advice on September 8.

Note: First injection given seventh day of disease.
Total quantity given, one hundred and seventy cubic centimeters.

In this case the only immediate effect of the leucocyte extract seems to have been in allaying toxic symptoms, in

that after injections vomiting ceased and nourishment was better taken.

CASE XXIV. — S. C., male, fourteen years, laborer. Bellevue Hospital. Service of Dr. Norrie.

Past history and family history irrelevant.

Present illness: Two days before admission to the hospital complained of pain in the abdomen and vomited. On the day following had high fever and his neck became stiff.

July 31, 1907. — Admitted to Bellevue Hospital. Temperature, 103.2°; pulse, 68; respiration, 22. During the early days of the disease the patient displayed an irregular temperature showing daily excursions, maintaining an average of about 103°.

August 4. — Throat examination suspicious of diphtheria, taken to Willard Parker Hospital.

August 7. — Re-admitted to Bellevue Hospital. Neck still stiff, temperature still fluctuating about 103°, Kernig's sign still present.

August 8. — Leucocyte extract, ten cubic centimeters, injected subcutaneously. Temperature drops to normal following injection, but there is no further change in condition of patient.

After August 8, daily injections of leucocyte extract of ten cubic centimeters each were given, each time markedly affecting the temperature but not noticeably affecting the general condition. There is, nevertheless, slight but distinct general improvement.

August 20. — Patient taken out of the hospital by relatives against advice. On date of discharge somewhat improved but not cured.

Note: First injection given tenth day of disease.
Total quantity given, one hundred and twenty cubic centimeters.

It was noticeable in this case that in spite of apparent severity of other symptoms the mental condition of the patient remained excellent throughout the disease. He was neither irrational nor did he himself complain of any of the suffering usually attendant upon his condition.

SUMMARY AND DISCUSSION.

In reviewing any group of clinical cases treated with a therapeutic agent, hitherto untried, conclusive judgment as to its value can be formed only upon a twofold basis. On the one hand, there is statistical evidence based on a comparison of large numbers of the treated with a similar series of those untreated; and, on the other hand, there is the extremely

valuable evidence of daily observation of the individual case, taking into account all the details of the clinical picture, and allowing for the favorable or unfavorable features of the disease as manifested in the individual patient.

Statistical evidence, though given in the final summary of the cases, can, at most, be of little value at present. The observations are too few for this, and in justice to the method of treatment it should be held in mind that most of the cases were of a very severe type, or came under treatment late in the disease when the infection had brought about a state of grave toxemia, great emaciation, and in many cases probably well-established anatomical changes.

An attempt to draw conclusions from a series of cases like the preceding twenty-four is, naturally, therefore fraught with many difficulties. The characteristically irregular course of epidemic meningitis weakens, to a great extent, the value of any opinion deduced from the evidence of the individual case, and final judgment must be reserved for statistical study based on larger numbers. An analysis of the preceding small group of cases, however incomplete, nevertheless reveals definite features which seem to argue for a favorable influence of leucocyte extract treatment upon the course of the disease.

Statistically considered, out of the twenty-four cases two were discharged from Bellevue Hospital against advice, and removed from the possibility of further treatment before the outcome of the disease could in any way be foretold.

Although improved somewhat at the time of discharge, they have been lost sight of and cannot fairly be included in any statistical summary. Of the remaining cases fourteen were discharged cured and without sequelæ. Eight cases died. Calculated in percentages, this yields the result of 63.6 per cent cured to 36.4 per cent fatal.

Reviewing the individual cases a little more in detail, it seems of interest to state that fifteen of these cases were under fifteen years and, of these, three died, leaving eighty per cent of recoveries with no sequelæ. Of the cases over fifteen years there were seven, five of which died. This result

may have been due to the fact that several of these adult cases were in extremis when admitted to the hospital. In spite of this, under treatment some of these cases showed a marked improvement and did not die before twenty-seven, seven, thirty-eight, eleven, and twenty-five days after treatment was begun. Of the children that were treated, but who died, one survived seventy-nine days, one sixty-two days and one, a baby of seven months, twelve days after treatment was instituted. (See table.)

RECORD OF CASES WHICH SURVIVED.

Case.	Age.	First Day of Disease.	Admitted to Hospital.	First Injection Leucocyte Extract.
II....	5 years.	April 29.	April 30.	2d day.
III....	6 "	" 25.	" 26.	3d "
IV....	8 "	May 9.	May 9.	2d "
V....	23 "	" 20.	" 29.	17th "
VI....	6 "	" 4.	" 5.	3d "
VII....	9 "	July 2.	July 4.	3d "
VIII....	11 "	May 13.	May 15.	20th "
IX....	7 months.	" 2.	" 4.	47th "
X....	5 years.	June 30.	July 8.	41st "
XI.....	4 "	Aug. 11.	Aug. 14.	5th "
XII....	9 "	March 16.	March 17.	53d "
XIII....	12 "	May 25.	May 25.	39th "
XIV....	26 "	Feb. 27 ?	March 6.	82d "
XV....	6 "	Sept. 20.	Private case.	4th "

RECORD OF CASES WITH FATAL OUTCOME.

Case.	Age.	First Day of Disease.	Admitted to Hospital.	First Injection Leucocyte Extract.	Died.	Lived after Treatment.
I. ..	15 years.	April 21.	April 22.	3d day.	May 17.	25 days.
XVI. ..	5 "	May 6.	May 7.	3d "	July 30.	79 "
XVII. ..	Adult.	?	" 21.	?	May 27.	7 "
XVIII. ..	"	?	" 30.	?	July 7.	38 "
XIX. ..	4 years.	June 6.	June 7.	2d day.	Aug. 8.	62 "
XX. ..	40 "	July 9.	July 16.	7th "	July 29.	13 "
XXI. ..	25 "	June 28.	June 29.	2d "	" 10.	11 "
XXII. ..	7 months.	Sept. 29 ?	Oct. 2.	6th " ?	Oct. 14.	9 "

It seems also of interest to note the fact that of seven cases in which treatment was begun subsequent to the seventh day of the disease, there was one hundred per cent of recoveries without sequelæ. Treatment had not begun in these cases until the lapse of seventeen, twenty, forty-seven, forty-one, fifty-three, thirty-nine, and eighty-two days after the first symptoms developed, and a reference to the histories will show that these patients were, as a rule, in grave condition.

In view of the many statistical studies of this disease during recent years, both abroad and in this country, these figures may be given without further comment.

Considered individually, the separate cases show much that is of importance both for the estimation of actual beneficial results and for the elucidation of the manner in which such results are probably produced.

Instances of immediate changes in the condition of the patient following the injection of leucocyte extract were noticeable no less in some of the fatal cases than in those that recovered. Such changes were often more marked after the first injection than after later ones.

Almost without exception there was an improvement in those symptoms which depend, largely, in this disease upon

the central nervous system: vomiting, delirium, stupor, and hyperesthesia were usually diminished or entirely allayed after one or two administrations of quantities ranging from five cubic centimeters to twenty cubic centimeters. The very promptness of the reduction of these symptoms makes it possible to exclude their having, in these cases, depended upon anatomical changes in or exudate pressing upon the nerve centers. This leads to the logical inference that underlying conditions were usually those of toxemia. The significance of this observation in man becomes more marked when taken in connection with the result attained by us in our animal experiments, in which the major action of the leucocyte extract seemed to be principally one of poison neutralization.

Marked reduction in the temperature following injections was noticeable in many of the cases. In some of these cases, however, the diminution of the fever was a temporary phenomenon, limited to the twenty-four or forty-eight hours immediately following the injection, a fact which also argues strongly for the idea of poison neutralization.

Attention may also be called to the fact that the spinal fluid often became markedly less turbid after the first injection, the organisms also gradually diminishing in numbers. It is not clear, however, to the writers that this was the immediate result of the leucocyte extract injections, but may rather have been due to a rapid refilling of the spinal canal subsequent to repeated lumbar puncture.

In those cases in which record was made of blood examinations, a reduction in the leucocytosis gradually took place as the toxic symptoms lessened.

No further analysis of the cases seems necessary and the validity of the observations and conclusions here given can be determined only by further clinical investigations.

[In conclusion the senior author takes great pleasure in acknowledging the invaluable services of his former assistant, Dr. David G. Allen, whose careful and conscientious observation and treatment of our first meningitis cases largely made possible the work here recorded.]

2. ON THE TREATMENT OF LOBAR PNEUMONIA WITH LEUCOCYTE EXTRACT.

INTRODUCTION. — Many attempts have been made to produce antipneumococcic sera, with the hope that they would prove of therapeutic value in the treatment of infections due to the pneumococcus.

The fact, however, that not one of these sera has come into general use may probably be taken as a fair proof that the benefits, if any, derived from their use have not been sufficiently obvious and regular to seriously commend them to the practitioners of medicine and surgery as a routine necessity in the treatment of pneumococcus infections.

It was natural, therefore, in view of this supposed inefficiency of immune serum, that we should try, with much interest and some hope of success, the effect of leucocyte extract upon infections due to the pneumococcus, and especially upon lobar pneumonia.

The histories and charts of the patients whom we have treated will be given in some detail and, when taken in connection with the results obtained in our experiments on animals, will no doubt prove the best guide to the individual analyst in forming a judgment as to whether our method of treatment, based primarily on theory, has been shown to have any foundation in fact.

CASE I. — Donald, male, thirty-five years. St. Luke's Hospital. Service of Dr. T. C. Janeway.

Past history negative.

Present illness: For one month patient has had a slight cough with profuse expectoration, occasionally blood streaked. During this time patient has been feverish and has had occasional night sweats.

Four days before admission to hospital, he awoke with intense pain in the left chest aggravated by moving. The pain was especially severe on coughing and deep breathing. Since that time has had high fever and has been occasionally irrational.

May 7, 1907. — Admitted to St. Luke's Hospital. Temperature, 104° F.; pulse, 124; respiration, 38. Over the left lung from the clavicle to the third space, in front, there is dulness, bronchial voice and breathing, and there are numerous subcrepitant and crepitant râles.

During May 8 and 9 the area of consolidation rapidly extends, finally covering the entire upper left lobe. The patient is in rather poor condition. Leucocytes, thirteen thousand.

May 10. — Leucocyte extract given, ten cubic centimeters subcutaneously. Following the injection there is a slight drop of the temperature, not more so, however, than on preceding day. There is no marked change in the general condition.

May 11. — Leucocyte extract, six cubic centimeters, injected subcutaneously. Following this injection there is a marked drop in the temperature from 105° to 102° F. There seems to be some improvement in the general condition of the patient.

May 12. — Two injections of leucocyte extract given, nine cubic centimeters each, one just before noon, the other about 6 P.M. The temperature, which was rising at the time the first of these injections was given, rises to 103.2° at four in the afternoon, then drops throughout the night, reaching 99.2° by the morning of the following day. Leucocytes, ten thousand five hundred; polynuclears, ninety-two per cent.

May 13. — The condition of the patient is very markedly improved. He is rational, his pulse which had reached 120 on the day before drops to 80, his respirations which had reached a maximum of 54 on the day before dropped to 34. Leucocytes, thirteen thousand; polynuclears, 95.5 per cent. Temperature remains low, not exceeding 99.4° throughout the day. Leucocytes at 2 P.M., twenty-one thousand two hundred.

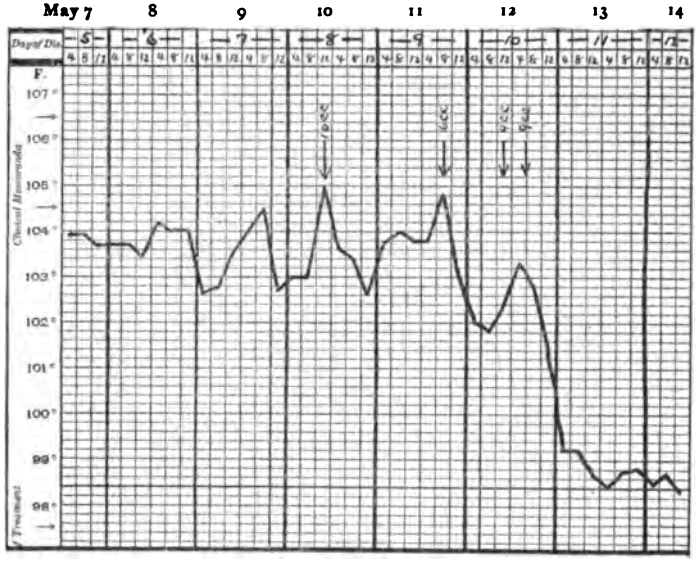
May 14. — The temperature of the patient on this day does not exceed 99.8°. The patient seems convalescent. Leucocytes, fifteen thousand; polynuclears, 82.3 per cent.

After this the patient grows rapidly better, physical signs clear up and after a short convalescence the patient is discharged from the hospital cured.

Note: First dose given on eighth day of disease.
Total quantity given, thirty-four cubic centimeters.

In commenting upon this case it is important to note that the previous history of the patient arouses considerable suspicion of a preëxisting tuberculosis. This suspicion is somewhat strengthened by the low leucocytosis observed during the active stages of the pneumonia. At the time treatment was commenced the condition of the patient was very grave, and recovery, while not impossible, was extremely uncertain. A survey of the chart leaves little doubt as to the influence of the injections of the leucocyte extract upon the temperature. It is also of considerable interest to note

that the leucocytosis of the patient increased rapidly and considerably after treatment was begun.



May 12. — Almost no chlorides in urine. Leucocytes, 10,500. Polynuclears, 92%. Lymphocytes, 8%.
May 13. — Leucocytes, 13,300. Polynuclears, 95½%. Lymphocytes, 4½%.
May 14. — 11 A.M., Leucocytes, 21,200. 2 P.M., 19,300. Polynuclears, 88%.

Lobar pneumonia. Case I. Donald.

CASE II. Mohan, male, adult. St. Luke's Hospital. Service of Dr. T. C. Janeway.

Family and previous history irrelevant.

Present illness: Four hours before admission, while at work, the patient had a shaking chill. His teeth chattered, his lips were blue, and he felt feverish. At the same time he began to suffer from pain in the lower anterior part of the right chest which was markedly aggravated by deep breathing.

May 10, 1907. — Admitted to St. Luke's Hospital. Temperature, 102° F.; pulse, 100; respiration, 26. Over the lower lobe of the right lung there are typical signs of consolidation. The sputum is blood streaked. The leucocytes are twenty-six thousand; polynuclears, eighty-nine per cent.

May 11. — The condition of the patient is unchanged, temperature rises, reaching 104° F. at noon. Leucocyte extract, six cubic centimeters, subcutaneously injected. Following this injection there is a sharp and steady

drop in the temperature reaching 98.8° on the morning of the following day.

May 12. — During the morning of this day temperature steadily rises, reaching 103° F. at noon. Leucocyte extract, ten cubic centimeters, injected in early afternoon. Temperature again drops, reaching 98° F. at midnight.

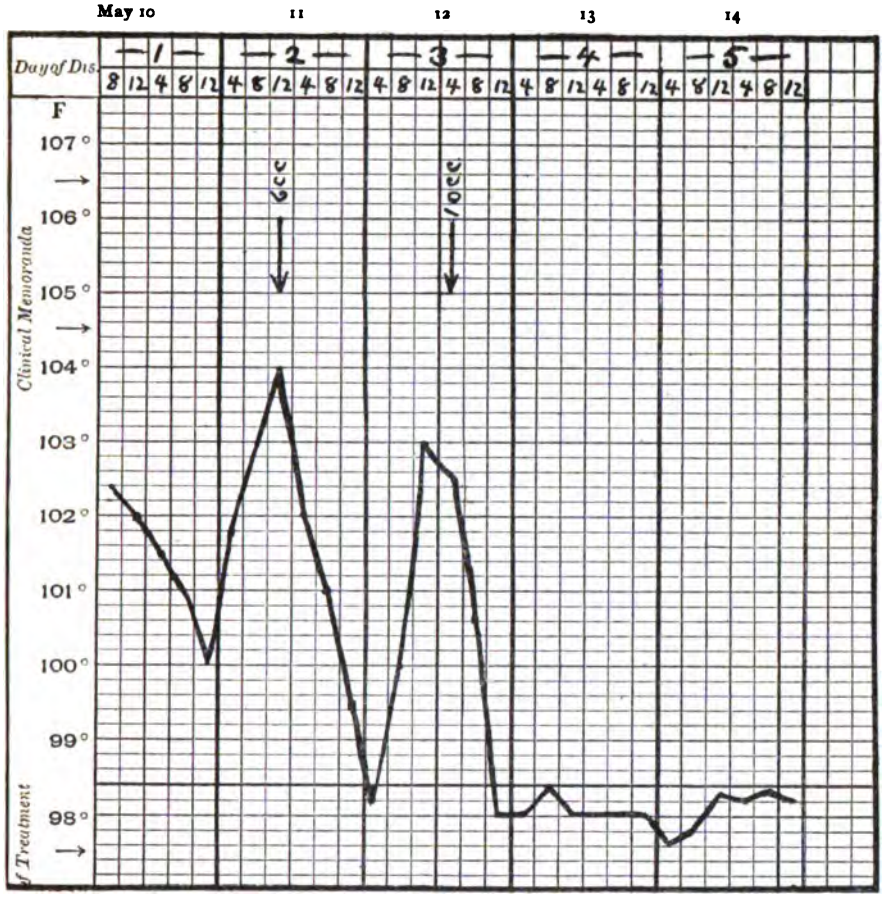
May 13. — The general condition of the patient is very much improved, breathing is easier, pulse and respiration have become normal.

