DIABETES MELLITUS

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DIABETES MELLITUS

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Stefan S. Fajans, M.D.
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PREFACE

The Fogarty International Center was established in 1968 as a memorial to the late Congressman John E. Fogarty from Rhode Island. It had been Mr. Fogarty's desire to create within the National Institutes of Health a center for research in biology and medicine dedicated to international cooperation and collaboration in the interest of the health of mankind.

The Fogarty International Center is a unique resource within the Federal establishment, providing a base for expansion of America's health research and health care to lands abroad and for bringing the talents and resources of other nations to bear upon the many and varied health problems of the United States.

As an institution for advanced study, the Fogarty International Center has embraced the major themes of medical education, environmental health, societal factors influencing health and disease, geographic health problems, international health research and education, and preventive medicine. Our commitment to the study of preventive aspects of human disease is expressed in the forthcoming Fogarty International Center Series on Preventive Medicine.

Improvement in the health status of the American people will depend, in great measure, on the design and application of programs which place major emphasis on the preventive aspects of human disease. Although health authorities generally agree with this thesis, there is need for more precise definition of effective methods and programs of prevention, financial resources required to implement these programs, and priorities to be assigned to research in preventive methodology. The need to assemble expertise in this field to elucidate mechanisms whereby the full impact of preventive medicine may be brought to bear on the solution of America's major health problems has been expressed repeatedly in public statements by leaders throughout the health field.

In response to this need, the Fogarty International Center initiated a series of comprehensive studies of preventive medicine in order to review and evaluate the state of the art of prevention and control of human diseases, to identify the deficiencies in knowledge requiring further research, including analysis of financial resources, preventive techniques, and manpower, and to recognize problems in application of preventive methods and suggest corrective action.

This monograph, Diabetes, is the product of diligent effort by numerous experts in the field whose contributions have been blended into a single volume by a skillful editorial committee under the chairmanship of Dr. Stefan S. Fajans and represents the fourth volume of the Fogarty International Center Series on Preventive Medicine. The subtle nature of diabetes mellitus and the broad spectrum of the clinical expressions of this disease has made precise definitions and determinations of prevalence difficult. Conservative estimates reveal that

approximately 400,000 Americans each year learn for the first time that they have diabetes, four million are presently affected and 40,000 are said to succumb to one of the complications of this disease. Ranking as the sixth commonest cause of mortality and accounting for the expenditure of at least two billions of dollars, diabetes mellitus is clearly a major health problem in this country. This monograph presents a detailed review and evaluation of the state of knowledge and research in the prevention and control of diabetes mellitus, identifies gaps in the knowledge of this disease for the purpose of defining research needs, and proposes way in which current knowledge may be applied more effectively to alleviate the burden of diabetes.

Milo D. Leavitt, Jr., M.D.
Director
Fogarty International Center

CONTRIBUTORS

Sol Aisenberg, Ph.D.
Space Science Division, Whittaker Corporation, Boston, Massachusetts

Ronald A. Arkey, M.D.
Division of Medicine, Mount Auburn Hospital, Cambridge, Massachusetts

Walter F. Ballinger II, M.D.
Department of Surgery, Washington University School of Medicine, St. Louis, Missouri

Bernard Becker, M.D.
Professor of Opthalmology, Washington University School of Medicine, St. Louis, Missouri

Peter Bennett, M.D.
Epidemiology and Field Studies Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, Phoenix, Arizona

Robert E. Bolinger, M.D.
Department of Internal Medicine, University of Kansas Medical Center, Kansas City, Kansas

Ronald A. Chez, M.D.
Pregnancy Research Branch, National Institute of Child Health and Human Development, National Institute of Health, Bethesda, Maryland

K.W. Chang, Ph.D.
Space Science Division, Whittaker Corporation, Boston, Massachusetts

Rex S. Clements, Jr., M.D.
Division of Endocrinology and Metabolism, Department of Medicine, University of Alabama Medical Center, Birmingham, Alabama

R. H. Egdahl, M.D.
Department of Surgery, University Hospital, Boston, Massachusetts

Paul S. Entmacher, M.D.
Medical Director, Metropolitan Life Insurance Company, New York City, New York

Frederick H. Epstein, M.D.
Professor of Epidemiology, University of Michigan, Ann Arbor, Michigan

Stefan S. Fajans, M.D.
Professor of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan

F. Robert Fekety, Jr., M.D.
Professor of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan

James B. Field, M.D.
Clinical Research Unit, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Daniel W. Foster, M.D.
Department of Internal Medicine, Southwestern Medical School, The University of Texas Health Science Center at Dallas, Texas

Norbert Freinkel, M.D.
Kettering Professor of Internal Medicine, Northwestern University, Chicago, Illinois

Frederick C. Goetz, M.D.
Division of Internal Medicine, University of Minnesota Medical School, Minneapolis, Minnesota

Jack Goldstein, B.A., B.M.E.
University of Southern California School of Medicine, Los Angeles, California

J. M. Hiebert, M.D.
Department of Surgery, University Hospital, Boston, Massachusetts

Ronald Kalkhoff, M.D.
Milwaukee County General Hospital, The Medical College of Wisconsin, Milwaukee, Wisconsin
R. C. Karl, M.D.
Washington University School of Medicine, St. Louis, Missouri
David Kipnis, M.D.
Professor of Internal Medicine, Washington University Medical School, Barnes and Wohl Hospital, St. Louis, Missouri

Harvey C. Knowles, Jr., M.D.
Department of Internal Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio
Robert A. Kreisberg, M.D.
Department of Medicine, University of South Alabama, Mobile, Alabama
Paul Lacy, M.D.
Department of Pathology, Washington University School of Medicine, St. Louis, Missouri
Bernard Landau, M.D.
Department of Medicine, Case Western Reserve University, Lakeside Hospital, Cleveland, Ohio
Arnold Lazarow, M.D.
Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota
Harold E. Lebovitz, M.D.
Division of Endocrinology, Duke University Medical Center, Durham, North Carolina
Rachmiel Levine, M.D.
City of Hope National Medical Center, Duarte, California
K. Lundbaek, Professor
AARHUS Universitet, Kommunehospitalet, AARHUS, Denmark
Fred R. McCrumb, Jr., M.D.
Fogarty International Center, National Institutes of Health, Bethesda, Maryland
Curtis L. Meinert, Ph.D.
University of Maryland School of Medicine, Division of Clinical Investigation, Baltimore, Maryland
Leona V. Miller, M.D.
University of Southern California Medical Center, Diabetes Section, Los Angeles, California
Daniel H. Mintz, M.D.
Department of Medicine, University of Miami School of Medicine, Miami, Florida
John A. Moorhouse, M.D.
Faculty of Medicine, The University of Manitoba, Winnipeg General Hospital, Winnipeg, Manitoba, Canada
Bryce D. Munger, M.D.
Department of Anatomy, College of Medicine, The Pennsylvania State University, The Milton S. Hersey Medical Center, Hershey, Pennsylvania
Ruth Osterby, M.D.
Patologist-Anatomisk Institut, Kommunehospitalet, AARHUS, Denmark
Leon D. Ostrander, M.D.
Professor of Internal Medicine, University of Michigan, University Hospital, Ann Arbor, Michigan
Daniel Porte, Jr., M.D.
Veterans Administration Hospital, Seattle, Washington
Thaddeus Prout, M.D.
Greater Baltimore Medical Center, Baltimore, Maryland
Arthur H. Rubenstein, M.D.
Department of Medicine, University of Chicago Hospitals and Clinics, Chicago, Illinois
John W. Runyan, Jr., M.D.
Division of Health Care Sciences, University of Tennessee, Memphis, Tennessee
D. W. Scharp, M.D.
Washington University School of Medicine, St. Louis, Missouri

Joseph Silva, M.D.
Assistant Professor of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan
Morton E. Smith, M.D.
Associate Professor of Opthalmology, Washington University School of Medicine, St. Louis, Missouri
J. Stewart Soeldner, M.D.
Associate Professor of Medicine, Harvard University Medical School, Joslin Research Laboratory, Boston, Massachusetts
D. F. Steiner, M.D.
Biochemistry Department, University of Chicago Hospitals and Clinics, Chicago, Illinois
Albert Winegrad, M.D.
George S. Cox Medical Research Institute, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

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INTRODUCTION

Diabetes mellitus has become a major public health problem when considered on the basis of its incidence, morbidity, mortality, and socioeconomic impact on the world's populations. The establishment of clear-cut objectives to guide a broad program of prevention and treatment of the disease and its complications is of primary importance.

Within the framework of the Fogarty International Center Series on Preventive Medicine, a six-member Organizing Committee was established to guide a review of problems in the field of diabetes mellitus. The charge to the Committee was to prepare a monograph which would (1) review and evaluate the present state of knowledge and research in the basic and the clinical aspects of diabetes mellitus; (2) assess the applicability of present knowledge to the prevention and control of diabetes, and (3) identify gaps in knowledge and areas requiring further research. The Committee asked contributors to the monograph to (1) make projections and recommendations for future needs in terms of manpower and techniques, and (2) consider the direction in which maximum efforts might be most fruitful in developing measures or means for the prevention of clinical diabetes and its complications.