After a rapid convalescence is discharged cured.

Note: First injection second day of disease.

Total quantity given, sixteen cubic centimeters.

This case is interesting primarily because of a sharp and rapid response of the temperature to the injections of the leucocyte extract. The occurrence of the crisis on the afternoon of the third day of the disease is a phenomenon sufficiently rare to encourage the opinion that it may have been at least in part due to the injection.



May 10. — Leucocytes, 26,000. Polynuclears, 89%. Lymphocytes, 11%.
May 11. — Leucocytes, 19,800. Polynuclears, 90%. Lymphocytes, 10%.
May 12. — Leucocytes, 19,700. Polynuclears, 94½%. Lymphocytes, 5½%.
May 13. — 11 A.M., Leucocytes, 6,800. 2 P.M., Leucocytes, 7,400. Polynuclears, 86%. Lymphocytes, 14%.
May 14. — Leucocytes, 5,600. Polynuclears, 48¾%. Lymphocytes, 50¾%. Eosin, ½%.

Lobar pneumonia. Case II. Sylvester Mohan.

CASE III. — Hart, male, adult. St. Luke's Hospital. Service of Dr. T. C. Janeway.

Family and previous history negative.

Present illness: Five days before admission to the hospital the patient slept in a draught, and on the following morning had a severe shaking chill which lasted for a few hours. The chill was followed by pain in the left chest, which was very severe and was increased by coughing and by deep breathing. Feeling too ill to go to work he went to bed and had, he believes, high fever, and perspired freely. Four days before admission he began to cough frequently and had reddish but scanty sputum. Since then he has felt very ill, has coughed a great deal, often so severely that a coughing spell brought on vomiting. He has suffered severely from pain in the left side, thirst, and dyspnea.

June 28, 1907. — Admitted to St. Luke's Hospital. Temperature, 104.4° F.; pulse, 125; respiration, 32. Patient has a flushed face, dilated nostrils, and pulsation of the veins of the neck. Over left lung in axilla from angle of scapula to the base there is feeble broncho-vesicular to bronchial breathing, bronchial whisper, and many fine râles. Leucocytes, sixteen thousand; polynuclears, eighty-three per cent. On day of admission leucocyte extract, ten cubic centimeters, injected. Following injection of leucocyte extract there was a rapid and marked drop in the temperature, reaching 101° F. on morning of following day.

June 29. — Temperature, which had touched 101° F. in the morning, began to rise rapidly, reaching 105.2° F. by four in the afternoon. Leucocyte extract, ten cubic centimeters, again injected. Following this injection the temperature again drops, reaching its lowest point at about midnight, 102.6° F.

June 30. — Between midnight and morning of this day the temperature again begins to rise, but does not exceed 104° F. Two injections of leucocyte extract are given of ten cubic centimeters each at 10 A.M. and at 6 P.M. Following the last injection the temperature again drops, reaching 101° F. by the following morning. Throughout this time the general condition of the patient is markedly improving.

July 1. — In spite of the fact that the temperature of the patient is still falling and his general condition steadily improving, another injection of ten cubic centimeters of leucocyte extract is given.

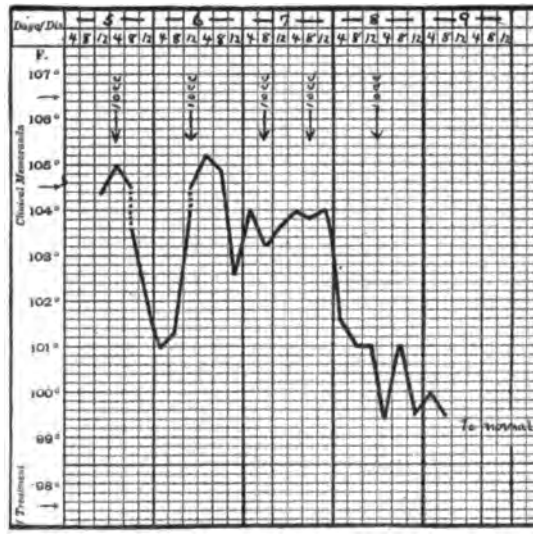
After July 1 with slight excursions the temperature sinks to normal and remains so. Convalescence is established and the patient steadily improves, being discharged cured from the hospital without further complications.

Note: First injection given fifth day of disease.
Total quantity given, fifty cubic centimeters.

The treatment in this case was not begun until the fifth day of the disease and no distinct shortening of its course can be claimed for the leucocyte extract. Nevertheless the

rapid response of the temperature to the injections of the leucocyte extract leaves little doubt in the minds of the writers that the injected substances distinctly influenced the infection of the patient.

June 28, '07.



Lobar pneumonia. Case III. Hart.

CASE IV.— Faulkner, female, fifty years. St. Luke's Hospital. Service of Dr. T. C. Janeway.

Family and previous history negative.

Present illness: Two days before admission to the hospital patient had a shaking chill. This was accompanied by pain in the left axilla and in the posterior chest. The pain was aggravated by deep breathing and coughing. On the day following she had severe headache, vomited and had high fever.

May 15, 1907. — Admitted to St. Luke's Hospital. Temperature, 102° F.; pulse, 120; respiration, 36. The patient seemed extremely ill and feeble. There were signs of consolidation in the left axilla. Leucocytes, eleven thousand four hundred; polynuclears, 88.5 per cent.

May 16. — The patient's condition is extremely grave. The temperature rises at noon to 104.2° F. There is much cyanosis. At noon leucocyte extract, ten cubic centimeters, is injected. Temperature drops following this to 101.6° F., but rapidly rises again.

May 17. — Temperature again fluctuating about 104° F. Two injections

of leucocyte extract given of ten cubic centimeters each. Patient's condition is apparently considerably worse. Leucocytes, twelve thousand one hundred; polynuclears, ninety-four per cent.

During the days following May 17, in spite of daily injections of leucocyte extract, the patient's condition grows rapidly worse and she is considered to be in extremis.

May 21. — Develops pericarditis, jaundice, and slight edema of the lungs. The leucocytes, which have been gradually increasing from day to day, now reach twenty-seven thousand; polynuclears, 93.5 per cent. In spite of severe condition patient seems to be holding her own.

Injections of leucocyte extract given at irregular intervals.

May 25. — Physical signs have been changing during the last few days and are now those of an abscess or small cavity in the upper lobe of the left lung. The temperature gradually falls and the general condition of the patient seems much improved.

Following May 25 the patient slowly but steadily improved. Finally discharged cured after prolonged convalescence.

Note: First injection given fourth day of disease.

Total quantity given, eighty cubic centimeters.

In this case attention is called to the fact that in spite of the extreme gravity of the patient's illness her mental condition remained good throughout. The leucocytosis which was low at first, thus giving for this disease a very unfavorable prognosis, steadily rose during the treatment with leucocyte extract. Finally, the localization of the infection in an abscess of the lung is additional evidence that in spite of the severity of the disease the resistance of the patient was not insignificant. Definite conclusions cannot, of course, be drawn from this observation. It is, furthermore, only fair to state that during the most extreme stages of the patient's illness her recovery was not looked for by the physicians in attendance.

Case V. — Syms, male, twenty-eight years. Roosevelt Hospital. Service of Dr. James.

Past history irrelevant.

Present illness: Three days before admission the patient had a sharp pain in the right side and felt ill. Two days before admission he began to cough and had reddish expectoration.

Dec. 16, 1907. — Admitted to Roosevelt Hospital. Temperature, 101° F.; pulse, 88; respiration, 36. The patient seemed quite ill. Examination of the lungs showed signs of consolidation over the lower lobe of

the right lung posteriorly. Leucocytes, twenty-four thousand. On day of admission temperature rose to 103.4° F. Leucocyte extract, twenty cubic centimeters, injected at 12.30 P.M. Following this injection the temperature dropped to 100° F.

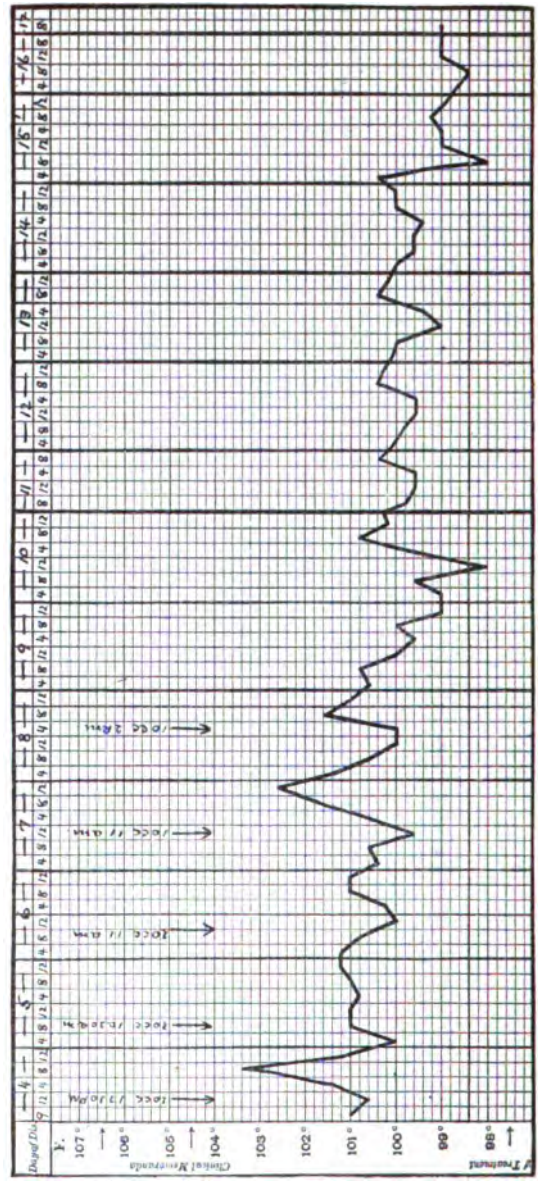
December 17. — Patient's temperature remained about 101° F., but patient himself seems profoundly intoxicated. Leucocyte extract, twenty cubic centimeters, given.

December 18. — Following last injection patient's temperature has again dropped to 100° F. and another injection of leucocyte extract is given, twenty cubic centimeters, because patient seems severely ill out of all proportion to his temperature.

After December 18 patient gradually improves and physical signs clear up. Two further injections of leucocyte extract were given, one on December 19 and one on December 20. The patient gradually improved, his temperature coming down by a slow lysis. Is discharged cured on January 1, after a rather prolonged convalescence.

Note: First injection given fourth day of disease.
Total quantity given, eighty cubic centimeters.

This case was never clearly one of true pneumococcus pneumonia, but was looked upon as possibly influenza. The temperature never exceeded 102° F., was very irregular and the apparent prostration of the patient was far more severe than the temperature would lead one to expect. It is of interest to note that after the first and fourth injections of leucocyte extract there was an initial sharp rise of temperature followed by an equally sharp drop. This observation, while somewhat confusing, is strikingly similar to the experience gained by the writers in animal experimentation where the injection of leucocyte extract and the improved condition of the infected animal were often followed by rise of temperature.


Dec. 16, '07.
Lobar pneumonia. Case V. Syms.

CASE VI. — Nicholaun, male, twenty-nine years. Roosevelt Hospital. Service of Dr. W. B. James.

Past history irrelevant.

Present illness: Two days before admission to the hospital the patient had a cough and a pain in the side. Did not feel very ill.

Dec. 7, 1907. — Admitted to Roosevelt Hospital. Temperature, 100.4° F.; pulse, 100; respiration, 24. On admission to the hospital the patient had signs of consolidation in the lower lobe of the right lung, posteriorly. Very soon after admission the temperature rose to 103.8°. Patient had cough, pain in the chest, and slight expectoration.

December 9. — Leucocyte extract, ten cubic centimeters, injected subcutaneously. At time of injection patient's temperature had reached 104.6° F. After injection it rapidly dropped to 102° F. on the following morning.

December 10. — Leucocyte extract, ten cubic centimeters, injected without very marked effect.

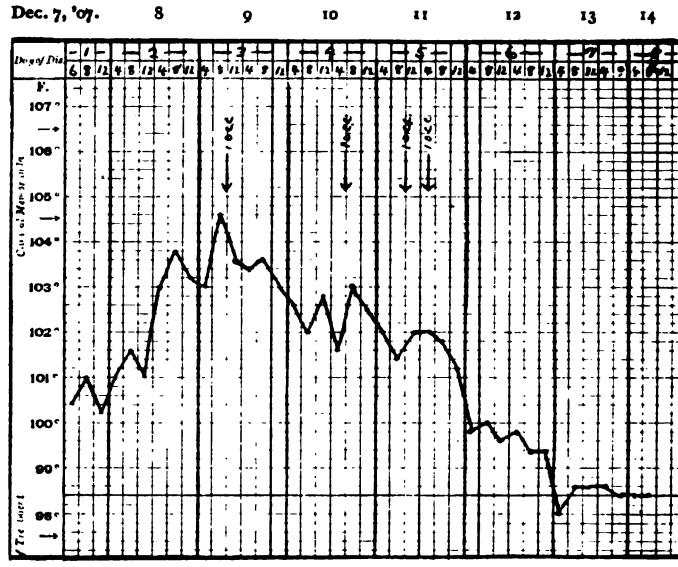
December 11. — Patient's condition about the same. Two injections of leucocyte extract of ten cubic centimeters each given, and temperature promptly drops, reaching 99.8° F. on following morning.

During December 12, sixth day of disease, temperature reaches normal and remains so throughout a short and uneventful convalescence.

Note: First injection given third day of disease.

Total quantity given, forty cubic centimeters.

Nothing very striking can be learned from this case. The case was never very severe and although sharp drops in temperature followed the injection of the leucocyte extract, the writers are reluctant to attribute this entirely to the leucocyte extract.



Lobar pneumonia. Case VI. Nicholaun.

CASE VII. — Innis, male, twenty years, porter. Roosevelt Hospital. Family and past history negative.

Present illness: Patient was perfectly well until four days before admission to the hospital. On this day he had a sharp shaking chill which lasted for fifteen minutes. Soon after this had a severe pain low down in the right chest. The following morning he noticed that his expectoration was blood streaked. He remained in bed with a cough and pain in the chest. Felt extremely weak.

Jan. 12, 1908. — Admitted to Roosevelt Hospital. Temperature, 105° F.; pulse, 104; respiration, 36. On admission to the hospital the patient had rapid respiration and dilatation of his nostrils. Examination of the chest showed dulness, bronchial breathing and nasal voice over the right lung behind from the angle of the scapula to the base, extending forward to the axilla. Over the same area there are many subcrepitant râles.

January 13. — Condition of patient is practically unchanged. Two injections of leucocyte extract are given, one of twenty cubic centimeters and one of ten cubic centimeters at 12 o'clock and 4 P.M. respectively. Following this the temperature drops sharply from 104.2° to 102° F. on following morning.

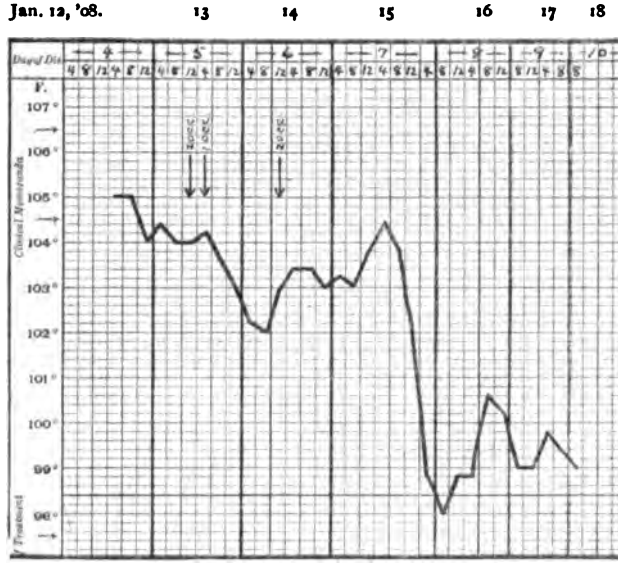
January 14. — Temperature again begins to rise, but general condition of patient is much improved. Another injection of leucocyte extract, twenty cubic centimeters, is given, following which there is no marked change in temperature.

January 15. — During day temperature drops abruptly, reaching 99.4° F. by midnight.

After January 15 patient's temperature, with a few transient rises to 100°, remains low and patient grows into a rapid convalescence. Discharged cured January 28.

Note: First injection given fifth day of disease.
Total quantity given, fifty cubic centimeters.

The noticeable feature of this case was the marked improvement in the toxemic condition of the patient after the injections and the unmistakable responses of the temperature.



Lobar pneumonia. Case VII. Innis.

CASE VIII. — This case while not one of pneumonia is yet one of pneumococcus infection, the lesion being a pneumococcus empyema. For this reason the case is included in the present series.

Uren, female, thirty years. St. Luke's Hospital. Service of Dr. T. C. Janeway.

Family and past history negative.

Present illness: Four weeks before admission the patient began to feel ill with pains all over the body. She had fever, sweated at night and felt chilly in the morning. These symptoms continued until nine days before admission when she awoke at 3 A.M. with a shaking chill and severe pain

in the lower left chest. At the same time she had high fever and began to cough. She noticed that her expectoration was blood streaked.

May 11, 1907. — Admitted to St. Luke's Hospital. Temperature, 102.8° F.; pulse, 116; respiration, 24. On admission patient had herpes about lips. Examination of the chest reveals flatness and bronchial breathing over left lower lobe behind. Over the same area there are many crepitant râles. Leucocytes, twenty-eight thousand three hundred; polynuclears, 85.5 per cent.

May 13. — Tapping of left chest yields fifty ounces of slightly turbid fluid.

After May 13 patient seems to show slight improvement, the signs in the chest changing to those of empyema. Empyema opened and in discharge are found pneumococci. From this time until May 24, the temperature is of a mild septic type, dropping occasionally to 100°, rising to 102° almost daily, occasionally to 103° F. The patient meanwhile is growing anemic and remains weak. Sinus in chest continues discharging.

May 24. — Leucocyte extract, ten cubic centimeters, injected subcutaneously. Following the injection temperature falls to normal and remains so for twenty-four hours.

May 25. — Leucocyte extract, ten cubic centimeters, injected.

May 26. — Temperature rises to 99.8° and ten cubic centimeters of leucocyte extract are again injected. Following this injection temperature drops to 97° on following day.

The discharge which had been continuous for the past few weeks now begins to subside, and soon dries up entirely. With the exception of an occasional transient rise the temperature remains normal, the patient gains strength, appetite returns, and rapid convalescence is established.

Note: First injection given twenty-second day of disease.
Total quantity given, thirty cubic centimeters.

In this case there was a condition of chronic infection of a mild type which, nevertheless, was sufficient to overcome the powers of resistance of the enfeebled patient. The rapid reaction after a few injections of leucocyte extract, noticeable in the temperature, in the cessation of the discharge, and in the marked subjective improvement in the condition of the patient, seems to indicate that the leucocyte extract sufficiently neutralized the toxemia in this case to permit the recuperation of the patient's own protective powers.

The seven cases of lobar pneumonia whose histories we have given are of course too few to be considered statistically, and offer, from this standpoint, no true basis for a definite opinion as to the value of the treatment.

When the cases are considered individually, the fact that the disease naturally terminates abruptly by crisis and is of irregular duration also makes it difficult, especially from simple inspection of the histories and the charts, for one to estimate accurately the part played by the leucocyte extract in the changes which follow its administration.

In the histories and the short critical notes following each history, we have, therefore, as has been seen, endeavored to indicate the changes in the patient — subjective and mental symptoms, temperature, and leucocytosis — which it seems to us might possibly be attributed to the action of the extract.

It is doubtful whether more definite and general conclusions than those stated in the critical notes should be drawn.

The uniformity, however, with which temperature changes followed the single injection of the extract in the lighter and less toxic cases, points strongly to a poison-neutralizing action on the part of the leucocytic substances. Similar observations made upon the more severe cases when treated with proportionately larger doses or more frequent injections — two within twelve hours — bears out this opinion, and such a conclusion as this finds support, also, in the unmistakable evidence of our animal experiments.

Other changes in the patients, such as in subjective symptoms, in the number of leucocytes, or in the limitation of the lesions, are but corollaries of the beneficial action indicated by the changes in the temperature, and are evidence of a more efficient operation of the agents of protection and a returning of physiological balance.

All these changes give the impression, at least, that we are dealing with an agent which further clinical tests will not unlikely prove of definite therapeutic value.

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BACTERIOLYSIS OF THE GONOCOCCUS AND OF THE MENINGOCOCCUS WITH NORMAL AND SPECIFIC IMMUNE RABBIT SERUMS. *

JOHN C. TORREY.

(*From the Department of Experimental Pathology, Loomis Laboratory, Cornell University Medical College, New York.*)

In previous communications, [1, 2] the writer has mentioned the fact that the serums of rabbits immunized to the gonococcus are rich in specific bactericidal immune bodies. The experimental evidence, which both justifies this assertion and also discloses the same attributes for the meningococcus, is to be found in the following pages.

It is generally agreed that the Neisser-Wechsberg plating method [3, 4] provides an accurate means of determining, quantitatively in vitro, the bactericidal potency of an immune serum. This technic has been used by Stern and Krote,[5] Laubenheimer,[7] Krote and Steinberg [6] and others in the detection of specific bacteriolysins in the serum of typhoid patients acutely ill or convalescent; by Töpfer and Jaffé [8] and by Neufeld and Hüne [9] in studies of the anti-bodies in the blood of patients sick or recovering from typhoid and paratyphoid as compared with those produced by experimental inoculations; by Wright,[10] by Shiga [11] and others in determinations of specific blood changes in man following protective vaccinations. These and various other investigations have shown that the typhoid, the paratyphoid and the dysentery bacillus stimulate the production of specific

* Received for publication Oct. 8, 1908.

bactericidal immune bodies, as revealed by in vitro tests, as do also V. choleræ and certain other vibrios. On the other hand, repeated attempts to demonstrate, by this method, the presence of these anti-bodies in serums immune to such Gram-positive cocci as pneumococcus, streptococcus and staphylococcus have led to negative results. As regards the Gram-negative diplococci, however, no experiments in accordance with this method have been reported, save a brief note by Neisser[4] in reference to the gonococcus and the preliminary statements of the writer. The positive results for the gonococcus and the meningococcus, described herein, aside from a purely scientific interest, may be of significance in connection with the serum-therapy of the diseases which they incite.

The susceptibility of these diplococci, especially the gonococcus, to unfavorable physical and chemical conditions renders accurate bactericidal tests with them a pathway beset with pitfalls. These experiments have been conducted for a year and a half, and a considerable part of that time has been employed in overcoming difficulties and developing a technic which, if rigorously followed, would lead to uniform and consistent results. As the method adopted is somewhat complicated and contains many details, which it is essential to observe, the various steps are described at length.

Methods. — According to the Neisser-Wechsberg technic a few drops of broth are added to each tube containing the dilutions of activated immune serum, seeded with a definite amount of bacteria, and also to the controls. Hüne[12] found that for B. typhosus and V. choleræ the optimum proportion of broth was one part to six parts of the whole mixture. Although this degree of enrichment is quite sufficient to sustain the vitality of these microörganisms, it is inadequate for the gonococcus. Experiments showed that each tube must be provided, not only with broth, but also with a definite amount of serum. The following mixture was finally adopted for the tube containing necessarily the minimum enrichment, viz.: the control of the seeding of culture. Serum broth was first prepared by adding one cubic centimeter of inactivated normal rabbit serum to fifteen cubic centimeters of "Thalmann's" broth (reaction + 1 to phenolphthalein). Of this rabbit serum broth, one cubic centimeter was added to three cubic centimeters of .9 per cent normal saline

solution. In this mixture, with serum broth present in the proportion of one to four, the strains of the gonococcus employed generally increased slightly in four to five hours, decreasing rarely and in negligible degree, and in eighteen hours multiplied sufficiently to cloud the mixture. The criticism might be raised that the use of this amount of inactivated normal serum for enrichment would influence the results obtained, by the introduction of other elements into the experiments, such as extraneous intermediate bodies. As regards the tests with the reactivated immune serums, a number of points, as for example the well-defined and consistent non-killing post-zones, show that this factor is of no significance. Certain control experiments with fresh normal serums, however, seemed to indicate that their bactericidal titer for the gonococcus may be increased, in slight degree, by the presence of this inactivated normal serum, but not so far as to interfere with the comparative value of the tests.

As is indicated in the following chart (Table I.) the two constant elements in all the mixtures employed in these tests are the one cubic centimeter of serum broth and the one-half cubic centimeter of the emulsion of the diplococcus in saline solution. The other elements (diluted active or inactive immune serum and diluted fresh normal serum) were added in one-cubic-centimeter amount, as the purpose of the test required, and .9 per cent salt solution in amount sufficient to bring the whole to four cubic centimeters. The object of this chart is the avoidance of needless repetition and by reference to it the constituents and their proportions in any test mentioned in the following tables may be determined.

TABLE I.

Chart showing the elements and their proportions as they occurred in tests mentioned in subsequent tables.

	Mixtures.	NaCl Solution .9 Per Cent.	Inactivated Immune Serum Dilution.	Fresh (Active) Immune Serum Dilution.	Serum Broth.	Fresh Normal Serum (Complement) Dilution.	Seeding Emulsion.	Total.
	Test of fresh (active) immune serum	1.5 cc.	—	1 cc.	1 cc.	—	.5 cc.	4 cc.
	Test of heated and reactivated immune serum	.5 "	1 cc.	—	" "	1 cc.	" "	" "
Controls.	Test of inactivated immune serum without complement	1.5 "	" "	—	" "	—	" "	" "
Controls.	Test of fresh normal serum (complement)	" "	—	—	" "	1 cc.	" "	" "
Controls.	Test of emulsion of bacteria used in seeding	2.5 "	—	—	" "	—	" "	" "

The immune serums were produced in rabbits according to methods which have been described in previous articles. In general the animals had received seven to nine inoculations at the time of bleeding, which was performed eight to ten days after the last inoculation. The serums were inactivated by heating in a water bath at 55° to 56° C. for one-half hour and kept on ice. No preservative was added and no trouble encountered from contamination.

For complement, a normal rabbit was bled from the ear about eighteen hours before the experiment and the clotted blood kept on ice until the time of use. The serum was diluted and added to the tubes as rapidly as possible. If a large number of tubes are to be supplied, the flask containing the diluted fresh normal serum should be packed in ice — a procedure which was found desirable in connection with the "fixation of complement" experiments.[16]

The cultures used in these tests have all been under cultivation on ascitic agar for over two years. The differences in virulence and rapidity of multiplication of the gonococcus strains were not marked. Mention is made of this fact because Hüne[18] has stated that virulent strains of B. typhosus and V. choleræ multiply faster and are more resistant than the avirulent to destructive agents in serum. It is probable that uniform results with freshly isolated cultures of the gonococcus would be obtained with great difficulty, because of their marked sensitiveness to unfavorable conditions.

A standard emulsion of the diplococci for seeding the tubes was obtained in the following way. Growth from an eighteen-hour ascitic agar culture was taken in amount sufficient to cloud very slightly five cubic centimeters of saline solution. It was necessary to vary the degree of cloudiness for various strains, on account of differences in the number of living cocci in a like amount of growth. Such variations precluded the seeding of the tubes with a fixed amount (by weight) of growth. This five-cubic-centimeter emulsion was then poured into a flask containing two hundred cubic centimeters of sterile .9 per cent saline solution, and the whole thoroughly shaken. Each half cubic centimeter would then contain from forty thousand to two hundred thousand diplococci. The process of seeding the tubes should not occupy more than ten minutes, as after that period the diplococci begin to die out more or less rapidly.

In making the mixtures, the required amount of saline solution (sufficient to bring the completed mixture to four centimeters) was placed in a five-inch test-tube. Next was added the serum broth, then the dilution of the serum to be tested and the complement and finally one-half cubic centimeter of the emulsion of diplococci. After each addition the tube was well shaken. The tubes containing the various mixtures were then placed in an incubator at a temperature of 36° to 37° C.

As it is necessary to use some such medium as ascitic agar in plating, the process is rather more complicated than is plating as ordinarily conducted. Agar, made according to the method of Thalmann and titrated

+ 1.2 to phenolphthalein, was tubed in eight-cubic-centimeter amounts. Just before the experiment these were melted and cooled to about 50° C., and to each was added three cubic centimeters of ascitic fluid heated to the same temperature. Until used the ascitic agar tubes were placed in a water bath with a temperature sufficient to prevent solidification. The mixtures were plated out after an incubation of four and one-half hours. A tube was thoroughly shaken and one-half cubic centimeter withdrawn and placed in a sterile petri dish. Over this was quickly poured the eleven cubic centimeters of ascitic agar, which had been cooled until only slightly warm to the hand, and the whole thoroughly mixed. For each tube in the experiment a fresh sterile one-half cubic centimeter pipette was used. It would seem that the plating out of one-half cubic centimeter from each of the seeded tubes, instead of a certain number of drops, as recommended by Neisser, is conducive to greater accuracy.

After the plates had solidified, they were placed (inverted) in the incubator, as were also the tubes containing what remained of the mixtures. Although in eighteen to twenty-four hours the colonies at times attained a sufficient size to permit counting, it should be deferred until forty-eight hours, as the stronger dilutions of the immune serums often exercise a marked inhibitory influence. Plates made from these tubes, apparently almost sterile in twenty-four hours, may show a multitude of colonies on the second day. Hüne [13] and others have encountered like inhibitory conditions in these plating experiments. Plates containing less than ten thousand colonies were counted with a reasonable degree of accuracy, above that number the figures given are in a larger measure approximate.

In investigations of this character controls are of the utmost importance. These should include: (1) a control tube indicating the number of bacteria seeded, from this tube platings should be made at once and again after the period of incubation to detect any numerical change in bacterial content; (2) a control tube indicating the degree of destructive action of the amount of fresh normal serum, alone, used to reactivate the immune bodies; (3) a control tube showing the degree of inhibitory action of the strongest dilution of inactivated immune serum alone; (4) plates controlling the sterility of the various elements in the experiment. Controls of sterility are not recorded in the tables as the inactivated serums and the fresh normal serums proved almost without exception to be sterile. The detection, in fact, of any contamination, occurring during the course of these experiments, was not difficult, thanks to the characteristic appearance of the gonococcus and the meningococcus colonies.

Bacteriolysis with the fresh serum of normal rabbits. — Many investigators have found that normal rabbit serum may be quite strongly bactericidal for certain bacilli and vibrios. This lytic action may be manifest for B. typhosus and B. paratyphosus in as high dilutions as 1–10 to 1–30,

and for V. choleræ asiaticæ at still greater weakening (Töpfer and Jaffè,[8] Buxton,[13] Hüne[12]), but for Micrococcus gonorrheæ and Diplococcus intracellularis meningitidis no accurate quantitative estimations have hitherto been reported. It is evident, however, that in order to conduct tests in the reactivation of serums immune to these diplococci, their degree of susceptibility to fresh normal serum itself should be determined.

As has been frequently reported, apparently normal rabbits differ greatly in the bactericidal potency of their fresh serums. Accordingly, it seemed advisable to set aside a number of normal animals and to titrate accurately the strength of their complement. Not only, however, were there found to be differences in the serum potency of individual rabbits, but also changes occurred from time to time in the same animal. During the fall, winter, and spring these variations were not marked, but with the advent of the hot summer months a decrease in strength of one-half to one-third was not uncommon. The irregularities at this time became so marked that the successful reactivation of immune serum was a matter of great difficulty. Cohn[14] has also noted that fresh normal serum is more strongly bactericidal in winter than in summer. This is due, he thinks, to the temperature of the air and not to food or other factors.

In the following table are given the results with the fresh serum of two normal rabbits: one (Rabbit No. 4) with a low bactericidal titer; and another (Rabbit No. 5) in which it is rather high.

TABLE II.

Bactericidal action of fresh normal rabbit serum on six strains of the gonococcus and on one strain of the meningococcus. Rabbit No. 4, weak; No. 5, strong.