It is obvious that effective preventive measures are difficult, if not impossible, where the etiology and pathogenesis are poorly understood. Nevertheless it was visualized that a cooperative venture representing several points of view might serve as a stimulus for accelerative research to gain new basic knowledge. The monograph would not serve as a textbook of diabetes mellitus but as a document prepared primarily for the use of professional and/or administrative personnel and planners of the National Institutes of Health, as well as a resource for legislative committees. It was the hope of the Committee that the report would be a valuable contribution to national health planning and would assist in the making of informed decisions about measures that might have significant impact in terms of public health. Accordingly, the Organizing Committee intended each chapter to include material which would give (1) background, (2) the current state of knowledge, (3) a realistic appraisal of what information must still be acquired through research now and in the future, and (4) how and why such information should lead to improved preventive medicine (a) immediately by application to patient care and (b) by long-range projection. It is apparent that some subject matters lend themselves better to this approach than do others.

The table of contents details the contributions and their authors. The various sections address themselves to the problem of the definition of diabetes, epidemiology, socioeconomic aspects of the disease, etiology and pathogenesis, acute complications of the diabetic state, long-term complications, new approaches to control and prevention of the disease, and manpower needs for research and patient care.

The Committee hopes that the monograph will fulfill the needs which prompted its formulation. The Committee is very much indebted to Dr. Fred R. McCrumb of the Fogarty International Center for his assistance in all aspects of the planning and preparation.

Organizing Committee and Editorial Board
November 1975

DIABETES MELLITUS

1

THE PROBLEM OF DIABETES MELLITUS

Stefan S. Fajans and Norbert Freinkel

"Idiopathic" diabetes mellitus[1] is a disorder of metabolism that, in its fully developed clinical expression, is characterized by fasting hyperglycemia, atherosclerotic and microangiopathic vascular disease and neuropathy. Hyperglycemia may become manifest years before the clinical recognition of vascular disease or neuropathy. Only a few decades ago a generally acceptable definition of diabetes mellitus would have stressed the presence of continuous hyperglycemia and glycosuria. Even today a few investigators and clinicians are hesitant to accept a definite diagnosis of diabetes in the absence of fasting hyperglycemia (i.e., continuous hyperglycemia). However, the great majority agree that diabetes mellitus may present clinically in a mild or asymptomatic form without fasting hyperglycemia and that this is the most common recognizable form of the disease. The typical vascular and neuropathic manifestations of diabetes may occur in patients with relatively mild carbohydrate intolerance and with normal fasting blood glucose levels. A definition of "carbohydrate intolerance" is difficult since there is no strict separation between normal and abnormal carbohydrate tolerance even in otherwise healthy, ambulatory young people. It is based on statistical rather than biological considerations. Even in most groups of first degree relatives of diabetic patients there is no sharp dividing line but a continuum between normal and abnormal carbohydrate metabolism.

There is common agreement that "idiopathic" diabetes is a disease in which an inherited susceptibility may play an important part. This susceptibility has its origin at conception and may exist for prolonged periods before additional pathogenetic factors cause the emergence of a recognizable abnormality of carbohydrate metabolism. The genetic defect may remain without clinical expression indefinitely. Thus, a definition of genetic diabetes mellitus should include stages in the natural history of the disease which presently cannot be recognized, since we lack a marker for "genetic diabetes."

Before discussing further some arbitrary definitions of the types and stages in the natural history of "idiopathic" diabetes, it is important to cite several recent findings and concepts.

The biochemical and clinical manifestations of the disease encompass a spectrum from the unrecognizable, to the recognizable but asymptomatic form of the disease, and to symptomatic diabetes with acute metabolic decompensation (ketoacidosis, hyperosmolar coma) or with chronic complications or associations (cataracts, complications of pregnancy, neuropathy, atherosclerosis, microangiopathy). Hyperglycemia and these complications are found in both juvenile-onset type and maturity-onset type diabetes. These two types of diabetes usually have been thought to represent only a quantitative difference in the defect in insulin secretion or action, and attendant sequellae. However, a growing body of evidence suggests the existence of heterogeneity of "idiopathic diabetes mellitus" in terms of (a) inheritance, (b) insulin responses to glucose in maturity-onset type diabetes, and (c) prevalence of vascular disease.

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[1]As distinguished from secondary types of diabetes or carbohydrate intolerance associated with a variety of well-defined genetic syndromes.

A difference in the inheritance of diabetes has been shown between the families of maturity-onset type diabetes in young people and families of patients with classical juvenile-onset type diabetes (1). This difference not only provides evidence of genetic heterogeneity but further indicates that there is a need for careful definition of the phenotype of diabetes in populations in which the genetics of diabetes is to be analyzed.

Genetic heterogeneity has been found also among sets of identical twins of which at least one had diabetes mellitus (2). Concordance of diabetes among the pairs of identical twins was very high (92 percent) among those in whom the age of onset of diabetes in the index twin was 40 years or more (mostly maturity-onset type), while concordance was found with a frequency of only 53 percent in those in whom diabetes was diagnosed under 40 years of age in one twin (mostly juvenile-onset type). This suggests that there is a difference in genetic as well as environmental factors in the etiology and pathogenesis of diabetes between these two groups of identical twins.

A difference in the inheritance between juvenile-onset and maturity-onset type diabetes may be associated with a difference in the frequency of occurrence of certain histocompatibility types or HL-A antigens (HL-A8 and/or W15) (3,4), and a difference in the frequency with which viral and autoimmune processes may be involved (3,4). In one group of patients with juvenile-onset type diabetes an increased susceptibility to beta cell damage by viral agents may be due to a defective immune response influenced by genes in the HL-A chromosomal region and leading to an autoimmune process (3,4). The presence of cell-mediated immunity to pancreas antigen was reported to be more frequent in patients with insulin-dependent than in patients with insulin-independent diabetes (3, 5). These findings support the concept that these two types of diabetes differ from each other and have been cited to indicate also that they are two different disease entities in etiology and pathogenesis (3).

In all prevalence figures based on population statistics, diabetes mellitus has been treated as a single disease entity. Juvenile-onset type, ketosis-prone diabetes can be associated with all the acute and chronic complications of the disease. Since it is associated with absolute insulin insufficiency and with the sudden appearance of typical symptomatology, it is usually cited as the prototype of diabetes. On the other hand, among the total population of known diabetic patients not more than 5 percent belong to this type, while the great majority can be characterized as maturity-onset type or ketosis-resistant diabetic patients.

The definitions of the stages in the natural history of diabetes mellitus have been based on the absence or presence and on the degree of abnormality of glucose metabolism (6). These have ranged from prediabetes or potential diabetes, where there is no recognizable abnormality of carbohydrate metabolism, to overt or clinical diabetes mellitus with gross fasting hyperglycemia. Prediabetes or potential diabetes is a conceptual state which can be recognized only in retrospect since we lack a specific marker for "genetic" diabetes and since there is heterogeneity of diabetes in terms of genetics. Thus, at the present time, we cannot determine what percentage of a general population harbors a genetic predisposition to diabetes providing the necessary base for environmental factors to precipitate the disease.

On the other hand, latent or chemical diabetes is the stage in which a diagnosis of diabetes can be made by the use of standardized laboratory procedures although the patient is completely asymptomatic. The majority of patients with maturity-onset type diabetes without complications

have asymptomatic, latent, or chemical diabetes.

Heterogeneity of insulin responses to administered glucose among nonobese patients with latent diabetes has been demonstrated and emphasizes that so-called "idiopathic" diabetes mellitus includes more than one disorder associated with hyperglycemia (6,7). Progression to insulin-requiring diabetes (some to ketosis-prone type) occurred only in individuals who had insulin responses which were delayed and subnormal, or lower than the mean responses of the control subjects. In contrast, none of the patients with "high" insulin responses, or with responses exceeding the mean of the control subjects, have progressed to insulin-requiring diabetes.

Heterogeneity in occurrence of significant vascular disease in diabetes also suggests that we are dealing with a syndrome that includes entities in which different pathogenetic factors (genetic, environmental) are at play. Among a group of classical, juvenile-onset type, ketosis-prone diabetics of more than 30 years duration and with continuous hyperglycemia ("poorly controlled") 20 percent of patients do not have clinically significant retinopathy or nephropathy (8). As reported from at least six clinics in five different countries, clinical evidence of microvascular disease, atherosclerosis, or neuropathy has been found in only 20-40 percent of insulin-requiring, ketosis-prone patients, who have survived diabetes for 20-40 years or more (9).

These concepts of the heterogeneity of "idiopathic diabetes mellitus" in terms of heredity, insulin responses to glucose, and occurrence of vascular disease underline the enormous task which lies ahead of us in trying to unravel the etiologies and pathogeneses of diabetes mellitus and its complications. The plural is used advisedly since it is becoming apparent that we are dealing not with a single disease but with a syndrome composed of multiple entities.