Dilutions of Fresh Normal Rabbit Serum.	Actual Dilution of Serum in Mixture.	Gonococcus Culture A, Plated in 4 hrs.	Culture B.	Culture C.	Culture G.	Culture H.	Culture I.	Meningococcus.
1. Rabbit No. 4, 1–2	1–8	0	1	2	125	12	100	1
2. " " 1–10	1–40	35	230	40	390	1,900	8,500	200
3. " " 1–25	1–100	700	3,150	120	25,000	20,000	20,000	3,000
4. " " 1–50	1–200	10,500	20,000	30,000	25,000	17,000	20,000	10,000
5. " " 1–80	1–320	25,000	20,000	35,000	25,000	20,000	20,000	10,000
6. " " 1–150	1–600	25,000	30,000	—	—	—	—	—
7. Seeding, Control	—	25,000	30,000	35,000	25,000	20,000	20,000	10,000

Control seeding, plated at once. A, 25,000; B, 40,000; C, 35,000; G, 15,000; H, 20,000; I, 20,000; meningococcus, 10,000.

Dilutions of Fresh Normal Rabbit Serum.	Actual Dilution of Serum in Mixture.	Gonococcus Culture A, Plated in 4 hrs.	Culture B.	Culture C.	Culture G.	Culture H.	Culture I.	Meningococcus.
1. Rabbit No. 5, 1–2	1–8	0	0	0	0	1	0	50
2. " " 1–10	1–40	0	0	0	300	7	160	250
3. " " 1–25	1–100	7	1	210	25,000	2,800	1,200	1,050
4. " " 1–50	1–200	35	70	400	28,000	3,150	20,000	2,800
5. " " 1–80	1–320	1,120	150	1,500	28,000	25,000	20,000	24,000
6. " " 1–150	1–600	10,000	30,000	30,000	—	20,000	—	—
7. Seeding, Control	—	22,000	30,000	35,000	28,000	20,000	20,000	35,000

Control seeding, plated at once. A, 22,000; B, 30,000; C, 35,000; G, 17,000; H, 20,000; I, 17,000; meningococcus, 15,000.

Two points in this tabulation are noteworthy. First, that these diplococci, in general, are decidedly susceptible to the bactericidal action in vitro of fresh normal serum, and second, that certain strains are much more sensitive than others. The more susceptible strains are A, B, and C; the hardier, G, H, I. This grouping is significant from the fact that previous experiments in agglutination [15] and in "fixation of complement" [16] have indicated the diversity of these two

sub-groups. The susceptible strains apparently are ten times more readily destroyed by fresh normal rabbit serum than B. typhosus, culture A suffering some destruction with No. 5 serum diluted 1–150, and with a still more potent rabbit serum at 1–300. The culture of meningococcus, used in these experiments, reacted to fresh normal serum in a way similar to the more resistant gonococcic strains G, H, and I.

Fresh immune serums. — Theoretically, one would expect that a serum immune to the gonococcus, when tested fresh in vitro and without extraneous complement, would have approximately the same bactericidal titer for this diplococcus as the average fresh normal serum. The accompanying tabulation (Table III.) of the results of a series of tests with the fresh serum from a rabbit undergoing inoculations with culture A substantiates this expectation. In this and the following tables it should be observed that the dilution of the serum, indicated, is really only one-fourth of the actual dilution in the experiments, because of the other elements in the mixtures (saline solution, serum broth, etc.).

TABLE III.

Bactericidal action of serum drawn at various periods from a rabbit undergoing inoculations with gonococcus A. Serum tested fresh.

Dilutions of Serum from Rabbit 386.	Normal Serum from Rabbit 386.	Serum drawn 6 Days after First Inoculation.	Serum drawn 8 Days after Third Inoculation.	Serum drawn 8 Days after Fifth Inoculation.	Serum drawn 9 Days after Seventh Inoculation.	Serum drawn 7 Days after Ninth Inoculation.	Serum drawn 10 Days after Fifteenth Inoculation.
1. 1–2	—	—	—	0	0	0	0
2. 1–5	—	1	0	0	0	1	0
3. 1–25	60	15	31	3,500	0	980	90
4. 1–50	140	210	1,560	11,000	0	6,790	15,500
5. 1–75	1,050	12,000	20,000	12,500	2	9,000	35,000
6. 1–100	11,000	25,000	28,000	15,000	22	10,000	25,000
7. 1–125	20,000	25,000	30,000	19,000	910	16,000	35,000
8. 1–150	25,000	25,000	30,000	19,000	5,600	17,000	35,000
9. 1–200	25,000	25,000	30,000	19,000	2,380	16,000	—
10. Control seeding	25,000	25,000	30,000	19,000	25,000	25,000	35,000

Up to the fifth inoculation there occurred a progressive drop in the specific bactericidal action of this fresh immune serum, a sudden rise after the seventh, followed by irregular increase and decrease to the fifteenth inoculation. It seems reasonable to suppose that these variations are due to changes in the complement content of the rabbit's blood rather than to fluctuations in the number of immune bodies. This is, evidently, true from the fact that immune bodies were found to be present in great abundance in the inactivated serum, drawn after the fifth inoculation, yet the killing titer of this serum, when active, was only one-fourth that of the normal. Again, the fresh serum after the seventh inoculation was far more bactericidal than after the fifth, and yet contained rather fewer immune bodies. According to the experiments of Buxton,[17] fresh immune typhoid and paratyphoid serum may or may not be specifically bactericidal, depending to a certain extent on the number of the inoculations. He found that after the first inoculation the "killing zone" might rise rather higher than with the normal serum, but with succeeding inoculations a prezone appeared, in which there was no bactericidal action, and this broadening finally reached the non-killing post-zone. Thus, after the fourth or fifth inoculation the fresh immune serum was not specifically bactericidal in vitro at any dilution. With fresh antigonococcic serum, apparently, the results are quite different, as no prezone has appeared at any stage of immunization and there is always a more or less extensive killing zone. In these respects this serum resembles fresh cholera immune serum. The low specific killing titer of these fresh immune serums is certainly not due to a poverty of immune bodies, but rather to the fact that in the graded dilutions a point is soon reached at which the complement is depleted to such an extent that it is entirely deflected by the plethora of immune bodies.

The serum of a rabbit inoculated nine times with meningococcus (Table IV.), when tested fresh, had certainly no stronger specific bactericidal action than normal serum. It

also reacted on gonococcus A to the same degree as fresh normal serum.

TABLE IV.

Bactericidal action of fresh meningococcic serum on the meningococcus and the gonococcus A.

Dilutions of Fresh (Active) Meningococcic Serum.	Culture Meningococcus, Plated in 4½ Hours.	Tubes in 24 Hours.	Culture Gonococcus A, Plated in 4½ Hours.	Tubes in 24 Hours.
1. 1–5	350	No growth.	50	No growth.
2. 1–10	280	" "	4	" "
3. 1–25	4,900	" "	9	" "
4. 1–50	3,500	Growth.	200	" "
5. 1–100	12,000	"	40,000	Growth.
6. 1–250	13,000	"	40,000	"
7. 1–500	13,000	"	40,000	"
8. 1–1,000.....	12,500	"	40,000	"
9. Control seeding ..	30,000	"	40,000	"

Control seeding, plated at once. Meningococcus, 5,000; gonococcus A, 20,000.

Reactivation of immune serums. — The Neisser-Wechsberg method for the quantitative determination of specific bactericidal immune bodies consists essentially in the use of graded dilutions of the inactivated immune serum in conjunction with a fixed amount of complementing serum. By far the most difficult feature in such experiments with the gonococcus is the determination and the employment of the proper amount of complement. If complement is present in too great abundance the specific action of the inactivated immune serum will be masked by the bacteriolysis of the fresh normal serum itself, while if the amount be too small the immune bodies will fail of reactivation. As Neisser and others have proved, for the optimum specific bactericidal action, complement and amboceptor must be in right relation.

Experiment to demonstrate the proper relation of complement and immune body. — Although von Nadoleczny [18] has found that the bactericidal properties of a fresh normal serum may stand in no direct relation to its complementing properties, in these experiments the one seemed to serve as a useful index of the other, viz., it was found necessary in reactivation to use a larger dosage of a normal serum weak in bactericidal properties for the gonococcus than of one strongly bacteriolytic. In order to determine, then, the amount of a fresh normal serum which would reactivate a given number of immune bodies and yet not, in itself, destroy more than a negligible number of the gonococci seeded, the following experiment was performed. To a constant dilution of inactivated immune serum and seeding of gonococcus A were added graded dilutions of fresh normal serum. Parallel with this another series was run with the same dilutions of complement, but with the immune serum replaced by normal saline solution. The results are given in Table V.

TABLE V.

Experiment to show the optimum ratio of complement to immune body.

Inactivated Immune Serum from Rabbit No. 361 Inoculated with Gonococcus A.	Complement Serum from Normal Rabbit No. 6.	Culture.	Colonies in 4½ Hours.	Tubes in 24 Hours.	Controls of Complement used in Test. Same Dilutions.	Colonies in 4½ Hours.	Tubes in 24 Hours.
1. 1–1,000....	1/40 cc.	A.	1	Very slight growth.	1/40 cc.	40	Good growth.
2. 1–1,000....	1/45 "	"	1	Very slight growth.	1/45 "	560	" "
3. 1–1,000....	1/50 "	"	2	Slight growth.	1/50 "	2,800	" "
4. 1–1,000....	1/55 "	"	1	Fair growth.	1/55 "	4,200	" "
5. 1–1,000....	1/60 "	"	4	" "	1/60 "	10,000	" "
6. 1–1,000....	1/65 "	"	17	" "	1/65 "	14,000	" "
7. 1–1,000....	1/70 "	"	70	" "	1/70 "	15,000	" "
8. 1–1,000....	1/75 "	"	36	" "	1/75 "	15,000	" "
9. 1–1,000....	1/80 "	"	1,260	" "	1/80 "	15,000	" "
10. 1–1,000....	1/90 "	"	1,190	" "	1/90 "	15,000	" "
11. 1–1,000....	1/100 "	"	6,300	Good growth.	1/100 "	15,000	" "
12. 1–1,000....	1/110 "	"	7,000	" "	1/110 "	15,000	" "
13. 1–1,000....	1/125 "	"	10,500	" "	1/125 "	15,000	" "
14. 1–1,000....	1/150 "	"	15,000	" "	1/150 "	15,000	" "
15. 1–1,000....	1/200 "	"	15,000	" "	1/200 "	15,000	" "

Control seeding, plated at once, 12,000; in four and one-half hours, 15,000.

A comparison of the figures in the fourth and seventh columns of this table indicate that activation was evident with a dilution of complement approximately three-fourths as high again as the dilution at which the fresh normal serum alone first failed to cause any diminution of the gonococci; and also that the dilution of complement best adapted for the detection of the immune bodies in this antigonococcic serum is at that point where the fresh normal serum alone begins to show a slight destructive action. As other like experiments confirmed this result, this optimum dilution was determined for the various normal rabbits and used in the activations.

The time element in the bacteriolysis. — It has been determined that three hours' incubation is sufficient for the bacteriolysis of cholera in experiments of this character, while with typhoid and paratyphoid bacilli it is advisable to carry out the plating after an incubation of about five hours. For the gonococcus the proper time of plating is dependent in a measure upon the amount of complement used in the reactivation of the immune serum; if so strong as to cause alone a marked destruction of the gonococci, the bactericidal action with the reactivated immune serum is complete within an hour; on the other hand, if present in so small amount as to have no bacteriolytic effect by itself, but sufficient to cause a certain amount of reactivation, the bactericidal action proceeds slowly and is still taking place at eight hours. If the complement is of such an amount as to cause optimum reactivation, but only slight destruction by itself, the plating may properly be carried out at some time after three hours. Four and one-half hours was chosen as giving a margin of safety.

Activated immune serums. — In the following tables (Tables VI. and VII.) are given the results of typical experiments with a reactivated gonococcic and a meningococcic immune serum. In the first column are placed the dilutions of the inactivated immune serum — each one-fourth the final dilution under the conditions of the experiment. In the second column is given the amount of fresh rabbit serum used for reactivation, and in the last two columns are described the appearance in twenty-four and also forty-eight hours of the various mixtures from which the platings were made. "Control complement" indicates the effect on the diplococci of the amount of fresh normal serum used in reactivation.

TABLE VI.

Bacteriolysis of gonococcus A by its specific serum.

Inactivated Immune Serum from Rabbit Inoculated with Gonococcus A, 8 times.	Complement Fresh Serum from Normal Rabbit No. 6.	Culture.	Colonies in 48 Hours.	Tubes in 24 Hours.	Tubes in 48 Hours.
1. 1–5	1/75 cc.	A.	10,000	Slight growth.	Good growth.
2. 1–10	" "	"	5,000	" "	" "
3. 1–25	" "	"	1,540	Very slight growth.	" "
4. 1–50	" "	"	296	" " "	" "
5. 1–100	" "	"	92	Slight growth.	" "
6. 1–500	" "	"	3	" "	" "
7. 1–1,000	" "	"	6	" "	" "
8. 1–2,000	" "	"	88	Good growth.	" "
9. 1–5,000	" "	"	280	" "	" "
10. 1–10,000	" "	"	700	" "	" "
11. 1–25,000	" "	"	4,200	" "	" "
12. 1–50,000	" "	"	5,000	" "	" "
13. 1–100,000	" "	"	6,300	" "	" "
14. Control Complement	1/75 cc.	A.	7,000	Good growth.	Good growth.
15. " Culture A Seeding.	—	"	15,000	" "	" "

Control seeding, plated at once, 12,000; media and sera, 0.

TABLE VII.

Bacteriolysis of a meningococcus culture by its specific serum.

Inactivated Immune Serum from Rabbit Inoculated with Meningococcus, 9 times.	Complement Fresh Serum from Normal Rabbit No. 6.	Culture.	Colonies in 5 Hours.	Tubes in 24 Hours.	Tubes in 48 Hours.
1. 1–5	1/20 cc.	Meningococcus.	9,440	Slight growth.	Fair growth.
2. 1–10	" "	"	4,200	" "	" "
3. 1–25	" "	"	1,260	" "	Good growth.
4. 1–100.....	" "	"	232	Very slight growth.	" "
5. 1–500	" "	"	172	" " "	" "
6. 1–1,000	" "	"	206	Fair growth.	" "
7. 1–2,000	" "	"	490	" "	" "
8. 1–5,000	" "	"	1,890	Good growth.	" "
9. 1–10,000	" "	"	5,000	" "	" "
10. 1–25,000	" "	"	7,000	" "	" "
11. 1–50,000.....	" "	"	7,000	" "	" "
12. Control Complement	1/20 cc.	Meningococcus.	7,000	Good growth.	Good growth.
13. Control Culture Seeding.	—	"	100,000	" "	" "
14. Meningococcus Serum 1–5...	—	"	22,000	" "	" "

Control seeding, plated at once, 12,000.

The bacterial counts in these tables, especially that with the gonococcus, parallel very closely those obtained in similar experiments with typhoid and paratyphoid. This particular gonococcic serum was rich in immune bodies, killing as it did an actual dilution of 1–40,000. As has been demonstrated by Neisser and Wechsberg[3] and others, the extent of the prezone is dependent upon the degree in which the immune bodies are present in excess of the complementing elements. In the first tube, apparently, as noted in other instances, they were so far in excess as to abort in a measure the bactericidal power of the fresh normal serum alone. Where immune body and complement are in optimum ratio (as in tubes 5 and 6), there occur the greatest

destruction of gonococci. Although the tabulation of the colonies on the plates is very similar to that obtained with typhoid, the tubes from which the platings were made differ after twenty-four hours' incubation from those in a like experiment with this bacillus. Whereas, in the optimum killing zone with typhoid immune serum the tubes may be sterile in twenty-four hours, with gonococcus serum this does not occur in that a few cocci are always left which multiply slowly until in forty-eight hours the tubes are clouded with growth. It is possible that the incomplete killing is due to the necessary use of comparatively weak complement.

With the inactivated serum of a rabbit well immunized to the meningococcus (Table VII.) the killing zone was nearly as well marked and practically as extensive as with the gonococcus A. No experiments in vitro have been reported hitherto, which prove that meningococcic serum may be rich in bactericidal immune bodies. In comparing this table with the one dealing with gonococcus, it should be observed that a larger amount of complement was necessary for the reactivation of the meningococcic serum, and further that this particular strain of meningococcus multiplied much faster in the culture control tube, during five hours' incubation, than any strain of gonococcus which was tested.