However, although genetic etiologies may differ, certain uniform metabolic consequences can be anticipated whenever blood glucose remains elevated too much and too long. For example, as reviewed elsewhere in this volume (Chapter 13, Winegrad and Clements), hyperglycemia effects an increased disposition of glucose by the polyol pathway. Such diversion of glucose could alter intracellular ion fluxes, hydration, reductive environment, and other metabolic parameters as yet undefined. Hyperglycemia can also induce selective increase of enzymes involved in forming the constituents of basement membranes (Chapter 13, Winegrad and Clements). Even more subtle physiochemical changes may result from hyperglycemia: e.g., serum osmolality appears to parallel hyperglycemia in *asymptomatic* maturity-onset diabetics (10). Presumably, therefore, thirst is not activated sufficiently to compensate for the osmotic contribution of the "extra" circulating glucose in this relatively stable population. Since all cells should function as perfect osmometers, one may infer that intracellular hyperosmolarity is a common concomitant of hyperglycemia and that the hydration of intracellular macromolecules may be compromised more frequently in diabetics than is generally appreciated (10). Regardless of the underlying genetic defect(s), any or all of the above consequences of hyperglycemia per se could contribute to the development of some of the anatomic lesions of diabetes.

In addition, certain untoward aspects of the diabetic state could be caused by secondary increases in the availability of hormones with potentially catabolic properties. For example, exuberant basal levels of growth hormone are more common in diabetics and paradoxical responses of growth hormone to glucose occur more frequently (see Chapter 13, Winegrad and Clements). Growing clinical experience suggests that these disturbances in growth hormone secretion may parallel the severity of the metabolic derangements rather than being characteristic of any single subgroup of

diabetics (11,12). Indeed, the abnormalities may be rectified in large measure by regulation of the diabetes (12). Failure of glucose to effect normal suppression of glucagon has also been reported in diabetic subjects (13). It is possible that this altered pattern of glucagon secretion may represent a primary component of genetic diabetes especially since the alpha and beta cells share a common embryological origin. Moreover, as of this writing, complete correction of glucagon dysfunction has not yet been observed in human diabetics at least during acute insulin therapy (13). However some secondary changes in glucagon secretion can be induced. Thus, the same pattern of seeming glucagon autonomy has been elicited in animals with experimental diabetes, and, under such circumstances, it can be reversed by treatment with insulin (14). Clearly, definitive answers concerning primary vs. secondary etiologies for abnormalities in the secretion of hormones other than insulin in genetic human diabetes must await experiences with insulinization programs that reproduce the normal multiphasic excursions of endogenous insulin more closely than conventional therapy (e.g., the "artificial pancreas"; islet transplants, etc.; see Chapters 19 and 20). However, on the basis of existing data, it seems likely that at least some of the disturbances in hormones such as growth hormone and glucagon are secondary to *acquired* changes in glucose recognition within the cells from which these hormones are secreted, or within those suprahypophyseal or peripheral neural centers which regulate their responsiveness to metabolic mixtures.

All the above represent pathophysiological phenomena which could be ascribed to hyperglycemia and disturbed glucose homeostasis per se. As such, they convey the tacit implication that simple correction of the abnormalities in blood sugar (and the relative insulinopenia) should also eliminate many of the complications of diabetes mellitus. However, despite a voluminous and controversial literature, that proposition has never been really tested. As cited above, none of our traditional forms of diabetes therapy have reduplicated the moment-to-moment fine tuning between circulating glucose and insulin that the normal beta cell provides. Moreover, it may be simplistic to view hyperglycemia in such unidimensional terms. Immediately following alimentation, metabolic objectives are geared to utilize ingested nutrients not only for fulfillment of prevailing oxidative demands and repair of antecedent metabolic deficits, but also for storage in anticipation of the longer intervals when exogenous fuels will be unavailable. With carbohydrate-containing "mixed meals," the prompt and appropriate acute increases in the availability of insulin, and the counterbalancing reductions in hormones such as glucagon, and usually growth hormone, make optimum anabolism possible (15). But the anabolism involves dietary protein and fat as well as carbohydrate, and *in an integrated and insulin-dependent fashion.* Thus, it is perhaps misleading to speak of "glucose intolerance," even though historical precedent and our diagnostic use of simple glucose challenges to reproduce the "fed state" (15) have made this term part of common clinical parlance. Since insulin affects anabolism of and from protein and fat, as well as carbohydrate, a logical extrapolation from "glucose intolerance" must be that *the complex integrated disposition of all dietary components is also "out of phase."* Supporting data are sparse, and may require greater analytical sophistication and more diagnostic studies with "mixed meals" rather than oral or intravenous glucose. It is not inconceivable that dose-response curves between insulin and individual food-stuff may differ so that given degrees of "glucose intolerance" may be accompanied by varying delays in the concurrent disposition of the amino acids, chylomicrons, and very low density lipoproteins of dietary origin. Similarly, "fasting hyperglycemia" cannot be viewed in terms of glucose alone. Normoglycemia after overnight fast presupposes that

basal elaboration of insulin has been sufficient to brake the recall of endogenous fuels in accord with prevailing oxidative demands. "Fasting hyperglycemia" therefore indicates that catabolism has been inappropriate, and by definition, such faulty catabolism involves stores of proteins and lipids, as well as carbohydrates.

In this regard, the changes in blood glucose that occur with aging warrant comment. Epidemiological experience has indicated that fasting blood glucose increases at the rate of "2 mg/100 ml per decade through the adult years;" "postprandial values change at the rate of 4 mg/100 ml per decade and those following a glucose challenge at 6-13 mg/100 ml per decade" (16,17). It is not yet certain whether this is a normal consequence of aging or because increased age unmasks those with a genetic predisposition to one of the forms of diabetes mellitus. Suffice it to say that within the framework of "normal criteria," some 50-60 percent of all sexugenarians and septugenarians may display abnormal "glucose tolerance" (17,18). Moreover, population studies have confirmed that hypertension and ECG abnormalities are more common in those with such asymptomatic elevations in post glucose values (see Chapter 14). Within the context of the metabolic considerations that are outlined above, one may infer that sluggish anabolism from ingested nutrients and less regulated catabolism of endogenous resources may obtain in fully half of the aged (17,18). It is tempting to speculate that some of the so-called "normal" concomitants of old age, such as atherosclerosis, may represent expressions of these metabolic abnormalities; and that in this regard, some of the lesions of the young and middle-aged diabetic represent a form of "accelerated aging." The observation that the regression coefficient between age and width of the basement membrane of muscle capillaries is twice as great in diabetics as in normals is compatible with this proposition (19). Thus, the apparent sparing of some diabetics from degenerative complications (8) might merely reflect a genetic resistance to the metabolic consequences of faulty postprandial anabolism and/or poorly integrated catabolism--i.e., a genetic propensity to longevity. In the least however, the metabolic view of all diabetes as a form of "accelerated aging" would imply that detailed analysis of the diabetic syndromes may yield not only new genetic insights but also more catholic clues concerning those degenerative changes which we have classically accepted as "age-related."

In Chapters 2 and 3 the prevalence of diabetes in terms of known hyperglycemia, the prevalence of macroangiopathy, microangiopathy, and neuropathy in those with diagnosed diabetes, as well as the social and economic aspects of the disease are given. Population statistics have rarely been obtained by examining populations particularly predisposed to the disease. It has been reported that in the families of young propositi with maturity-onset type diabetes, 85 percent of the parents and 53 percent of siblings had maturity-onset type diabetes as well (1). Furthermore, it has been shown that in the majority of patients with this type of diabetes there may be no, or only slow, progression of carbohydrate intolerance over many years or decades and that diabetes may remain unrecognized for similar periods of time (6). Several population studies have demonstrated an impressive correlation between the prevalence of asymptomatic hyperglycemia and the prevalence of atherosclerotic cardiovascular disease (20-22). We do not know in what proportion of patients with cardiovascular disease the disease is secondary to or associated with diabetes mellitus. We are also lacking large scale longitudinal prospective studies of individuals with asymptomatic diabetes in early life to determine the frequency of emergence of overt diabetes or of complications such as neuropathy, atherosclerotic cardiovascular disease,

retinopathy, or nephropathy in middle or advanced age. Such studies are essential if we are to assess the magnitude of the problem which diabetes poses in terms of morbidity and mortality and if we are to devise effective prophylactic procedures in the future.

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EPIDEMIOLOGY

2

DIABETES MELLITUS: THE OVERALL PROBLEM AND ITS IMPACT ON THE PUBLIC

Harvey C. Knowles, Jr., Curtis L. Meinert, and Thaddeus E. Prout

It is the purpose of this report to give estimates of the long-term problems of diabetes facing this country today. The estimates given will deal mainly with the epidemiology of diabetes and hyperglycemia in the United States, time lost from activity because of diabetes, the risks of atherosclerosis and hypertension in the known diabetic population, the significance of hyperglycemia in the atherosclerotic population, the risks of small blood vessel disease in the known diabetic population, and the problems of neuropathy and of pregnancy in diabetes. Finally, the impact of society, needed areas of research and applied treatment, and the support necessary will be discussed.

THE EPIDEMIOLOGY OF DIABETES

Epidemiology of diabetes in the United States is unclear in many aspects. First, the prevalence of diabetes is not known with certainty. Differences in testing procedures and criteria for diabetes have led to variations in reported results. Nevertheless, crude estimates of prevalence can be made, and prevalences by measurements of blood sugar levels or by interview or medical history in population samples are listed below.