The agglutination factor. — Soon after the publication of the Neisser-Wechsberg method, the criticism was advanced that agglutination might be a factor in the results obtained. This objection has been found invalid by Lipstein[19] and others, but as the gonococcus is especially prone to spontaneous agglutination and is quickly clumped in comparatively high dilutions by immune serum, it seemed desirable to anticipate any criticism, based on this ground, of the soundness of these experiments. That agglutination, spontaneous or otherwise, is a negligible factor is proved by at least three considerations:

(*a.*) Fresh immune serum, at a dilution of 1–1,000, caused no decrease in the number of the gonococci, but when this same serum is reactivated, at this dilution the plates

were practically sterile and yet the degree of agglutination was necessarily the same in each instance.

(*b.*) In a previous article [15] it has been shown that strain A and strain G interagglutinate with their respective antiserums in as high a dilution as 1–200 and yet reactivated A serum causes no decrease in the number of G colonies, nor G serum of A colonies.

(*c.*) Finally, in a parallel experiment with the same inactivated immune serum (Table VIII.), the typical "killing zone" occurred only in the series in which there was reactivation with fresh normal serum, hence the agglutination factor was of no significance.

TABLE VIII.

Experiment showing that agglutination is a negligible factor.

Inactivated Immune Serum from Rabbit inoculated with Gonococcus A 7 times.	Complement Fresh Serum from Normal Rabbit No. 8.	Culture.	Colonies in 4½ Hours.	Tubes in 24 Hours.	Same Immune Serum, but *without* Addition of Complement. Colonies in 4½ Hours.	Tubes in 24 Hours.
1. 1–2.....	—	A.	—	—	3,220	Very slight growth.
2. 1–5.....	1/100 cc.	"	3,640	No growth.	6,300	Slight growth.
3. 1–10.....	" "	"	210	" "	7,800	" "
4. 1–25.....	" "	"	15	Very slight growth.	13,700	Good growth.
5. 1–100....	" "	"	9	" " "	15,500	" "
6. 1–500....	" "	"	10	" " "	23,800	" "
7. 1–1,000..	" "	"	76	Fair growth.	18,500	" "
8. 1–2,000..	" "	"	288	Good growth.	—	—
9. 1–5,000..	" "	"	5,000	" "	16,800	Good growth.
10. 1–10,000.	" "	"	5,000	" "	21,800	" "
11. 1–25,000.	" "	"	13,000	" "	—	—
12. 1–50,000.	" "	"	11,000	" "	—	—
13. Control Complement.	1/100 cc.	A.	12,000	Good growth.	—	—
14. Control Culture A Seeding.	—	"	19,000	" "	22,400	Good growth.

Control seeding, plated at once, 10,000; same, 14,500; media and sera, 0.

Inhibitory action of inactivated immune serum alone. — Hüne [12] has described a weak but clear action of specific serums, without complement, in some experiments with typhoid and cholera and also in stronger dilutions with serums which were not specific. The latter, he thought, might be due in part to the phenol used as a preservative. From the figures in the sixth column of Table VIII. it will be seen that this inactivated serum immune to gonococcus A, without complement, caused some decrease in the number of colonies in a dilution as high as 1–10. Other inactivated serums, immune to strain H, have proved lytic without the addition of complement in a much more marked degree at this dilution, but only specifically. In these instances the action cannot be ascribed to a preservative as none was used. Several possible explanations of this lytic action suggest themselves, such as the presence of a complementing substance of a more stable nature, the bringing into play by the stronger dilutions of serum of an autolysate similar to that described by Flexner [20] for the meningococcus, or the presence of an inhibitory substance in the serum of the same nature as that produced in growing culture. At any rate it should be noted that the decrease in the count occurs alone in the prezone region and the phenomenon can play no part in the killing zone of the reactivated serum.

Inter-bactericidal action of various immune serums. — The question arises whether a reactivated anti-serum immune to one strain of the gonococcus will prove bactericidal for all other strains of this diplococcus; in other words, whether the immune bodies raised by various members of this group are homogeneous or heterogeneous. The same query is pertinent as regards the relationship from this standpoint of the gonococcus and the meningococcus. The following tables (IX., X., XI., XII., XIII.) elucidate these relationships as they have been found, by repeated tests, to exist between four strains of the gonococcus and between these and a single strain of the meningococcus. Each table contains the

results of the interaction of a single culture with each of the five anti-serums. Attention is directed to the amount of fresh normal serum (complement) employed with the several cultures. This is indicated in each case in conjunction with the "complement control" figures. It will be noted that it was necessary to vary the amount of complement. Guided by the experiments demonstrating that fresh normal serum in amount sufficient to cause in itself a slight destruction of the diplococci seeded was ample for the proper reactivation of the immune bodies, the dose of complement was so varied. As a result of this method of standardization, the delicate gonococcic cultures A and C received only about one-fourth as much complement as the more resistant strains G and H and also as this particular strain of the meningococcus. Whether or not this variability may be due to differences in the receptors of these strains, there is no decisive evidence. Attention is also directed to the fact that these strains of the gonococcus multiplied in the control tubes only slightly, if at all, in four or five hours.

TABLE IX.

Tests with gonococcus culture A against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4½ Hours.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.
1. 1–5.....	10,000	25,000	11,500	15,000	6,000
2. 1–10.....	5,000	9,000	—	—	—
3. 1–25.....	1,540	5,000	10,500	15,000	4,000
4. 1–100.....	92	200	12,500	15,000	9,500
5. 1–500.....	3	112	14,000	15,000	22,000
6. 1–1,000.....	6	328	11,500	15,000	7,500
7. 1–2,000.....	88	—	—	—	—
8. 1–5,000.....	280	14,000	14,000	15,000	8,000
9. 1–10,000.....	700	14,000	—	—	—
10. 1–25,000.....	4,200	25,000	—	—	—
11. 1–100,000.....	6,300	—	—	—	—
Control with Complement.	Normal Rabbit No. 6, Serum 1/75 cc.	Normal Rabbit No. 2, Serum 1/100 cc.	Normal Rabbit No. 3, Serum 1/100 cc.	Normal Rabbit No. 3, Serum 1/125 cc.	Normal Rabbit No. 8, Serum 1/100 cc.
	7,000	29,000	12,500	15,000	5,500
Control Culture Seeding.	15,000	30,000	18,000	16,000	10,000
Control Seeding, plated at once.	12,000	25,000	16,000	14,000	8,000

TABLE X.

Tests with gonococcus culture C against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4¾ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4¾ Hours.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.
1. 1–5.....	4,200	24,000	4,200	50,000	12,000
2. 1–10.....	1,050	19,000	—	—	—
3. 1–25.....	152	15,500	5,000	50,000	12,000
4. 1–100.....	140	315	5,000	50,000	12,000
5. 1–500.....	3,290	215	5,500	50,000	12,000
6. 1–1,000.....	9,800	175	7,000	50,000	12,000
7. 1–2,000.....	18,000	—	—	—	—
8. 1–5,000. ...	19,000	560	8,400	50,000	12,000
9. 1–10,000....	19,000	6,000	—	—	—
10. 1–25,000....	19,000	17,000	—	—	—
11. 1–50,000...	19,000	15,000	—	—	—
Control with Complement.	Normal Rabbit No. 6, Serum 1/75 cc.	Normal Rabbit No. 2, Serum 1/100 cc.	Normal Rabbit No. 3, Serum 1/100 cc.	Normal Rabbit No. 3, Serum 1/125 cc.	Normal Rabbit No. 3, Serum 1/125 cc.
	15,000	26,000	6,000	45,000	12,000
Control Culture Seeding.	25,000	30,000	5,500	50,000	18,000
Control Seeding, plated at once.	24,000	28,000	6,500	50,000	18,000

TABLE XI.

Tests with gonococcus culture G against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4½ Hours.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.
1. 1–5.....	14,500	4,500	0	16	5,000
2. 1–10.....	—	—	0	30	—
3. 1–25.....	8,400	6,000	0	2,800	5,700
4. 1–100.....	12,000	4,500	1	17,000	5,000
5. 1–500.....	12,000	6,500	350	18,000	5,000
6. 1–1,000.....	14,000	7,800	840	18,000	4,480
7. 1–2,000.....	—	—	1,680	14,000	5,000
8. 1–5,000.....	—	7,500	2,170	18,000	—
9. 1–10,000....	—	—	3,300	18,000	—
10. 1–25,000....	—	—	3,200	18,000	—
Control with Complement.	Normal Rabbit No. 1, Serum 1/30 cc. 14,000	Normal Rabbit No. 6, Serum 1/20 cc. 5,500	Normal Rabbit No. 4, Serum 1/20 cc. 3,500	Normal Rabbit No. 4, Serum 1/20 cc. 18,000	Normal Rabbit No. 7, Serum 1/20 cc. 5,000
Control Culture Seeding.	28,000	9,000	10,000	19,000	6,000
Control Seeding, plated at once.	20,000	7,500	7,000	—	5,000

TABLE XII.

Tests with gonococcus H against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4½ Hours.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.
1. 1-5.....	2,900	3,500	1	280	5,700
2. 1-10.....	—	—	4	106	—
3. 1-25.....	2,170	2,800	15	102	5,000
4. 1-100.....	3,220	1,640	280	210	5,000
5. 1-500.....	2,870	2,800	2,240	1,300	5,000
6. 1-1,000	2,730	4,000	4,200	3,850	5,000
7. 1-2,000.....	—	—	5,320	8,500	—
8. 1-5,000.....	2,800	—	8,500	10,500	5,000
9. 1-10,000 ...	—	—	8,680	11,500	—
10. 1-25,000....	—	—	9,200	14,000	—
Control with Complement.	Normal Rabbit No. 6, Serum 1/25 cc. 3,500	Normal Rabbit No. 7, Serum 1/25 cc. 4,900	Normal Rabbit No. 6, Serum 1/30 cc. 8,500	Normal Rabbit No. 4, Serum 1/25 cc. 14,000	Normal Rabbit No. 7, Serum 1/35 cc. 5,000
Control Culture Seeding.	5,000	8,000	11,000	21,000	6,720
Control Seeding, plated at once.	4,200	7,000	9,000	18,000	11,000

TABLE XIII.

Tests with meningococcus against the various immune serums.

Dilutions of the Immune Serums.	Result with Serum Immune to Meningococcus. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus A. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus C. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus G. Colonies in 4½ Hours.	Result with Serum Immune to Gonococcus H. Colonies in 4½ Hours.
1. 1-5.....	13,000	750	700	8,960	10,000
2. 1-10.....	12,000	—	—	—	—
3. 1-25.....	4,000	5,600	2,800	18,000	17,000
4. 1-100.....	1,400	12,000	5,000	30,000	50,000
5. 1-500.....	210	15,000	5,500	100,000	50,000
6. 1-1,000.....	220	15,000	5,600	100,000	50,000
7. 1-2,000.....	420	—	—	—	—
8. 1-5,000.....	1,950	15,000	7,000	100,000	50,000
9. 1-10,000....	2,700	—	—	—	—
10. 1-25,000....	7,500	—	—	—	—
Control with Complement.	Normal Rabbit No. 8, Serum 1/30 cc.	Normal Rabbit No. 3, Serum 1/40 cc.	Normal Rabbit No. 6, Serum 1/20 cc.	Normal Rabbit No. 3, Serum 1/30 cc.	Normal Rabbit No. 3, Serum 1/30 cc.
	11,000	14,000	7,000	33,000	30,000
Control Culture Seeding.	30,000	50,000	35,000	100,000	100,000
Control Seeding, plated at once.	4,300	5,500	11,000	14,000	10,000

The essential point brought out in these experiments lies in the demonstration that the gonococcic strains A and C produce, in rabbits, immune bodies which cause mutual bacteriolysis, but none which react with strains G and H; and further, that G and H raise such anti-bodies reacting on one another to some extent (the action of H serum on culture G is slight), but none for strains A and C. Again, that all four of these antigonococcic serums were slightly destructive for the meningococcic culture. Finally, that this strain of the meningococcus produced an anti-serum strongly bactericidal for itself, but in no degree on any of the four gonococcic strains. It is, also, to be observed that

cultures G and H stimulated apparently the production of fewer immune bodies than A, C, or the meningococcus, as is indicated by the comparatively low specific bactericidal titer of their anti-serums and the complete absence of prezones.

In a previous article [16] on the "fixation of complement" with serums immune to these same strains, it was demonstrated that cultures A and C reacted alike, as did also to a certain extent cultures G and H, but that there was no interaction between these two pairs. This result is interesting in that it indicates, at least, a parallelism in specificity between the "fixation of complement" and these bacteriolytic experiments. Whether, however, we may say that these two processes are identical as regards the bacterial amboceptors concerned is left for further experimentation and will be discussed in another place.

The gonococcus group. — Culturally and morphologically the gonococcus group is apparently homogeneous. Such differences as may appear between various strains in their aptitude to adapt themselves to an unfavorable environment (culturally) are of slight significance, as these are variable and merely matters of degree. As regards their reaction to strains there seems to be a uniformity within the group to the smallest detail. Their enzymatic activities are feeble and probably uniform. In fact it is apparently only within the field of these more delicate serum reactions that heterogeneity may be observed. First, by a study of agglutination [15] with specific serums radical differences were evident between certain strains; a diversity, which was again manifest in the delicate "fixation of complement" test,[16] and finally confirmed by these bacteriolytic experiments.

Although agglutination seemed to disclose a surprising amount of variation within the group, certain cultures reacted practically alike and fell within two or more sub-groups. In the subsequent experiments my aim has been to determine how radically as regards other serum reactions two of these sub-groups differed from one another: the one sub-group represented by cultures A, B, and C, or let us say Type I.;

and the other by cultures G, H and I, or Type II. These differences, as determined, may be summarized as follows:

(*a.*) These two types raise certain agglutinins which are common, but their specific agglutinins are entirely distinct.

(*b.*) In the fixation of complement the cultures grouped under Type I. manifested a practical identity as regards antigen and anti-body; and the cultures of Type II. showed a similar uniformity among themselves. Yet there was little or no interaction between the members of these two sub-groups, indicating, thereby, the existence of a radical difference between the antigens and the anti-bodies derived from and with these two types.

(*c.*) Again, the cultures of Type I. are decidedly more sensitive to the bacteriolytic action of fresh normal rabbit serum than those of Type II.

(*d.*) Finally, the bactericidal immune bodies raised by the members of Type I. are inter-active among themselves, but are inactive with those of Type II.; and the reverse is true as regards the Type II. cultures.

We find, then, certain strains of the gonococcus which are alike in antigen, in the anti-bodies which they call forth, and in their receptor apparatus, but also others which are totally different. Is it necessary to count such subtle differences important or may they, from a practical standpoint, be disregarded? Is such heterogeneity of fundamental significance in the serum-therapy of gonococcic or other infections? Unfortunately we are still almost entirely in the dark as to the mode of action of bactericidal serums, when introduced into the body. That the bactericidal immune bodies specifically active in vitro against certain micro-organisms are of no significance in bacteriolysis in vivo has not been proved,* and until such proof is forthcoming it is only reasonable to suppose that these immune bodies may be active agents in some curative serums. Elsewhere

* Töpfer and Jaffè,[8] among others, hold the view that the bacteriolytic process in vitro and in the animal body are not identical. They found during sickness with typhoid that in vitro test high and the Pfeiffer lower, but during convalescence Pfeiffer high, protecting strongly, and in vitro feeble.

certain evidence has been given by the writer[2] which indicates that this may be the case with antigonococcic serum. Granting for the time being that bactericidal immune bodies, as revealed in test-tube experiments, are of significance in the curative process, these results are of practical importance in that they show:

(*a.*) That a serum produced with one strain of the gonococcus may be ineffective if the patient harbors a strain of this diplococcus belonging in a different sub-group. This being so it is desirable to determine the number of these sub-groups and to employ as many of them as possible in the preparation of a therapeutic serum. Shiga's[21] decidedly efficient anti-dysenteric serum now contains antibodies for all of the five main groups of B. dysenteriæ.

(*b.*) That certain strains of the gonococcus are very readily destroyed by fresh normal serums and by their specific anti-serums, while others succumb less readily to such lytic agents. It is possible that this fact is of some weight in determining the transitory character of certain cases of gonorrheal infection as compared with the chronic nature of others.

CONCLUSIONS.

1. Certain strains of gonococci are very sensitive to the bactericidal action of fresh normal rabbit serum, while others are decidedly more resistant.

2. Bactericidal immune bodies are readily produced in rabbits by inoculation with the gonococcus and also by the meningococcus.

3. A serum immune to one strain of the gonococcus may be entirely inactive in vitro against another strain.

4. All of the inactivated antigonococcic serums (four) tested in these experiments were slightly bacteriolytic for a certain strain of meningococcus. An antimeningococcic serum contained no bactericidal immune bodies for four strains of the gonococcus.

5. These experiments indicate that there is a parallelism

in the specificity of the results obtained by the "fixation of complement" method and in vitro bactericidal tests. They likewise confirm the conclusion, previously expressed, that the gonococcus group is heterogeneous.

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I. — A NEW PARA-DIMETHYL-AMIDO-BENZALDEHYDE TEST FOR INDOL.*

II. — CHOLERA-RED REACTION AS AFFECTED BY MIXED CULTURES.*

E. N. Tobey, A.M., M.D.

(*From the Bacteriological Laboratory of the Harvard Medical School.*)

I.

In the year 1900 Ehrlich first described the para-dimethyl amido-benzaldehyde reaction with indol and used it as a urinary test. In 1906 Böhme used the reaction to determine the presence of indol in bacterial cultures. The method employed by Böhme consists of two solutions made up as follows:

Solution 1. — Para-dimethyl-amido-benzaldehyde, 4 parts.
Alcohol (96 per cent), 380 parts.
Concentrated hydrochloric acid, 80 parts.

Solution 2. — A saturated watery solution of potassium persulphate (as an oxydizing agent). To about ten cubic centimeters of the bouillon culture of the organism add five cubic centimeters of solution 1, and then five cubic centimeters of solution 2, and shake the mixture. The presence of indol is indicated by the appearance of a red color, which becomes deeper on standing. Five minutes is sufficient in all cases for the completion of the reaction.

Böhme found that the test is ten times as sensitive as the sulphuric-nitrite test, and does not fail in concentrated solutions of indol as the older test does. Marshall also found this new test far more sensitive than the old.

In applying the test of Böhme, I soon found that one cubic centimeter of each solution would accomplish just as much as five cubic centimeters of each solution. Then on adding solution 1 with a dropper to a tube containing a growth of indol-producing organism, I noticed that at the

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junction of the upper layer of reagent with the medium below, a bright red ring was formed. This color disappeared on shaking, and solution 2 was then necessary to get the color.

To get a good idea of the efficiency of the test as compared with that of the old sulphuric-nitrite test, I employed it in six different media, using twenty-nine varieties of bacteria. Growths of one, two, three, five and twelve days were used. The old method employed was as follows: Ten drops of sulphuric acid were added to the culture and the tube shaken, then one cubic centimeter of a .02 per cent solution of sodium nitrite was added carefully drop by drop, and the red ring looked for at the junction of the two layers of fluid. The new method consisted in adding to the culture drop by drop one cubic centimeter of solution 1 of Böhme, and the ring sought at the junction of the two layers. In order to determine the accuracy of this ring method without solution 2, the tubes were later shaken and solution 2 added, but in no instance was there any difference in the reaction.

The results obtained by the testing of the twenty-nine bacteria by the old and new methods are as follows:

Organism.	Old.	New.
1. B. typhosus	—	—
2. " paratyphoid A	—	—
3. " " B	—	—
4. " fecalis alkaligenes	—	—
5. " coli communis	+	+
6. " " " (H.)	+	+
7. " " " (W.)	+	+
8. " Escherich (McConkey)	+	+
9. " Emmerich (McConkey)	+	+
10. " dysenteriæ (Shiga)	—	—
11. " " (Flexner)	+	+

	Organism.	Old.	New.
12.	B. acidi lactici	+	+
13.	" pyogenes fetidus	+	+
14.	" enteritidis (Gaertner)	—	—
15.	" aerogenes	+	+
16.	" pneumonia	—	—
17.	" hog cholera	—	—
18.	" proteus vulgaris	+	—
19.	" pestis	—	—
20.	" icteroides	—	—
21.	" mucosus capsulatus	+	—
22.	" saccharolyte (Rivas)	—	—
23	" fusiformis (Kral)	—	—
24.	Sp. Milleri (Kral)	+	+
25.	" Finkler-Prior	+	+
26.	" Deneke	—	+
27.	" rubrum	—	—
28.	" cholera	+	+
29.	" Metschnikovi	+	+

It will be seen that the only differences are in B. proteus, B. mucosus capsulatus and Sp. Deneke. B. typhoses, B. paratyphoid A and B, and B. hog cholera were grown for twelve days, but no indol found by either method. Sp. Deneke showed the new method to be more sensitive than the old, because it gave a reaction in twenty-four hours, whereas forty-eight hours were required for a faint reaction by the other method. The B. mucosus capsulatus of this laboratory has not been carefully worked out, so it cannot be compared with previous descriptions. The new method, however, showed no indol in a twelve-day growth, although the old method gave a reaction in a three-day growth.

B. proteus vulgaris is described by Chester as producing indol, and the reaction by the old method is certainly well marked. A forty-eight-hour growth, a control tube of which gave a marked reaction, was distilled. The distillate was then tested by the old method, but no ring was seen, although indol, if present, should come over in the distillate. This shows there is a substance in some cultures which, when the old method is employed, will give a reaction like indol.

Marshall, in comparing the old method with the new, does not give his way of doing the old method. The results he gets that are different according to the method consist in getting by the old method a positive reaction in cultures that are usually described as non-indol-producing. He does not mention the results other observers have obtained with different makes of peptone, or with different races of bacteria. The positive reaction he obtained with such a bacterium as B. typhosus may be due to the above-mentioned reasons. He also gives a five-day growth as customary when using the old method, although his table shows that he was able to get a positive reaction by the old method, even with non-indol-producing bacteria, in twenty-four to forty-eight hours.

The six different media employed are as follows:

1. Sugar-free bouillon prepared with B. coli communis, and having salt.
2. Sugar-free bouillon prepared with B. coli communis, without salt.
3. Sugar-free bouillon prepared with B. saccharolyte (Rivas), and having salt.
4. Sugar-free bouillon prepared with B. saccharolyte (Rivas), without salt.
5. Dunham's peptone solution.
6. Peptone solution (one per cent) without salt.

In making the above, it was noticed that when salt was added a cloudiness occurred and after sterilizing a precipitate was thrown down, so that the media had to be filtered again. All media was titrated to .5 per cent acid with phenolphthalein as an indicator.

In the six hundred tubes used and with the twenty-nine

different bacteria employed, no appreciable difference in growth was observed, except in the peptone solution without salt, when the growth was always scanty and sometimes absent. Sodic chloride (one-half per cent) has, therefore, no appreciable effect in sugar-free meat bouillon, but is necessary when peptone alone is used.

No difference in the reaction was observed with bouillon prepared either with B. coli communis or with B. saccharolyte (Rivas), but as the latter produces more gas and does not produce indol, it seems better to use this in preference to the other.

The intensity of the color is greater in sugar-free bouillon when Böhme's original method is used. When the ring methods of both the sulphuric-nitrite and the para-dimethyl-amido-benzaldehyde tests are employed, a faint reaction may be missed in a sugar-free bouillon. This occurred twice in one lot of one hundred and seventy-four tubes.

Experience here seems to indicate that transplants made directly from cultures kept in too cold a room fail to show indol, or give a much fainter reaction than when grown from cultures kept at room temperature. In the above experiments all transplants were made from agar cultures grown twenty-four hours in the incubator.

Witte's "Peptonum siccum pro bacteriologie" of recent manufacture was used in all media.

Para-dimethyl-amido-benzaldehyde is not expensive and is easily obtained.

CONCLUSIONS.

1. The quickest, simplest, and most accurate method for determining the presence of indol is by using the ring method with para-dimethyl-amido-benzaldehyde as described above.

2. Solution 2 of Böhme is unnecessary, if the ring method is employed.

3. In using the ring method, Dunham's peptone solution should be used.

4. Bacteria will not grow well in a one per cent peptone solution unless .5 per cent sodic chloride is added.

5. The presence or absence of .5 per cent sodic chloride does not affect growth in sugar-free meat bouillon.

6. The time of growth required to ascertain the presence of indol is the same in the old method as in the new.

7. Certain cultures produce a substance which gives an indol reaction by the old method, but which does not come over in the distillate as indol does.

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II.

As there is difficulty in getting the cholera-red reaction in cultures made from the feces of cholera-infected patients, unless a pure culture of the spirillum of cholera is obtained, it occurred to me to find out what organisms affected the reaction. Accordingly, a number of tubes of Dunham's peptone solution were infected with a pure culture of the spirillum of cholera, and to each was added some of the growth of a pure culture of one other organism. The mixed culture was then allowed to grow twenty-four hours when it was tested by adding ten drops of concentrated sulphuric acid.

It was found in five trials that four different races of B. coli communis, B. pyogenes fetidus, B. acidi lactici, B. mucosus capsulatus and B. enteritidis of Gaertner always prevented the reaction. B. typhosus, B. fecalis-alkaligenes, B. icteroides, B. dysenteriæ (Flexner), and such spirilla as Sp. Milleri, Sp. Deneke, and Sp. Finkler-Prior did not affect the reaction.

Plates were then made from a twenty-four-hour growth of a culture containing both the spirillum of cholera and B. coli communis and both organisms recovered. The colon bacillus had not destroyed the cholera organisms nor apparently affected their growth.

A MODIFICATION OF THE TECHNIC OF COMPLEMENT FIXATION.*

ALFRED H. CAULFEILD.
(*Assistant Pathologist Toronto General Hospital.*)
(*From the laboratories of the Pathological Department.*)

Complement fixation or deviation lends itself to the investigation of etiological relationships between infecting agents (antigens) and the resulting antibodies found in the sera of patients suffering from various conditions. It is now recognized as a valuable aid to the diagnosis of syphilis, parasyphilis and other conditions, and stands in forensic medicine as a more delicate procedure in the detection of human albumen in blood clots, etc., than the precipitin test.[1]

Under the name of Die Wasserman-Neisser-Brucksche Reaktion Bei Syphilis it has attracted most attention, and in this condition is being extensively employed. The large number of cases reported and the different standpoints of investigation have given new conceptions regarding the interpretation which can be attributed to the active quantities of the luetic antibody detected in the various stages of the disease. These results have affected also the idea regarding the relation and interaction of the so-called antigen, luetic virus, and the resulting production of antibody. In other words, what was formerly regarded as a simple union of the antigen, obtained by a saline .5 per cent carbolic extract of a positive, syphilitic fetus, and the ambocepter has been shown to be something different from this. In the place of the antigen apparently some substance formed by the body cells, which also may rarely be present in other conditions or normally, acts when it was supposed only the antigen could form a union with the amboceptor.[2] The extent to which complement deviation is being employed practically and experimentally is best evidenced by the medical journals of to-day.

However, the object of this article is not to deal with the

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literature, or give a series of cases, but to present a modification of the technic. That usually employed necessitates one or one-half cubic centimeter as a unit for the various sera and extracts. This means that about ten cubic centimeters of blood must be obtained by puncture of a vein each time a case is investigated. In carrying out daily or frequent investigations on the serum of the same patient, which under certain circumstances seems advisable, such difficulty is experienced in obtaining the requisite amount of serum that a method using cubic millimeter quantities as a unit would be an advantage. For this reason an effort was made to devise a capillary pipette method.

An ordinary capillary pipette (such as is used for opsonic estimations) showed that no loss took place in the delicacy of the reaction. Thus, with sheep erythrocytes, inactive anti-sheep-erythrocyte-rabbit serum, and dilute normal guinea-pig serum drawn separately up to a mark and mixed, the reaction took place as in a test-tube. By laying the pipettes on their side in an opsonic incubator a negative hemolyses showed as a thin red line running the length of the pipette. Subsequent sedimentation of the corpuscles on standing the pipette upright brought out very plainly the extent of the hemolyses.

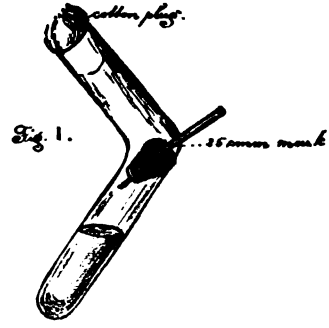
In each line of a protocol at least five equal quantities have to be brought together. The first three are incubated for an hour, and the remaining quantities subsequently added, thus:

Luetic ext. .15 x, Y serum .2, comp. 1, erythrocytes 1, hemolysin 1.

The test is essentially a quantitative one, so that not only must equal masses of the different solutions be brought together in one pipette, but the unit for each pipette must be the same. As graduation of each pipette would not be feasible, the following procedure was adopted:

Taking fifty cubic millimeters as a unit, small glass tubes containing exactly fifty cubic millimeters in about two and one-half to three centimeters length were obtained by drawing out a glass tube and measuring off this content by the

use of an exact fifty-cubic-millimeter quantity of mercury. The convenient spacings occupied by the quantity of mercury were first marked off, and subsequently subdivided and tested by a twenty-five-cubic-millimeter quantity before being accurately cut off. Now, if one has a small hole blown in the side of a sharply bent test-tube close to the bend, one of these fifty-cubic-millimeter tubes can be fitted into it by means of a cork with a punched hole (Fig. 1). Any contents of the bent tube will through gravitation and capillary attraction completely fill the cubic millimeter tube by a tilting and slightly rolling motion of the test-tube. The contents remain in the small cubic millimeter tube on righting, and can easily be drawn off with a capillary pipette and teat. This quantity can now be used to mark off the unit on the pipette.



I have the bent tubes plugged with cotton in both side and end holes and sterilized. The fifty-cubic-millimeter tubes passed diagonally through a cork can be boiled in saline solution before using; the side plug can be taken out and any contents poured in through this opening which is at once plugged by the cork carrying the cubic millimeter tube.

As complement is used in every tube, it is only necessary to have the dilute fresh normal serum contained in such a unit test-tube. After incubation of the first three quantities, as for example:

Luetic extract, heated serum, complement,

both erythrocytes and hemolysin in the same fifty-cubic-millimeter unit quantities must be added. This can be done by using another unit tube or by having previously marked off a fifty-cubic-millimeter space on the pipette a short distance above the tip which will have been sealed before incubation of the first quantities. This is most readily accomplished by marking each pipette before use with a glass pencil about four centimeters from the tip, while, at the same time that the fifty-cubic-millimeter content is marked off from the tip ("b" to "c" in diagram 2), the quantity can be drawn up to the first mark ("a" in diagram 2) and the point "d" found.

Fig 2.

Some difficulty may be experienced in getting control of the contents without expelling after the first incubation. If the tip be drawn out into a fine filament, and the teat slightly exhausted by compression, control can readily be obtained by breaking the filament which acts as a throttle. The tip can now be cut off at point "a" and the contents drawn up to "d" (a to d having a fifty-cubic-millimeter content), and mixed with the other quantities. If the separating air bubble be not too large, mixture takes place quite readily, and it is not necessary to expel the contents at any time during the test. The pipette is again sealed and incubated, after which sedimentation in the cool allowed to take place.

As a routine procedure I use my standardized luetic and normal extracts in two strengths; for example:

Luetic extract .1, Y sera .15, complement 1.
Luetic extract .2, Y sera .3, complement 1.

In the above when the luetic extract is used in a .1 and .2 strength I first of all dilute the original extract to a twenty per cent solution corresponding to the .2 strength, and the

serum to a thirty per cent solution. In the pipette, when the luetic extract and sera are used in a .1 and .15 strength respectively, I utilize the twenty-five-cubic-millimeter mark of the fifty-cubic-millimeter tube to give me half the amount, and use an equal amount of saline in drawing these into the pipette. With the idea of saving time and amount in preparing the dilution of the different heated sera to be tested I have adopted a graduated capillary pipette. For example, if the heated serum for the test is to be used in a .15 and .3 dilution I use a pipette with one hundred and eighty and six hundred cubic millimeter graduations. If the serum be drawn up to the one hundred and eighty mark and diluted with saline to the six hundred mark one gets six hundred cubic millimeters of a thirty per cent solution, which is more than twice the amount necessary for the test. The actual amount with control being probably 50×3 plus $50 \times 3/2 = 225$ cubic millimeters.

The features of this technic which seem open to criticism are twofold:

1. The possibility of contamination.
2. Action from the preceding quantity on the different solutions taking place from the tip of the pipette.

With due care I do not think the first possibility is any more likely to take place than in the test-tube method, and certainly it never seemed to appear in any of my reactions. Often the pipettes of a complete protocol of several sera have been kept for over three weeks without any change appearing in the end reaction. As a means of permanently rendering a capillary pipette sterile a curl can be put in it as used by Wright in his method of estimating bacteriolyses.[3] The second objection theoretically seems to be a very powerful factor. At first I kept a flask of sterile saline solution in which I dipped and washed the tip of the pipette after each quantity. Repeated trials without this and comparison with the ordinary test-tube method have convinced me that this is not necessary if one is at all observant of this point while making the test. The important part of the technic

seems to me to lie in the exact standardization of the extracts and sera used rather than excessive care during manipulation.