			Cases per 1000		
Source	Year	Age	Known	Newly Discovered	Reference
Oxford, Massachusetts	1946-47	15+	11	13	(91)
Tecumseh, Michigan	1959-60	20-79	18		(69)
Nat'l Health Exam Survey	1960-62	18-79	18		(62)
Sudbury, Massachusetts	1964	15+	11	8	(71)
Nat'l Health Interview Survey	1965-66	all	14.5	8	(28)
Nat'l Health Interview Survey	1967	all	16.1		(64)
Pima Indians	1966	15+	158	168	(9)

Despite variations in population sampling and in use of different criteria for diagnosis of diabetes, a common prevalence of approximately 2 percent emerges. Excluded from this estimate and included for contrast is the prevalence observed in the Pima Indians in Arizona. Just why the Pima population should have such a high frequency is unknown. The estimate of 2 percent in the non-Indian studies would yield a diabetic population of approximately 4.0 million in the United States if a total population of 200 million is assumed. Of the 4.0 million, it is likely that 2 to 3 million are now known, and one-half to one million have diabetes and remain to be diagnosed. The prevalence of diabetes in children is apparently one in one thousand. About 389,000 new cases are reported yearly for the total population for all ages. It is estimated that there are about 5.5 million other persons in the United States destined to develop diabetes. Taking these figures into account, one can arrive at a total figure of approximately 10 million persons in the United States who have diabetes known, diabetes unknown, or will develop diabetes. These groups consist respectively of approximately 1.6, 0.6, and 2.8 percent of the population.

Estimates by interview have been made of the ages of known diabetics in the United States. United States Public Health Services (USPHS) information obtained in 1965 and 1966 indicated

that at the time, 79 percent of male and 83 percent of known female diabetics were at least 45 years old (28). Five percent were age 24 or less. Below age 45, the relative frequencies by sex appear equal, but after age 45, the rates for females are higher.

Another poorly understood area of epidemiology concerns the age at diagnosis of diabetes (63). Eight percent of cases are diagnosed at age 24 or before, 22 percent from 25 through 44, 50 percent from 45 through 64, and 20 percent at age 65 or older. Only 4 percent reported their diagnosis to be made before age 15.

Although it would seem that genetics play a role in the development of diabetes, the factors accounting for pancreatic failure at different times of life are anything but apparent. Observations in other countries suggest that movement from rural to urban areas leads to an increased risk of diabetes. Increases in intake of fat and sucrose, as well as obesity, have been implicated as influential factors, but simply a high caloric intake may be a common denominator (87). Of particular interest at the minute, are the observations of a relation of development of juvenile-type diabetes with overt insulin failure to viral epidemics (54). The problem of genetic versus environmental influences on the development of diabetes and complications is well described in the studies of Pyke on diabetes and retinopathy in identical twins (82).

In 1965, 33,000 deaths were listed as due to diabetes, and the disorder listed as the eighth leading cause of death (28). In 1972 and 1973 respectively, 39,000 and 36,000 deaths were so listed in the provisional statistics, and the disorder had advanced to the sixth cause (66). These figures may be conservative, however, for many deaths related directly or indirectly to diabetes may be attributed to other causes. Although a change in the coding classification system used for mortality statistics occurred between 1967 and 1968, from the Seventh to the Eighth Revision of the International Classification of Diseases, the increase cannot be attributed to this since the comparability ratio for diabetes mellitus between the two classifications was 0.9971 (67). The growth of the population and its changing age distribution have some effects, of course, on the diabetes mortality picture, but neither of these factors can account for the increase during this period. Both the crude and the age adjusted mortality rates have increased (66,67).

In regard to survival with diabetes, the most extensive studies are those dealing with long-term follow-up of patients seen initially at the Joslin Clinic. Entmacher et al. in 1964 reported on 17,654 cases seen at the Clinic (31). Of great importance was the decline in deaths from ketoacidosis from 14.5 percent before 1930 to 1 percent in 1960. Vascular deaths rose in the same time from 46.8 to 76.6 percent. Tuberculosis deaths declined from 5.5 to 0.3 percent.

Hirohata et al. calculated the cumulative survival rates in patients seen at the Joslin Clinic since 1939 and living in Massachusetts (42). The rates were expressed as percentages relative to those in Massachusetts life tables and are given according to age at first clinic visit and anniversary of follow-up.

Age (years)	Anniversary (years)	Males	Females
0-19	5	99.6	96.9
	15	97.4	95.4
	25	82.6	83.2
20-39	5	100.0	96.7
	15	96.7	92.1
	25	82.6	86.3
40-59	5	97.0	96.6
	15	83.8	78.0

Age (years)	Anniversary (years)	Males	Females
40-59	25	75.0	55.1
60-79	5	98.4	88.4
	15	77.1	62.8
	25	62.9	35.1

The survival rates declined with duration of known diabetes. After age 40, they declined more rapidly, and those of women declined faster than those of men.

A third follow-up study of cases seen at the same clinic was conducted by Kessler (47).

	SMR	P
All causes	1.93	<0.001
Cardiovascular disease	1.84	<0.001
Cerebrovascular disease	1.17	<0.05
Nephritis	1.27	>0.1
Tuberculosis	1.51	<0.05
Cancer	0.95	>0.1
Accidents	1.03	>0.6

Standardized mortality ratios (SMR), defined as the observed divided by the expected death rates, differed significantly for all deaths and for those due to coronary artery disease (CAD). Death rates from cerebrovascular disease and tuberculosis were probably higher. No increase in deaths from renal disease could be identified. It is to be remembered, however, that the causes are those given in death certificates.

DISABILITY AND COST OF DIABETES

The findings of the National Health Interview Survey of the year 1965-1966 indicated that 562,000 diabetics were disabled in some way from diabetes (28). There were 38.8 million days of restricted activity due to diabetes, 19.9 million of these days being spent in bed. The disability days due to diabetes per year per diabetic were as follows:

	Restricted Activity Days		
	Bed Days	Other	Total
Men	6.7	5.2	11.9
Women	7.5	8.1	15.6

Eighty-two percent of the diabetics made visits to physicians in the year, and they averaged 6.6 visits each. In regard to hospital patients having diabetes, 1.7 percent of hospital discharges in 1971, exclusive of obstetrical and newborn discharges, had diabetes given as the first listed final diagnosis (65). This rate is equivalent to a discharge rate of 21.3 per 10,000 population. If a diabetes prevalence of 2 percent is assumed, then there are each year about 10 hospitalizations for diabetes per 100 diabetics in the population. Some of these persons may have more than one hospital episode in the year. In general, disability days due to all illnesses were 3 times more frequent among diabetics than those estimated for the United States population generally (63).

The same source provided also useful information for that time period (1965-1967) on the cost of diabetes to the American economy (28), although these figures need upward revisions in terms of present cost levels. The total was almost 2 billion dollars, with the division as follows:

	Millions
Estimated losses in earnings	1,344
Hospitalization	170
Nursing home care	45
Physician visits	90
Surgical fees	11
Insulin and oral drugs	148
Needles, syringes, etc.	8
Sugar tests (blood and urine)	51
Research	19
TOTAL	1,886

It is of interest that even with physician fees coming to 101 million dollars yearly, about half a million diabetic patients, more than one-fifth of the total known diabetics, stated that they had not consulted a doctor during the course of the year. Those who did see a physician made, on the average, 6.6 visits during the year.

LONG-TERM COMPLICATIONS

Unfortunately, reliable information on rates of developing long-term complications of diabetes are not at hand. Data on the natural history of disease have been collected from death certificates, hospital clinical and autopsy records, or obtained by surveys at clinic visits. These approaches provide crude estimates only, since they may deal with populations that happen to be available. Retrospective examination of records is an approach anything but satisfactory to obtain accurate facts. Prevalence surveys of living patients deal only with survivors. The use of prospective studies is a significant step forward in description of the course of diabetes, but so far there have been few efforts to measure events in diabetes precisely and express data in life table fashion (45). Even so, these few studies are limited to the populations which happened to be available when the studies were initiated. *What is urgently needed is a random sample of a diabetic population, as well as subjects with asymptomatic hyperglycemia, observed prospectively over a 20-year period with measurements of response variables made with sufficient precision to allow for data interpretation.*

Atherosclerosis is the most common debilitating hazard faced by the diabetic patient. Indeed, coronary artery disease (CAD) is the most common cause of death in the middle-aged, stable-type diabetic. Bell, in his classical studies of diabetics at autopsy, listed cardiovascular disease as the major or contributing cause of death in 49 percent of 1,555 necropsies (7). Of the vascular deaths, CAD accounted for 18.5 percent, peripheral atherosclerosis for 12.7 percent, and cerebrovascular disease for 7.7 percent. In a control group of 4,419 apparently nondiabetic autopsies, CAD was judged responsible for 24 percent of deaths. Of 5,009 deaths at the Joslin Clinic in the years 1960-1968, 77 percent were believed due to CAD (13). In contrast to this, vascular disease was held accountable for only 54 percent of all deaths in the United States in 1966 (93).

Thus, in all studies, atherosclerosis stands out as a major health hazard in the natural history of diabetes mellitus, and CAD is the leading cause of death. Accordingly, CAD will be discussed in some depth.

CORONARY ARTERY DISEASE

Three recent reviews of coronary artery disease (CAD) in diabetes have appeared which contain references to most of the studies made in the past 30 years (14,33,86). The lesions differ little from those in the nondiabetic population and will not be discussed here. In the studies referred to, CAD prevalence varied from a low of 18 percent (7) to a high of 75 percent (81) in a study where a dye injection technique was used. On the average, the frequency of CAD at autopsy in the diabetics was 2.5 times that in the nondiabetics.

Of less certain value are comparative data from surveys for CAD in living diabetic patients.

Author			Group	Pathology	Age	No.	Percent
Liebow	(56)	1955	Diabetic	CAD	10-90	383	42
				MI			7
				AP			10
Bryfogle	(17)	1957	Diabetic	CAD	>40	394	56
				AP			12
Anderson	(2)	1961	Diabetic	CAD	23-88	100	55
Liebow	(57)	1964	Diabetic	CAD	40-70	58	33
UGDP	(49)	1970	Diabetic	CAD	20-79	1017	9.5
Kannel	(44)	1961	Framingham	CAD	30-62	4469	1.6
Epstein	(32)	1965	Tecumseh	CAD	16-70+	5129	4.1

CAD - coronary artery disease
MI - myocardial infarction
AP - angina pectoris

Prevalences at a given time have been reported as 42 and 33 percent by Liebow et al. (56, 57), 55 percent in a black population by Anderson et al. (2), and 56 percent by Bryfogle and Bradley in the Joslin Clinic (17). In the University Group Diabetes Program the frequency of CAD as evidenced by ECG change or history of angina within one year of diagnosis of diabetes in a middle-aged, stable-type of population was 9.5 percent (49). For comparison, the frequency of CAD in total populations in the Framingham study was 1.6 percent (44) and in the Tecumseh study 4.1 percent (32). It is of interest that recent observations in the Pima Indians, a race with an extraordinarily high prevalence of hyperglycemia (see above), the prevalence of CAD in the hyperglycemics was only 5.3 percent in contrast to 3.3 percent in the normoglycemics (10). The prevalence of angina pectoris in diabetics has been reported as 7 and 12 percent by Liebow et al. (56) and Bryfogle and Bradley (17), respectively.

Certain items of interest come to light on reviewing the above studies. First, the sex differential of a higher risk of CAD in males in the general population is not apparent in the diabetic samples. Diabetic women, prior to the menopause, have a prevalence equal to men of the same age. Indeed, there is some suggestion that after age 50 women might even be at greater risk (34). Second, CAD appeared to progress and cause symptoms at a younger age in the diabetic population . This premise is not yet certain, however, since sudden deaths from coronary occlusion in young men are not uncommon. Third, several items originally believed to add to the risk of CAD in the population at large do not seem to hold in the diabetic population. For example, in the majority of studies quoted above, there was insufficient evidence to substantiate obesity as a risk factor. In the studies of Pell and D'Alonzo there was a higher mortality rate in obese diabetics, however (75). Herein, the authors felt that the added risk was due to obesity and not a potentiating effect on diabetes. The role of elevated cholesterol as a risk factor for CAD in the diabetic was not evident in the Framingham study (35). Finally, the relation of CAD to

severity of diabetes as judged by insulin dose, is uncertain. In the Framingham study, an increased mortality in diabetics taking insulin occurred only in the women. Indeed, a clear relation between elevated blood sugar per se and the development of CAD has not yet been established.

Three events do seem to increase the risk of CAD, however. First, all studies demonstrate a higher prevalence in patients with long duration of known diabetes. The known duration is a poor indicator of actual duration, however, since in many of the middle-aged, stable-type diabetic patients prone to CAD, hyperglycemia may have existed without symptoms for many years prior to diagnosis of diabetes. Second, as has been found in the whole population, all studies of hypertension and CAD in diabetes have reported an increased prevalence of CAD when hypertension is present. Pell and D'Alonzo felt, however, that similar to the effect of obesity discussed above, the added risk was from hypertension per se and not from potentiation of the diabetic effect (75). Third, it has been proposed that there is overlap of diabetes and Type IV hyperlipidemia, that latter condition often being accompanied by atherosclerosis (11). The exact prevalences of elevated serum triglycerides and pre-beta band staining in the diabetic population are not clear at this time (80).

Of particular importance in the course of myocardial infarction of the diabetic is the high immediate mortality and the low 5-year survival rate in those recovering from the initial attack.

Author		Year	Immediate Mortality No.	Immediate Mortality Percent	Five Year Survival No.	Five Year Survival Percent	Total Survival Percent
Katz	(46)	1949	57	51			
Robinson	(78)	1952	39	31			
Cole	(24)	1954	63	33	42	43	28
Bradley	(12)	1956	83	58	40	40	17
Liebow	(57)	1964	38	26			
Partamian	(72)	1965	205	38	127	38	24

Observations of hospitalized patients revealed immediate mortalities of 33 to 58 percent in initial attacks. In the control observations in four of these studies, the mortality was 20 to 28 percent, suggesting a better immediate survival in the nondiabetic. Of even more significance is the decrease in 5-year survival of those not dying in the first attack. In three studies, the survivals were only 38, 40, and 43 percent. When these death rates are combined with those of the initial attacks, the total 5-year survival rates are 28, 17, and 24 percent, respectively. In other words, a diabetic experiencing his first known myocardial infarction has roughly a 20 percent chance of being alive 5 years later.

Data on the frequency of CAD in the younger insulin-dependent diabetic population unfortunately are meager and may be obscured by inclusion of other forms of myocardiopathy. Preliminary observations on the course of vascular disease in juvenile diabetes, with diagnosis made at age 16 or before, suggest that the risk of microangiopathy precedes that of macroangiopathy, and that those at risk may die of renal failure before the clinical appearance of CAD (51). After the 30th year, the risk of small blood vessel disease decreases, but at this time the risk of atherosclerosis increases. Whether this new risk is related more closely to age or to duration of known diabetes cannot be determined at this time.

In summary, CAD in diabetes is characterized by a high prevalence, an equal risk by sex, occurrence at an earlier age, and a high death rate at the time of attack with a low 5-year

survival rate. It occurs mainly in the middle-aged, stable-type of diabetic, and the high prevalence makes it the most frequent and hazardous risk in the diabetic population. CAD is unusual in juvenile-type diabetes, but may become more prevalent as longevity increases in the juvenile diabetic population.

PERIPHERAL VASCULAR DISEASE

Peripheral vascular disease, characterized by atherosclerosis and obliteration of the arteries in the legs, is very common in the diabetic population. Monckeberg's sclerosis, calcification of the vessel media, is also common, but is asymptomatic because the vessel lumen remains patent.

Warren observed a frequency of 34 percent of peripheral arterial disease at autopsy in diabetics at autopsy (86). Bell found approximately 13 percent of the diabetic population had peripheral gangrene at death (7). In clinical studies, prevalences of 58 and 59 percent have been observed (16,53). In the Framingham study the morbidity of intermittent claudication in the diabetics was 4.5 times that expected (35).

Peripheral atherosclerosis occurs at a younger age in the diabetic population than in the general population. Similar to the observations on CAD, there does not appear to be a relation to severity of diabetes, though one would say it was more common with extended duration. Nor can a relation to severity of insulin failure as judged by insulin dosage be established. Studies have indicated that following amputation of one leg, the other leg is involved in 50 percent of cases in 2 years, and in 95 percent in 5 years (36). Moreover, only 36 percent of amputees may be alive in 5 years (90). Juvenile type diabetics with diagnosis before age 16 seem rarely to develop arterial occlusion below the knee. Monckeberg's sclerosis is very common in the juvenile population, however, and White found an 83 percent prevalence of medial calcification in long-standing juvenile diabetes (88).

CEREBROVASCULAR DISEASE

Whether or not there is increased frequency of cerebral atherosclerosis in diabetes is unsettled. Warren and LeCompte have suggested that it may be uncommon simply because diabetics often die with CAD before cerebral sclerosis can develop (86). Grunnet found two-thirds of 107 diabetics to have at autopsy evidence of cerebral sclerosis, but control data were difficult to acquire (38). Bradley states that 12 percent of diabetics may die from cerebrovascular accidents, a prevalence not different from that in the total population (13). Bell ascribed 8 percent of diabetic deaths to be from cerebrovascular disease as ascertained at autopsy (7). On the other hand, the morbidity of cerebrovascular disease in the diabetics in the Framingham study exceeded the expected by a factor of 2.4 (35). It would seem unlikely that the different prevalence of atherosclerosis in the diabetic and nondiabetic population would exclude the cerebral arteries, and that the concept of early death from other diabetic complications could explain the lack of overt evidence for an increased frequency.

HYPERTENSION

In this discussion, hypertension is referred to as that known as "essential" and is apart from the hypertension associated with renal disease.