The time involved is less than that by the test-tube technic providing one has had some experience in manipulating a capillary pipette and teat. For laboratories with slight accommodation for animals it offers the advantage of allowing one to collect sufficient amounts of guinea-pig complement without destruction of the animal. The titer of one's rabbit-anti-erythrocyte serum can at any time be estimated, and a larger quantity drawn off (by bleeding an ear vein) when that is found at a convenient strength. A few cubic centimeters as stock lasts I find as long as its value remains. In estimating the strength of the hemolysin a convenient method is to add ten cubic millimeters of the sera to one, two, three, four, etc., cubic centimeters of saline solution which gives one 1/100, 1/200, 1/300, etc., dilutions.

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TYPHOID MENINGITIS: CULTIVATION OF BACILLUS TYPHOSUS FROM MENINGES AND MESENTERIC LYMPH NODE IN A CASE OF GENERAL PARESIS, WITH NOTE ON EXPERIMENTAL TYPHOID MENINGITIS IN THE GUINEA-PIG.*

E. E. SOUTHARD, M.D.

(*Assistant Professor of Neuropathology, Harvard Medical School, and Pathologist and Assistant Physician, Danvers Insane Hospital.*)

AND

E. T. F. RICHARDS, M.D., C.M.

(*Assistant in Pathology and in Neuropathology, Harvard Medical School.*)

(*From the Pathological Laboratory of the Danvers Insane Hospital and the Huntington Laboratory of the Harvard Medical School.*)

I. We have to report a case of typhoid meningitis. The case presents certain unusual features. The bacillus typhosus was obtained in pure culture from the meninges and from a mesenteric lymph node, but was not present in the heart's blood at autopsy. There was no anatomical sign of typhoid fever except mesenteric lymph node swelling. The absence of typhoid lesions from the intestinal wall was especially noted by reason of the peculiar isolated lymph node swelling and was striking because the intestinal wall was actually thinner than normal. Such thinning of the intestinal wall is a not infrequent feature in advanced general paresis of the insane, of which the patient was a victim.

It appears that typhoid meningitis has occurred almost uniformly as an incident in typhoid fever of intestinal type. Every case of true suppurative typhoid meningitis with autopsy, as tabulated by Cole,[1] of Baltimore, has proved to be coincident with intestinal lesions. Cole has gathered twelve such cases from the literature and has added a thirteenth case from the Johns Hopkins clinic. Wentworth's case of a child of four years, from whose meninges the

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bacillus typhosus was cultivated by lumbar puncture, died, but unfortunately was not autopsied.[2]

The present case might have given rise to the suspicion of a primary typhoid meningitis, had it not been for the positive mesenteric lymph node culture. We were led to the latter finding partly through noting the condition of the nodes at autopsy and partly through our study (not yet published) of infection with Bacillus coli communis along the same path. The present finding came as a surprise in a set of routine cultures from cerebro-spinal fluids and lymph nodes.

Not only was there no sign of typhoid fever at autopsy, but also during life the terminal symptoms were inextricably massed with phenomena of general paresis. The patient had for years been under medical care and no symptoms even remotely resembling those of typhoid fever had ever been noted. There were no gall stones. Unfortunately no Widal reaction was performed with the patient's blood either before or after death. The isolated organism was clumped by anti-typhoid rabbit serum 1–50,000.

II. CLINICAL HISTORY. — The details of the present case follow: A salesman of thirty-one was admitted to the Danvers Insane Hospital June 2, 1906, and died there Nov. 29, 1907. The patient contracted syphilis at nineteen. He married at twenty-two and had two children, still living. There were no miscarriages. Difficulty with bowels and bladder followed marriage. Patient was treated for bladder disease by Dr. Abner Post. At twenty-eight patient lost control of limbs and was confined to the house about two months, under the care of Dr. L. R. G. Crandon. Three months later he had regained partial control of his limbs, but had lost all control of his sphincters. After another month he had returned to work, but did not work steadily and seemed to have lost ambition. In the summer of 1905, his mind became obviously altered. He grew indolent and extravagant and given to buying expensive and useless articles. Loss of interest in things followed, together with loss of memory for recent events, lack of insight into illness,

delusions of persecution by wife, irascibility followed quickly by crying. Before admission to hospital, he was euphoric, drawling and tremulous in speech, sprawling in penmanship, alternately depressed and exalted in manner. Knee-jerks were absent, gait ataxic, pupils stiff to light.

The family history was negative with respect to insanity. All the family are reported as nervous. A brother died of peritonitis at twenty-eight, a sister of pneumonia under twenty. Another brother and sister are living. Father and mother died of heart trouble at about sixty-seven and sixty respectively.

The patient was at high school one year and was a fair student. Considerable tobacco was used, and some alcohol. Intoxication denied. There was no history of typhoid fever or other acute disease.

The patient on admission was sallow, poorly nourished, and flat-chested, with a slight lateral curvature. There was slight dulness over right apex in front and in right upper back. Voice sounds were increased over right apex in front and over whole right back. The right chest showed bronchial respiration throughout. The respiration in front of right chest was of an interrupted character. The liver seemed moderately enlarged. The urine showed a very faint trace of albumin. There were a few small nodes in right groin and a scar on dorsum of penis.

NEUROLOGICAL EXAMINATION. — Slight swaying in Romberg position. Slight tremor of protruded tongue and extended fingers. Pupils irregular, left slightly larger than right. Left pupil reacted to light consensually, but not directly. Right pupil reacted very slightly to direct light, not consensually. Knee-jerks and Achilles jerks absent. Ankle clonus absent. Abdominal and cremasteric reflexes brisk. Sharp and dull points were recognized in the legs with numerous mistakes.

Vocal and facial tremor. Speech slow and drawling. Test phrases repeated well if care was taken. Consciousness clear. Orientation perfect. Calculating ability preserved. Many words omitted in writing. Penmanship clear but

shaky. Hallucinations absent. Memory of recent events poor. Associations of a logical or defining type.

Patient denied various statements in commitment papers and had little or no insight into the mental side of his disease — slight euphoria.

After a month's observation the patient was removed to a quiet ward and set to work a few days in the scullery. One night he began to yell as if assaulted and said later that he had an idea that he was going to die. Before three months had passed he had become untidy, disorderly, and imperfectly oriented.

The general degeneration continued rapidly. One week before death the temperature rose to 103° F. and the patient succumbed to what seemed clinically like a bronchopneumonia. Unconsciousness two days before death.

Note with respect to history of typhoid. — Inquiries of his physicians, wife, employer, and brother tend to show conclusively that the patient never had a disease even remotely resembling typhoid fever.

CLINICAL SUMMARY. — Male without history of typhoid fever. Syphilis at nineteen. Marriage at twenty-two (two healthy children, no miscarriages). Disturbance of bladder and rectum. Successful in business (salesman) till twenty-eight, when advancing tabes confined him to bed for a time. At thirty mental signs of general paresis developed. Death at thirty-two after an acute illness of a week.

III.

The autopsy was performed sixty-seven hours after death by Drs. E. E. Southard and M. M. Canavan. Following is a copy of the protocol:

Well developed, emaciated white male; one hundred and sixty-five centimeters long. Lividity of dependent parts. Rigidity of limbs and neck. Pupils: left, 2.5 millimeters; right, four millimeters. Abdominal fat, one centimeter deep. Decubitus absent. Penis and scrotum show reddish discoloration.

Peritoneal cavity. — Walls not remarkable. Omentum thinly clad with fat. Mesenteric lymph nodes slightly enlarged, one measuring 2.5 x 1.5 x .5 centimeter. Diaphragm arches to fifth space on both sides.

Pleural cavities. — Both free from adhesions. Visceral surface of the *right* lower lobe shows delicate early fibrin, in small readily detachable strips. Pleural cavities contain a slight amount of fluid.

Pericardial cavity. — Slight excess of fluid. Pulmonary vessels give no evidence of ante-mortem clot.

Heart. — Weight, two hundred and ninety-five grams. Epicardial fat moderate in amount. Muscle firm and red, without evidence of increase of interstitial tissue. Endocardium of auricles and of right ventricle normal. Endocardium of left ventricle mantled with a thin layer of connective tissue, especially well marked towards the aortic valve. Valves normal, except mitral, which shows a few spots of yellowish softening both upon the edge and at various points in the curtain. Chordæ tendineæ normal. Chambers contain cruor clot. Coronary arteries show very faint, small areas of yellowish thickening, the most marked of which lies about two centimeters from the origin of the descending coronary.

Measurements: T. V., 11.5; P. V., 6.5; M. V., 7.5.
A. V., 7.5; L. V., 1.3; R. V., .4.

Lungs. — Weight, eight hundred and ninety grams, left; seven hundred and eighty grams, right. Edematous, congested, dark and semi-solid behind. The posterior portions of both lower lobes show areas in the midst of the congestion which are of a grayish pink color, and leave a granular surface on section. These measure from .5 centimeter to three centimeters in diameter. Pieces from the posterior portion of the lower lobe sink in water. A moderately foul odor from the cut surface of the lower lobe. Bronchi reddened and velvety. Vessels not remarkable. Bronchial lymph nodes small.

Spleen. — Weight, one hundred and eighty grams. Border shows numerous indentations. Capsule of the usual thickness. Substance not pulpy. Substance shows innumerable small oozing hemorrhages. Malpighian bodies somewhat larger than normal. Trabeculation not remarkable.

Gastrointestinal tract. — Esophagus not remarkable. Stomach small; walls thin and without rugæ. The remainder of the tract shows walls which are possibly somewhat thinner than normal, but show no other evidence of lesion. No lesion to correspond to the enlarged mesenteric lymph node was fouud.

Liver. — Weight, one thousand five hundred and eighty-five grams. Border sharp. Substance of the usual firmness, or a trifle increased. Lobulation distinct. Color grayish brown. Gall bladder normal.

Pancreas. — Of a pinkish color; gives no evidence of lesion.

Adrenals. — Show softened interiors.

Kidneys. — Weight, three hundred and fifteen grams. Capsule of the usual thickness, strips with considerable ease from a reddish gray surface. Cortex of normal thickness, shows in places slight grayish opacity, suggesting fat. Pelves contain a slight amount of slightly turbid fluid, but the mucosa shows no evidence of acute inflammation. Ureters normal.

Bladder contracted, with thickened walls. Ureteral orifices patent. Prostate not enlarged. Posterior portion of the right lobe contains a pea-sized abscess cavity from which wells up yellowish gray pus. The aortic arch shows a slight degree of sclerosis with faintly puckered lines radiating from a point opposite the coronary orifice. This lesion extends about six centimeters. The rest of the aorta is normal.

Head. — Skin exceedingly loose, and the whole skull cap thinned. The diplœ are absent. Adhesion with dura easily separated. The dura somewhat thickened, but not distended. Along the longitudinal sinus extensive calcareous granulations adhere to it. The longitudinal sinus does not contain blood, and the inner surface is normal in color. The pia is extensively thickened and opaque and a general subpial exudate exists which is more marked over the vertex where it lifts the pia from the brain surface to the extent of three centimeters in Rolandic, superior frontal, intraparietal, and mesial precentral sulci on each side. The arteries at base are free from atheroma. The temporal lobes are much bound down by adhesions, as is the cerebellum. Post-mortem softening is evident. The hemispheres show no asymmetry, but the frontal convolutions are markedly atrophic. The corpus callosum is united to the cortex by old adhesions and has to be dissected away from it. Lateral ventricles contain some slight amount of cloudy fluid, and the pia among vessels is opaque. Some granulations in ependyma. Brain weight, one thousand three hundred and five grams. Pons and cerebellum, one hundred and ninety-five grams.

Cord. — Dura much thickened, and the pia corresponds to its appearance in brain with a like exudate. Cross-section of cord shows sclerosis of posterior columns.

ANATOMICAL SUMMARY.

Acute conditions:

Hypostatic pneumonia, with early serofibrinous pleuritis and without lymph node swelling; enlargement of mesenteric lymph nodes; acute cerebrospinal leptomeningitis; multiple small hemorrhages of spleen.

Other findings:

Scar of penis; sclerosis of aortic arch (Heller's type?) and slight coronary arteriosclerosis; calvarium thin and dense; dura mater thickened and adherent to calvarium; calcified arachnoidal villi; chronic cerebral and cerebellar leptomeningitis; atrophy of frontal lobes; granular ependymitis; sclerosis of posterior columns of spinal cord; emaciation; unequal pupils; slight parietal fibrous endocarditis, slight mitral sclerosis; gastrointestinal atrophy; chronic cystitis; chronic abscess of prostate.

This picture seems to accord in most of its details (1) with the sequelæ of syphilis, (2) with the characteristic findings in general paresis of the insane, and (3) with a terminal acute condition.

IV. BACTERIOLOGY. — Cultures upon agar plates were made from the heart's blood, from the largest of the swollen lymph nodes, and from the pus of the subpial region of the vertex.

The heart's blood culture yielded a single colony, which proved to be Staphylococcus pyogenes albus. No further work was done with this organism, which is possibly related with the rich staphylococcal flora of the lung of this case.

The mesenteric lymph node and cerebrospinal fluid cultures both yielded many colonies of an organism which proved to be Bacillus typhosus.

The organisms identified as Bacillus typhosus are motile, Gram-negative bacilli, usually slender, though of variable size and length. They grow upon agar as moist white (bluish white, grayish white, occasionally slightly yellowish white) translucent (occasionally opaque) colonies, varying from pin-point to one millimeter in diameter. The organisms fail to produce gas on glucose agar, produce no indol, and do not liquefy gelatine. Milk is not coagulated, but becomes slightly acid. The acidity diminishes after five days. Growth on potato colorless.

Parallel cultures upon the same batch of media were carried out with known cultures of Bacillus typhosus, Bacillus coli communis, and Bacillus paratyphosus α and β, given us by Prof. H. C. Ernst. The organism of this case paralleled in all respects the known culture of Bacillus typhosus.

The serum of a rabbit immunized against Bacillus typhosus by Dr. F. P. Gay clumped our organism in dilutions as high as one to fifty thousand.

V. MICROSCOPIC FINDINGS. — The microscopic examination of the tissues added a few features to the anatomical diagnoses:

Slight pigmentation of heart fibers; pneumonia, staphylococcal, with bronchial plugs of bacteria; slight chronic splenitis; focal necroses of liver; slight chronic interstitial nephritis; slight cloudy swelling of kidney.

Following is the result of microscopic examination of tissues in trunk:

The heart showed, in a section through the wall of the left ventricle, a trivial degree of perinuclear pigmentation affecting occasional fibers only. Pigmented fibers are more frequent in the internal layers of muscle, adjacent to the thickened endocardium. The endocardial thickening is almost purely fibrous and mononuclear cells are conspicuously absent. No lesions like those figured by Mallory[3] (Plate LVII., Fig 18) were found, or any lesions pointing to the effect of typhoid toxines.

Sections from lower lobe of lung show a very rich flora of mixed character, dominantly coccal. Many alveoli appear to have served as culture tubes for the richest possible growths of cocci, often in chains. The bronchi are sometimes completely plugged with masses and sheets of organisms suggesting staphylococci. In the bronchi and in the middle of some alveoli the masses are possibly the effect of multiplication post-mortem. Phagocytosis for these bacteria by polynuclear leucocytes is rarely seen. A few bacillary forms were seen, but there is no evidence that they are typhoid bacilli. The question of phagocytic cells, the effect of specifically typhoid multiplication, as raised by Mallory in discussing a case of typhoid fever complicated by fibrinous pneumonia,[4] is not brought up in the present case. The phagocytic cells present are pigment laden. A few instances of cells phagocytic for red blood corpuscles were seen. There are a number of instances of small hemorrhages into the alveoli, and about these accumulations of blood corpuscles the growth of bacterial masses is especially rich. Fibrin is not a prominent element in the diffuse cellular and serous exudation, but may be found in a few places. There seems no reason for regarding this pneumonia as anything but a recent hypostatic pneumonia with very prominent bacterial picture, and certainly no reason for regarding it as of typhoidal origin.

The framework of the spleen, particularly in a zone beneath the capsule, consists of trabeculæ which are set

unusually close, and the reticulum of the pulp in this region is prominent. The pulp spaces contain a moderate number of phagocytic cells, which contain as a rule blood corpuscles. The spaces are as a rule not unduly dilated. Lymphoid and plasma cells are moderately numerous, but polynuclear leucocytes are occasional only. Giant cells of bone marrow type are rare findings. Fairly numerous ill-preserved mitotic figures occur in some parts of the pulp, perhaps in the plasma cells. The lymph nodules show no marked change. Small hemorrhages are prominent and frequent in the pulp. There are no organisms in association with these hemorrhages.