There have been numerous studies to determine if hypertension is more frequent in the diabetic population. The usual criticism of poor standardization of measurement, unsatisfactory

sampling of the diabetic population, and lack of suitable control data can be applied in all. Nevertheless, there is the clinical impression that hypertension is more common than exptected and that women and the elderly may, in particular, be at risk for elevated blood pressure. A recent study of DuPont employees indeed supports the view of an increased prevalence in the middle-aged stable-type diabetic population (74). Listed below are prevalences of hypertension observed in various diabetic groups.

Author		Year	Patients	No.	Percent
White	(88)	1956	Juvenile diabetes, 15+ years	879	53
Bryfogle	(17)	1957	Clinic, all ages	394	36
Pell	(74)	1967	Industrial employees	662	37
Liebow	(56)	1955	Clinic, all ages	383	43
Anderson	(2)	1961	Clinic, all ages, (black)	100	59
Brandman	(16)	1953	Clinic, all ages	264	24
UGDP	(49)	1970	Clinic, stable type diabetes at diagnosis	1017	31

All the studies deal for the most part with the general diabetic population except for that of White, which concerns only long-standing juvenile diabetes (88). The frequencies given range from 24 to 59 percent, the differences being due to variations in age at time of measurement and in acceptable blood pressure levels for hypertension. In most instances, women appeared to be at a greater risk than men. Accelerated or malignant hypertension has not been reported as a common event in diabetes. Control data on nondiabetic patients was unavailable or inadequate except for the study by Pell and D'Alonso (74).

HYPERGLYCEMIA IN CORONARY ARTERY DISEASE

In recent years attention has been directed to the prevalence of hyperglycemia in populations with vascular disease. By hyperglycemia is meant elevated blood sugar, but not necessarily to the level of diabetes. Unfortunately, the definition of hyperglycemia varies from author to author. In 47 patients with various forms of severe atherosclerosis, Waddell and Field found the OGTT to be "normal" in only 15 percent (84). Bartels and Rullo observed abnormal glucose tolerance in 77 percent of a group of patients with peripheral vascular disease and no known diabetes (5). Most investigations of blood sugar levels in atherosclerosis have been made in CAD, however. Nine studies of oral glucose loading, particularly in the recovery phase of myocardial infarction yielded hyperglycemia ranging from four to 77 percent in frequency. Again, many of the studies were hindered by lack of satisfactory control data, and often they were carried out at varying periods after a myocardial infarction.

The significance of hyperglycemia in the atherosclerotic population remains to be determined. There is no question of the increasing risk of atherosclerosis in known diabetes where hyperglycemia has been established previously. The reversed situation of mild hyperglycemia in the atherosclerotic population allows for speculation on the relation of hyperglycemia and atherosclerosis. Knowledge of whether or not a cause and effect relation occurs will be of the greatest importance in the direction of efforts to curtail vascular disease in diabetes.

RETINOPATHY AND BLINDNESS

Disorders of the eyes with loss of visual acuity are a common and serious complication of

diabetes. Recent reviews are those of Bradley (15), Leopold (55), and Caird (21).

In 1935 Waite and Beetham reported their findings of eye abnormalities in 2,002 diabetics and observed retinal hemorrhages in 18 percent and exudates in 10 percent (85). Since then, many prevalence studies of retinopathy in clinic populations have appeared, with frequencies ranging widely because of differences in ages and durations of diabetes in the patients. In juvenile diabetes with disease known of 10 or more years, frequencies of 19 to 100 percent have been reported and are listed in the review of Knowles et al. (50). Those with the lowest rates, 19 percent (25) and 23 percent (43), are of interest because they included patients with diabetes diagnosed before age 10. At the other extreme, White found lesions in 90 percent of juvenile diabetic patients with 30 or more years of known duration (88). All these surveys are hardly comparable, however, because of the variations in patient ages and especially in durations of known diabetes.

Burditt et al. has attempted to assemble published prevalence data in relation to age at diagnosis and duration of known diabetes (18). In general, the frequency of retinopathy increases with duration regardless of age at diagnosis, with frequencies ranging from 42 to 82 percent at 24 years duration. In one long-term prospective study of juvenile diabetes, a somewhat lesser frequency of 62 percent at 25 years duration was found (51). This is a lower figure than that given by White (88) and may be related to the population studied. The former data are from a complete population observed from the time of diagnosis of diabetes, whereas many of the other studies are of referred populations which may be biased by reasons of referral. Finally, it is of interest that in the juvenile diabetics, the risk of retinopathy appears to decrease after 30 years (51).

The prevalences of retinopathy given above include all degrees of retinopathy from simple red dots to neovascularization with gross hemorrhage and scarring. Neovascularization, or malignant retinopathy, is more common in the long-term younger insulin dependent diabetic. In a study of a group of 847 patients with malignant retinopathy, Root et al. found over 80 percent of cases to be in the age range of 20 to 59 (79). White, in her studies of diabetes with onset in childhood, found a frequency of 53 percent at ages 30-39 with an increase to only 58 percent in the 40 year age group (88).

Visual impairment usually occurs in a few years after the appearance of new blood vessel formation. The time for progression to blindness is quite variable, some cases progress rapidly in a year, some remain stationary, and others, estimated to be 10 percent in number by Beetham (6) and Davis (27), show regression of lesions. In Beetham's series of 351 cases observed over 4 years, 30 percent had become legally blind, 7 percent totally blind, and 10 percent had improved (6). Retinopathy in the older person appears to progress to gross visual impairment at a more rapid rate than in the younger person, as exemplified in the data of Caird et al. (20).

The impact of diabetic retinopathy on public health lies in the number of patients in the total population developing visual impairment from diabetes. The prevalences of blindness for all causes in the United States have not been clearly established because the reporting of blindness is not compulsory. Nor is there certainty as to the exact cause of blindness in many individuals. Blindness in diabetics is estimated to be 10 to 28 times as frequent as in the general population (21). One source of valuable statistics on the cause of blindness is that of the Model Reporting Area for Blindness Statistics which indicates that diabetic retinopathy is among the top four causes of blindness (61). Diabetic retinopathy accounted for 12 percent of new cases of blindness added to this register in 1966. The other three common causes of blindness

were maculopathies, lenticular problems and glaucoma; each of these may have diabetes as a major contributor. Blindness from diabetic retinopathy occurred at a younger age than blindness from the other three causes. The average age at the start of blindness due to diabetic retinopathy in the total diabetic population was 60 years compared with an average age of 78 for patients with macular degeneration, 72 years for patients with cataract, and 73 years for patients with glaucoma (61).

Diabetic retinopathy is reported to be the most common cause of newly reported blindness in the age group 41-60 years and the second most common cause in the age group 21-40 years, second only to congenital defects (40). The National Society for the Prevention of Blindness estimates that 44,660 individuals were blind from diabetes in the United States in 1962 and that 4,480 persons became blind from diabetes during that year (40).

Thus, when blindness is viewed in all of its many aspects, it is clear that:

a. Diabetes is the major *systemic* illness causing blindness.
b. Diabetes is the major *contributing factor* to blindness for reasons other than that due to diabetic retinopathy alone.
c. Diabetic patients have from *10 to 28 times* the amount of blindness found in the normal population.
d. Diabetes is the *leading acquired cause* of blindness in individuals in the most productive years of their lives.

Although diabetic retinopathy rarely appears early in the course of juvenile diabetes, usually being evident at 15 to 20 years after the diagnosis of diabetes, it becomes an almost inevitable consequence of diabetes with onset at an early age. Caird has estimated that chances of visual impairment or blindness sufficient to give rise to difficulty with employment rises with age from 3 percent in those under 30 at diagnosis of diabetes to 32 percent in those over 60. One can thus appreciate the economic cost to society (21).

The pathologic changes in the eye often are associated with changes elsewhere in the body. For example, relationships between patient survival and severity of retinopathy have been studied by Thomasen (83). In groups of patients matched for age and sex, the observed mortalities for the nondiabetic populations and the diabetics with microaneurysms only were 86 percent and 90 percent, respectively, in a 7-year period. On the other hand, percent survival in diabetic patients with more severe retinopathy during the same period was 41 percent for hemorrhages and/or exudates and 32 percent for malignant retinopathy, respectively. Certified causes of death in 660 cases of proliferative diabetic retinopathy studied at the Joslin Clinic were, in order, nephropathy, coronary occlusion, arteriosclerosis, and cerebral vascular accident (15). The relationship between the severity of retinopathy and glomerular disease in renal biopsies is well known. The triopathy of retinopathy, neuropathy, and nephropathy occurring in the same patient and increasing in percentage with the duration of diabetes is also well established.

The cluster of these pathologic events is of great importance, for it emphasizes the systemic nature of the pathology of diabetes, the relentless progression of this disorder in young patients, and the increasing frequency with which other major disabilities such as neuropathic ulcerations of the feet, chronic renal failure, or coronary heart disease may accompany significant retinopathy. Many of the diabetic blind have lost their vision in the third and fourth decade of life, a time when they are most productive. There is loss of income with

personal hardship that the diabetic family, as well as the diabetic patient, suffers. Not of the least in importance is the cost of services to the blind that society bears.