The liver shows a few focal necroses of the type described by Mallory[5] as characteristic of typhoid fever and ascribed by him to occlusion of liver capillaries by phagocytic cells derived chiefly from the spleen and intestine by embolism through the portal circulation. These necroses are in this liver infrequent. They occur in various parts of the liver lobule. The portal canals show in a few places evidence of accumulation of phagocytic cells, but neither these nor lymphoid and plasma cells can be considered as prominent features of the liver in this case. The fat content of the liver is low. There is small evidence of liver cell pigmentation.

The kidney fails to show the characteristic occlusion of veins in the pyramids with phagocytic cells, as described by Mallory in typhoid fever.[6] There are a few ill-marked foci of interstitial overgrowth in some parts of the renal cortex. Acute changes are represented only by a trivial degree of cloudy swelling and by destructive changes in a few isolated tubule systems (granular disintegration with tendency to formation of hyaline droplets). Some of the cells lining tubules of the pyramids contain collections of brown pigment granules.

The adrenal gland shows marked central and focal injection, a few foci of mononuclear cells in interstitial tissue, and post-mortem changes.

Prostate. — The walls of the abscess show great numbers

of plasma cells. The interior shows numbers of very large phagocytic cells, containing chiefly fat. Some of the cells contain pigment. Polynuclear leucocytes are demonstrable only in relation to dead cells of some parts quite broken down. In certain places the destruction seems to have centered upon ducts and to be spreading outward therefrom; but in other places groups of plasma cells are found surrounding intact ducts. Corpora amylacea are abundant. Some intact bodies are found free in the interior of the abscess. No bacteria were demonstrated in the abscess.

Bladder. — No bacilli were demonstrable on the mucosa.

THE NERVOUS SYSTEM. — Tissues from many parts of the cerebrum and cerebellum, from the spinal cord, and from ganglia and nerves were examined by general methods and by the Weigert and Marchi methods.

The examination confirmed the clinical diagnosis of general paresis and of tabes, since there was not only an extensive chronic encephalitis, with the usual lymphocytic and plasma-cell exudate and irregular gliosis, but also a well-marked posterior column sclerosis, not unusual save in its extreme degree.

It might be surmised that some difficulty would arise in distinguishing the effects of paretic meningoencephalitis from those of the more recent typhoidal process. The well-known tendency of typhoidal processes to escape polynuclear exudation, at least until frank necrosis has set in, gave rise to the idea that the two mononuclear pictures — that of general paresis and that of typhoidal processes — might be confusing.

The picture presented by the meninges was scarcely what might be expected. Although numerous mononuclear phagocytic cells are everywhere found, yet the predominant picture is that of a polynuclear exudation.

The polynuclear leucocytes occur in greatest numbers in the tissue spaces, especially in the meshes of the lumbar arachnoid and in the spaces of the frontal and paracentral pia mater. In the lumbar region of the spinal arachnoid wide

fields occur in which the cells are almost one hundred per cent polynuclear leucocytes. In places phagocytic cells occur, and in a few fields, even in the open tissue spaces, the number of phagocytic cells may rise to fifty per cent. Edema is a considerable feature in the meninges. Fibrin is found chiefly in the cerebral meninges and appears in numerous delicate strands in the tissue spaces.

One function of both large mononuclear and polynuclear cells seems to be phagocytosis for fatty materials. This can be well demonstrated by the Marchi impregnations, which show both types of cell to be fat-containing.

VI. Mallory suggested in 1898 that "careful bacteriological and histological study of the various complications of typhoid fever, and especially of cases of meningitis claimed to be due to the typhoid bacillus alone, may throw additional light on the character of the lesions produced by the typhoid bacillus."[7]

Mallory's histological evidence indicates that the essential lesion of typhoid fever is a proliferation of endothelial cells. The proliferation is diffuse but more marked in the lymphoid tissue of the intestine and mesenteric lymph nodes. The histological evidence points to the production by the typhoid bacillus of a soluble toxine. Mallory believes that this toxine is produced within the lumen of the intestine and diffuses along the lines of absorption through the lymphatics and along the lines of distribution through the circulation.

Strictly speaking, therefore, the presence of the typhoid bacillus is not necessary within the tissues of the body for the production of the essentially typhoid lesions. The toxine alone could set up processes which, so far as we can see, might terminate in the same miliary necroses, due to endothelial-cell infarctions, that characterize typhoid fever as it occurs.

How then are to be explained cases of abscess, pneumonia, and meningitis, reported as due to Bacillus typhosus? Has the typhoid bacillus under some circumstances a truly pyogenic character? Mallory's material permitted the

study of spleen abscesses and pneumonic lung in which the typhoid bacillus was present.[8] Study of the tissues inclined to the belief that the typhoid bacilli in the spleen abscesses had merely found there a favorable medium for growth and that the main effect of the bacilli in the pneumonic lung had been to complicate a lobar pneumonia with the proliferation of cells in the alveolar walls. The general conclusion is, therefore, that the typhoid bacillus has not been proved to be a pus-producing organism. The focal and purulent lesions of typhoid fever are either instances of infected infarcts due to typhoid toxine and subsequently invaded or occupied by bacteria (often by the typhoid bacillus itself) or else instances of focal lesions due to other causes than typhoid toxine and subsequently invaded or occupied by bacteria.

The position of typhoid meningitis is therefore almost crucial in this issue. With so simple a tissue as the meninges, as MacCallum[9] has again emphasized, the nature of the pure effects of the typhoid bacilli or their toxines should be made clearer. MacCallum has studied a case of typhoid meningitis coming to autopsy in the Johns Hopkins clinic. His conclusions follow the general trend of Mallory's argument. MacCallum finds nothing peculiar in the histological study of typhoid meningitis, unless it be the relative abundance of large phagocytic cells, found particularly about the veins, but also scattered through the tissues.

Although not studied in the light of Mallory's data, the cases of Ohlmacher[10] must be reviewed in this connection. Ohlmacher had reported in 1897 two cases of typhoid meningitis from the Cleveland City Hospital.

Ohlmacher's cases both died in the fourth week of recognized typhoid fever and both showed the usual intestinal lesions. The first case was delirious in the last week of life and several times passed urine involuntarily. In the second case there were no symptoms by which the date of meningitis could be fixed; the patient was either delirious or comatose throughout the thirteen days of his hospital stay. The descriptions of exudate found indicate the predominance in the first case and in parts of the second case of

proliferated endothelial cells and mononuclear leucocytes. But one block from the first case was examined. Two blocks from the second case showed abundant and closely packed polynuclear leucocytes with fragmenting nuclei together with endothelial cells; a third block showed a predominance of endothelial and mononuclear cells, resembling the appearances of the first case.

The second of Ohlmacher's cases seems to have been severer than the first, since it showed an acute endarteritis. There are no notes in the cases indicating phagocytosis of the bacilli, which were abundant in the fibrinous and cellular exudate and in the lymph spaces of the first case and existed in countless myriads in dense lines in the meningeal lymphatics or scattered among the cells of the second case. It is noteworthy that the two regions of the second case which showed polynuclear leucocytosis were the regions which showed endarteritis.

It will be worth while to summarize MacCallum's findings, since his appears to be the first thoroughly examined case since Ohlmacher's.

Microscopic examination in MacCallum's case showed histological appearances identical throughout the cerebral, basal, cerebellar, and spinal meninges, varying only in intensity. Fibrin was considerably developed, but the picture is largely cellular. "In certain areas, apparently where the process is most intense, there are abundant accumulations of polymorphonuclear leucocytes. Here the disintegration of the tissues is most evident, and the leucocytes themselves are frequently fragmented and in an advanced stage of degeneration." MacCallum, however, regards these polymorphonuclear leucocytes as reacting to the typhoid infection and not as brought out chemiotactically by tissue necrosis.

Besides these foci of polymorphonuclear leucocytes, the meninges contained great numbers of mononuclear cells, among which are mentioned cells resembling lymphocytes, plasma cells, and all transitions from lymphocyte-like cells to certain very large irregular mononuclear phagocytes

having a large vesicular nucleus and abundant ground-glass-like pink protoplasm.

The arteries showed lifting of endothelium by exudate in a form of arteritis not specific for typhoid meningitis but also shown in pneumococcus and streptococcus meningitis.[11, 12, 13] Hektoen's earlier work on tuberculous meningitis may be reviewed in this connection.[14]

It is the relative abundance of the large phagocytic cells which distinguishes typhoid meningitis from other forms. But, although attention has been focused upon the high content of mononuclear phagocytic cells in typhoid meningitis, the occurrence of these cells does not appear to us so remarkable as that of numerous polynuclear leucocytes evoked by Bacillus typhosus.

MacCallum's finding of polynuclear leucocytes in the meninges corresponds well with ours, but the cells in MacCallum's case occurred more in certain foci where disintegration of fixed tissues was most evident.

It seems, then, that the data of Ohlmacher's and MacCallum's cases as well as of our own point to the possibility that the typhoid bacillus, under certain circumstances and notably in the meninges, may act as a pyogenic organism. A case of purulent typhoid meningitis, recently reported by Henry and Rosenberger,[15] appears to resemble ours in the cell-contents of the meninges. This case is described as showing a large number of polynuclear and mononuclear cells, but the large phagocytic cells did not predominate.

With respect to this point it may be noted that MacCallum's case showed the course of a typhoid fever over three weeks long until death, complicated by cerebral symptoms at least two weeks in duration. Henry and Rosenberger's case lasted nine days. Our case had acute symptoms, probably due to bronchopneumonia, for a week, and unconsciousness lasted but two days. Moreover, whereas MacCallum's case, in common with most of those reported, possessed the usual lesions of typhoid fever, the other two cases are less easily to be pressed into the class of typhoid fever. The case of Henry and Rosenberger showed lesions possibly

consistent with early typhoid fever (acute catarrhal enteritis with enlargement of Peyer's patches and mesenteric lymph nodes), but scarcely consistent with extensive toxic effects elsewhere. Our own case showed one feature in common with typhoid fever, enlargement of mesenteric lymph nodes, but there is little evidence that typhoid toxine has produced extensive effects in this case. It would seem simplest, therefore, to take the phagocyte-content as indicating roughly the duration of the disease and the degree of endeavor to rid the meninges of necrotic material. There seems to be very little evidence from any of these cases that the typhoid bacillus has produced in the meninges a special accumulation of phagocytic cells.

It will be remembered that Mallory's hypothesis concerning the specific proliferation of endothelial cells in typhoid fever maintains that this proliferation is due to the toxine of the bacillus and not to the direct action of the bacillus itself. Mallory could not demonstrate any relation between the situation of the organisms and the character of the lesions.

In typhoid meningitis we may be dealing not with the effects of typhoid toxine alone, but also with the direct effects of the bacilli themselves. Polynuclear leucocytosis in the meninges appears to be a more characteristic feature than the accumulation of large phagocytic cells.

VII. EXPERIMENTAL TYPHOID MENINGITIS IN THE GUINEA-PIG. — The hypothesis is raised, by a consideration of the data of Ohlmacher's, MacCallum's, Henry and Rosenberger's, and our own cases, that the typhoid bacillus may in the meninges produce an exudation of polynuclear leucocytes. This effect of the bacillus, if demonstrable, would be of a different order from the proliferative stimulus of the typhoid toxine, though the effects of both bacillus and toxine may be together visible in any one region, as a combination of large phagocytic elements and polynuclear leucocytes.

Since the issue in a fatal human case is clouded by possible preëxistent phagocytes and by the well-known tendency

of the meningeal tissues to exhibit large phagocytic cells in every kind of tissue necrosis, it seemed desirable to see what the typhoid bacillus might do in a clear field.

The best results in this direction appear to have been obtained by Tictine [16] of Odessa, using rabbits, guinea-pigs, and susliks. Tictine trephined and made subdural inoculations. Abscesses at the site of inoculation were produced in rabbits.

Bacillus typhosus was recovered in the brain as late as twelve days after inoculation. Inflammatory edema occurred within twenty-four hours and suppuration within three days. The experimental meningitis of the suslik took a slower course than in the rabbit and showed marked individual variations. Bacillus typhosus was recovered from suslik brain as late as thirteen days after inoculation. The guinea-pigs used succumbed in twenty-four hours. Blood infections, as indicated by positive cultures in the spleen, were not infrequent in Tictine's animals.

In order to parallel results with work on other organisms [17, 12, 13] we preferred to use the guinea-pig. To avoid the direct effects of cerebral trauma we employed the method of orbital injection after cocainization, already employed in work with other organisms.[12, 13]

We have been able to recover Bacillus typhosus in small numbers from the base of the guinea-pig brain as late as ten days after orbital inoculation. The brain of a guinea-pig similarly inoculated and killed after fourteen days proved to be sterile.

Numerous colonies of B. typhosus were cultivated from the base of the brain five days after inoculation. Thereafter, if our series represents general conditions, the bacilli quickly diminish in number in the guinea-pig.

The same culture (derived from the present case) injected in small quantity into the peritoneum kills overnight, producing a profuse exudation containing polynuclear leucocytes. Such a guinea-pig contains B. typhosus in the heart's blood, but the brain is apt to be sterile.

On the other hand, with our orbital inoculations, we have

in no case produced a septicemia with B. typhosus. Probably, therefore, our histological findings in the nervous system are not to be taken as phenomena of a general infection.

The histological study of tissues from a series of guinea-pigs inoculated by way of the orbit with B. typhosus shows that the lesions produced are mild though quite definite. In general it may be said that the critical phase of the disease produced comes later than with the pyogens studied in previous papers. The base of the brain from the fifth to the seventh day shows the most acute lesions, consisting in a diapedesis of blood globules and an exudation of polynuclear leucocytes, together with serum.

Inoculations with Micrococcus aureus, previously reported, showed the acute inflammation at its height in three days. The pneumococcus inoculations gave more variable and less acute lesions, in the sense that mononuclear elements are admixed with polynuclear leucocytes comparatively early, and the height of the polynuclear exudation comes about the fourth day. The streptococcus inoculations correspond in main features with the pneumococcus inoculations.

In the typhosus inoculations, mononuclear elements arrive in the vessels and in the pial meshes by the seventh day.

On the tenth day the exudate has diminished in quantity, the cells and albuminous materials are found fused together in a seminecrotic mass including pyknotic polynuclear cells, and the vessels no longer give evidence of the arrival of fresh exudative cells.

By the fourteenth day the disease is virtually past, and traces only of old exudate are found. Sections from various parts of the nervous system stained for fat (Marchi) demonstrate few or no fatty changes until the seventh day, but are thereafter demonstrable in considerable quantity throughout the decline of the exudative disease. At fourteen days there are still many fibers showing black markings, and at this time numerous cells phagocytic for fat have made their appearance. The most extensive blackenings occur in cranial nerves. Thus it is interesting that the locus of severe fatty change is identical with that of most voluminous cellular

exudation, but the time-relations of the two processes differ, the fatty change running behind the exudation by some days.

Parallel inoculations have been made with B. coli and paratyphosus α and β. These will be reported in a comparative review at a later date.

VIII. Conclusions.

The points of the present paper are as follows:

1. A classical case of taboparesis, with previous history of syphilis, but without history of typhoid fever, succumbs after a week's acute illness to bronchopneumonia and to purulent cerebrospinal meningitis.

2. A typical strain of Bacillus typhosus was isolated in pure culture from a swollen mesenteric lymph node and from the meningeal pus. The blood failed to yield Bacillus typhosus. There were no typhoidal lesions in the intestines.

3. The meningeal exudation contained polynuclear leucocytes in great numbers. This finding, in connection with the older findings of Ohlmacher, W. G. MacCallum, and Henry and Rosenberger, leads to the hypothesis that Bacillus typhosus within the meninges may exert a directly pyogenic action. Should this hypothesis be upheld, the direct action of the bacillus stands in sharp contrast to the proliferative effects of the typhoid toxine described by Mallory in the intestine, lymph nodes and elsewhere in the viscera. The indications are, therefore, that Bacillus typhosus may have two separate effects, the one produced by a diffusible toxine (Mallory) characteristically in the intestinal tract and the other produced in the meninges either by direct local action of the bacilli or through an endotoxine, due to destruction of the bacilli.

4. In confirmation of the results of Tictine, Bacillus typhosus was experimentally found to inflame the meninges of guinea-pigs. In accordance with the hypothesis stated above, guinea-pig brains proved to show an exudation containing many polynuclear leucocytes. Mononuclear elements arrive by the seventh day after inoculation.

5. Research is desirable to determine whether the local action of Bacillus typhosus in the meninges is, or is not, of endotoxic type.

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