CATARACTS

In their early extensive studies, Waite and Beetham found a prevalence of cataracts of 50 percent in 1,732 diabetic patients (85). The prevalence in a control group of 526 apparently nondiabetic patients was 57 percent. McGuinness also found no increase in diabetics (59), but Caird is of a contrary opinion (21). It is generally accepted that cataracts are unduly common in the juvenile diabetic population, and Knowles et al. list reported frequencies ranging from 2 to 47 percent depending on ophthalmoscopic or slit lamp examination (50). Cataracts in the juvenile population are often of the metabolic or "snowflake" type, while those in older populations usually are the "senile" type and similar to those in the nondiabetic. The frequency in women appears to be somewhat higher than that in men between ages 40 and 70.

OCULAR PRESSURE

It is not certain if primary glaucoma exists in the diabetic population in increased frequency (15). In observations of primary open-angle glaucoma, Armstrong et al. found, however, a frequency of 4.8 percent in 393 adult diabetics in contrast to 1.8 percent in 280 controls (3). Intraocular tension is reported to be higher in the diabetic population, especially in juvenile diabetes (15). The occurrence of secondary glaucoma following proliferative retinopathy is well known and is found in about 10 percent of those with vitreous scarring.

RENAL DISEASE

Renal disease in diabetics is a serious hazard to patient activity and longevity, particularly in the young diabetic. Recent extensive reviews have been published by Balodimos (4) and Rifkin (76). The most common lesions are diabetic glomerulosclerosis and arteriolonephrosclerosis. Pyelonephritis occurs also and is occasionally accompanied by medullary necrosis. An increased frequency of asymptomatic urinary tract infection has been claimed but conflicting evidence has not supported this. The lesions incapacitating the diabetic subject are glomerulosclerosis and occasionally pyelonephritis. Other common forms of chronic renal disease do not appear to occur in diabetes beyond that expected in the general population.

Glomerulosclerosis may exist to some extent in all diabetics. At the moment the diagnosis is made by clinical course and by light microscopy at renal biopsy or autopsy. It is not always possible to delineate early mesangial lesions. At autopsy prevalences ranging from 15 to 82 percent have been reported with the mean prevalence being about 30 percent (4). Bell, in his study of 1,465 autopsied diabetics found 19.5 and 30 percent frequencies of glomerulosclerosis in men and women respectively (8). In each sex, two-thirds of the cases were of the diffuse type only, and one-third included nodules as well. Kimmelstiel reported an autopsy frequency of 17 percent of nodular glomerulosclerosis (48). Heptinstall reported either diffuse or nodular lesion occurred at autopsy in 50 percent (41). In general, there have been more female than male cases.

Warren and LeCompte state that renal failure caused death in 12 percent of diabetic cases at the New England Deaconess Hospital (86). In a study of the causes of death in 6,800 patients seen at the Joslin Clinic between 1956 and 1964, 389, or 6 percent, died of nephropathy (4). There were 208 men and 181 women, and almost half of the cases were of juvenile type with diagnosis made before age 20. Indeed, life insurance data indicate that death from renal disease is

17 times more frequent in the diabetic population than in the nondiabetic population (31).

In living diabetics, Bryfogle and Bradley estimated the prevalence of glomerulosclerosis to be 10 percent in a clinic population of diabetic patients of all ages (17). The younger diabetic population is very much at risk for glomerulosclerosis, however, and the prevalences in those with known diabetes of 10 or more years duration range from 4 to 100 percent (50). In her classical studies of long-term juvenile diabetes, White quotes the frequencies of glomerulosclerosis as evidenced by proteinuria to range from 18 percent at 15 to 19 years' duration to 63 percent at 35 to 39 years' duration (88). In a recent report of prospective observations of 167 juvenile diabetics followed beyond 10 years, the cumulative risks of proteinuria at 20, 25, and 30 years' known duration were 26, 37, and 48 percent, respectively (51). These figures are less than those given by White and could be explained again by the populations studied.

Data on survival with proteinuria have been developed by Caird (19). He found that at 5 years, the survival rates were of diabetics with proteinuria, 65 percent; diabetics without proteinuria, 73 percent; and the control population, 83 percent. Pell and D'Alonzo also published data on the relation of proteinuria and survival (75). In diabetics in an industrial population, they found that the 10-year death rates of diabetics with and without proteinuria were 39 and 23 percent, respectively, in comparison to a control rate of 10 percent. Finally, it should be noted that in juvenile diabetics, proteinuria is very unusual before 10 years' duration of known diabetes, and similar to the risk of retinopathy, is unlikely to appear de novo after 30 years of known duration (51). In line with this, White observed in 73 juvenile diabetics of 40 years' duration that though 18 had evidence of nephropathy, 12 had pyelonephritis (4). When azotemia appears in the juvenile diabetic, death follows usually within 3 years (52).

Urinary tract infection poses another renal problem for the diabetic patient. Studies of asymptomatic urinary infection in the diabetic population have been made with conflicting results (4). Studies of pyelonephritis made at autopsy suggest a higher rate in diabetes, with prevalences of 7 (78), 12 (29), and 36 percent (86) being reported in contrast to control rates of 2 and 3 percent. Variation in the criteria used for histologic diagnosis of chronic pyelonephritis likely accounts for the different prevalences.

The relation between urinary infection and pyelonephritis is not clear. For example, in a study of renal biopsies in 80 advanced insulin-dependent diabetics undergoing pituitary ablation for progressive retinopathy, 7 percent were found to have chronic pyelonephritis and 12 percent had positive urine culture (39). No relation was found, however, between urine culture, tissue culture, and biopsy appearance. Finally, it is estimated that medullary necrosis, a potentially lethal renal lesion, is present in 4 to 9 percent of autopsies of diabetics (1).

NEUROPATHY

Diabetic neuropathy implies abnormal function of peripheral nerve pathways in the diabetic patient. Almost any pathway, somatic or autonomic, motor or sensory, can be involved. The disturbed functions can be ones of increase or decrease, and the course can be fluctuant or steady and end in permanent dysfunction or remission. The relation of neuropathy to other aspects of the diabetic syndrome is not understood and will not be discussed here.

Recent reviews of neuropathy have been given by Colby (23) and Ellenberg (30). The variability of its course makes it almost impossible to determine its frequency, and it is likely

that most diabetics at some time or other experience some degree of abnormality. Indeed, with very precise nerve conduction measurements, it might be possible to disclose dysfunction in all diabetics. In earlier reviews, prevalences based on gross evaluation varied from 0 to 93 percent (37). Danowski et al. examined 374 consecutive clinic patients and failed to find an ankle reflex in 30 percent, decreased vibratory sense in 44 percent, and painful neuropathy in 9 percent (26). The difficulties in judging the excess of the milder neuropathic symptoms and signs in a diabetic population is apparent in the study of Mayne in England (58). He evaluated 220 diabetics and 110 control subjects of comparable age and sex ratio and found similar symptoms and signs in the controls, though of significantly less frequency.

The loss of productivity time in the diabetic because of neuropathy cannot be determined. In the long-term juvenile diabetic population, about 10 percent will be incapacitated at a given time with pain, trophic ulcer, ataxia, or diarrhea. The loss of position sense makes it all the more difficult for the patient with failing vision to ambulate. Orthopedic corrective measures have been developed for trophic foot ulcers, but little else is available to alleviate the other neuropathic syndromes.

PREGNANCY

It is estimated that diabetes is listed as a hospital discharge diagnosis with pregnancy in about 1 in 300 discharged pregnant patients (89). This figure is conservative because many patients with unknown gestational or transient diabetes may be overlooked. At the Cincinnati General Hospital in 1972, there were 66 admissions of patients with diabetes among 3,082 pregnancy admissions, a frequency of 2.1 percent. In 1971 the frequency was 1.7 percent. These figures fall probably below the actual rates in view of the failure to diagnose gestational diabetes.

For present purposes, diabetes in pregnancy will be looked on as either known prior to pregnancy, or developing during pregnancy with remission after completion of pregnancy. This latter type has been called gestational diabetes (70). Wilkerson and O'Sullivan conducted oral glucose tolerance tests in 752 unselected pregnant women without known diabetes (92). Using criteria for the diagnosis in the population at large, the authors noted prevalences of abnormal tests of 4.8 percent in the second trimester and 7.3 percent in the third trimester.

In a study of glucose tolerance after delivery in gestational diabetes, O'Sullivan found 7 percent of gestational diabetics to remain diabetic after completion of pregnancy, and 66.7 percent to develop established diabetes in 5 and a half years (70). The 7 percent remaining diabetics could have had diabetes established prior to pregnancy and therefore would not be classified according to the strict criteria of gestational diabetes, where it is assumed diabetes did not exist before pregnancy.

Pregnancy can influence carbohydrate metabolism in ways which will not be apparent in the nondiabetic person, but which will affect glucose tolerance in the patient with prediabetes or established diabetes. There is antagonism to insulin with failure of compensation by the pancreas of prediabetic women and resulting rise in insulin requirement in established diabetic women. There is little evidence for residual effects in the diabetic mother, however. It is not believed that diabetic glomerulosclerosis is worsened, though background retinopathy can increase and decrease in pregnancy, and rapidly progressing neovascularization has rarely been

reported to occur in pregnancy. The natural history of diabetic retinopathy in pregnancy has not yet been fully explored, however.

In regard to the effects of diabetes in pregnancy, there is no evidence for infertility nor for an increase in fetal loss in the first trimester. It is possible, however, that the frequency of second trimester spontaneous abortion is greater. Most of the problems take place in the third trimester, and are exemplified in a study of 705 diabetic pregnancies made in Cleveland in 1964 (60). The percent abnormalities observed were compared to the nondiabetic pregnant population.

	Nondiabetic Percent	Diabetic Percent
Toxemia	4.4	20
Hydramnios	0.3	20.4
Lethal defects	0.5	2.0
4,000 gm or >infant	0.7	37
Perinatal death		18.3

In regard to the effects of diabetes on the fetus, there are increased risks of excessive weight from fat accumulation, congenital defects and stillbirth, and on the delivered infant of prematurity, serious hypoglycemia, hyperbilirubinemia, and respiratory distress syndrome. These conditions are discussed elsewhere.

Good management of diabetes during pregnancy, coupled with selection of optimal time of delivery by inducement or section, have done much to give a favorable outcome. The survival rate of the viable infant will vary according to the stage of diabetes. Data collected on outcome of 870 diabetic pregnancies treated at the Joslin Clinic give an overall fetal survival of 90 percent (89). The studies of Oakley (68) and Pedersen (73) in particular, emphasize the value of diabetic control.

In essence, the impact of pregnancy on the diabetic woman is significant in that there is pregnancy, fetal and infant morbidity beyond the expected, though there is little damaging effect on the mother. Considerable time and effort are required by several physicians, as well as the patient, to assure the best outcome possible. Improved medical, obstetrical, and neonatal management have done much to increase fetal and infant survival. Two problems which so far defy correction are the spontaneous delivery initiated too early for infant survival and stillbirth. The first can be attacked through further advances in premature care. The second will require research into the causation.

DELIBERATIONS

The high prevalence, inconveniences of treatment, risk of debilitating consequences, and decreased longevity qualify diabetes mellitus as a public health problem of high priority. Insulin became available in 1922, and the event was hailed as the solution to the problems of diabetes. Not appreciated at the time, however, was that interference in normal activity could be produced by treatment and short-term control complications, nor that physical failure and shortened life span could result from long-term complications. The public looked on insulin as the panacea, and the mistaken concept remains today that the diabetic is handicapped only by his treatment annoyance and occasional episodes of uncontrolled diabetes. It has been the purpose, so far, to describe the status of the diabetic patient in the last decade. Emphasis from the epidemologic standpoint has been placed on the deficiencies in prevention and treatment in all areas of

diabetes. It is hoped that efforts can be stepped up to fill gaps in knowledge in cause and prevention, and in corrective treatment where cause is unknown and prevention not feasible.

To begin with, the estimate of 2 percent prevalence of diabetes is probably near the truth. Nevertheless, there are no prospective studies of glucose tolerance in selected populations other than that of the USPHS studies initiated in 1947 in Oxford, Massachusetts. Unfortunately, at the time the methods were crude. A similar study with standardized glucose tolerance testing and long-term follow-up would be of help to predict the future diabetic. In fact, of great value at the minute would be agreement on a single testing approach with criteria for the diagnosis of diabetes, well knowing that the criteria may be changed as information accumulates. Also, the development of standardized screening criteria for use in everyday medical practice to determine diabetes or need for glucose tolerance testing would be most helpful. Studies to give data in these areas could be initiated by national agencies and appropriate departments of schools of medicine and public health. The ultimate value would be to mark the unknown diabetic before he seeks medical attention for an event which might have been preventable, such as ketosis and foot infections. Finally, such studies would be helpful in defining the sex ratio in diabetes, knowledge of which would be helpful in investigations of the causes of different aspects of diabetes.

The reasons for the development of diabetes are anything but understood. As mentioned before, the aggregation of diabetes in families has led to a general view that a genetic influence must be present for diabetes to develop. The studies of Pyke of identical twins with strong family history of diabetes becoming concordant for diabetes in a short time and developing retinopathy in contrast to twins with less family history and less concordance for diabetes and retinopathy, support the concept of both genetic and environmental influences (82). Observations of the development of glucose intolerance made prospectively with notation of life events in families with strong diabetic history could yield information related to the course of insulin failure. Clarification of the mechanism of inheritance plus determination of influential environmental factors would be the first step towards development of a prevention program in diabetes.

The number of days of normal activity lost from uncontrolled diabetes are small in the insulin independent population, but large in those taking insulin. Theoretically, significant ketosis and hypoglycemia should never occur. Such a goal is unattainable, but increase in physician and patient education could do much to prevent wasted time. Studies of the reasons for time lost and effects on family, occupation, and environment could disclose areas where more intensive education is needed. Applied research to improve education could benefit markedly the economics of the young diabetic with fluctuant diabetes. It is likely that the 20 days per year of hospitalization per 200 diabetic patients could be lessened.

It is in the area of vascular disease that the diabetic develops most often his debilitating complications, however. Although the clinical vascular syndromes are well described, their incidence rates and natural history are not. To clearly define these, one would need a population of diabetics to follow prospectively for 25 years. Without parameters of the course of events, it is very difficult to evaluate treatment regimens of questionable effects, and it is unlikely that a treatment not requiring a clinical trial will be available in the near future. The only available studies of prospective data are one in juvenile diabetes (51) and one in stable type (49), both of which have too few patients to determine other than gross incidences and trends. It is

unfortunate that large diabetic centers in this country have not used prospective approaches starting 20-some years ago so that natural history data now would be becoming available hopefully for future intervention studies.

Large gaps of knowledge exist concerning diabetic atherosclerosis. As mentioned before, cardiovascular disease is the leading serious hazard facing the diabetic. Many population studies of hyperlipidemia and cardiovascular disease are being conducted in lipid centers in the United States, but they are not directed toward diabetes as a subject. Peripheral atherosclerosis markedly affects the older diabetic. It is possible that diabetic microangiopathy is present, and detailed histologic studies in extremities are needed, similar to those carried out in the eye, kidney, and muscle. Similarly, neuropathologic observations are needed to determine if cerebral atherosclerosis is increased, and if other vascular pathology is present. Of particular interest is the high prevalence of hypertension in diabetes apart from that of renal origin. Investigations of the pathogenesis are needed to determine if there is difference from essential hypertension in the nondiabetic population.

The relation of microangiopathy to the course of diabetes remains one of the greatest of unsolved problems. Diabetic retinopathy is a leading cause of blindness before age 50, and renal failure is the most common cause of death in the juvenile diabetic. Yet the cause of microangiopathy is unknown. Indeed, it is not known if mechanisms are the same in the development of retinopathy, glomerulosclerosis, and muscle capillary basement thickening. More extensive studies on the metabolism of diabetic vessels are urgently needed to disclose the factors causing the structural alterations. The natural history of retinal neovascularization is far from clear, and it would be extremely helpful to disclose factors affecting its course as seen in spontaneous regressions, pregnancy, and perhaps after pituitary ablation. At the moment, there is no proven treatment of value for retinopathy. Pituitary ablation has been largely abandoned, and photocoagulation is still in a trial stage. Other approaches to the problem of retinopathy must be developed. Of greater importance is the renal lesion of diabetes, for this is the most serious hazard for the young diabetic. Currently there is no conservative approach to its treatment, but preliminary experience with transplantation suggests that it may be successful in diabetes. Support for this should become available for Centers with large juvenile diabetic populations.

The pathogenesis of neuropathy will require intensive investigation. Current studies of the metabolism of neuronal tissue must be extended to find where the pathologic process fits in the general chemical derangements of diabetes. Orthopedic and physical medical and rehabilitative management can be advanced for the neurotrophic foot ulcer. The neurogenic bladder must be relieved, and corrective if not preventive approaches be developed for diarrhea, ataxia, and the peripheral somatic neuronal syndromes.

The complications of pregnancy and the newborn of the diabetic mother have been lessened considerably by more exact treatment of diabetes, accurate time of delivery, and in particular, by marked increase in the skills of newborn care. Nevertheless, 5 to 10 percent of cases result in perinatal loss. Research into the mechanisms of premature delivery, stillbirth, and the respiratory distress syndromes are desperately needed. In addition, a more intensive search should be made for the gestational diabetic, for she well may represent the commonly occurring female-middle-aged, stable-type diabetic.

There has been extensive controversy as to whether the complications result from insulin

failure and its consequences, whether they develop apart from inadequate insulin effect, or whether there are combinations thereon. Many observers believe from clinical observation that patients with more complete normalization of the blood sugar have fewer complications. Induced diabetes in animals has produced small blood vessel lesions resembling human diabetic microangiopathy. Furthermore, enzyme activity accounting for thickened glomerular basement membrane in alloxan diabetic rats has been defined. On the other hand, no satisfactory clinical trials demonstrating that control of diabetes diminishes the risk of vascular complications have been made. Also, it is not certain if the experimental vascular lesions in animals are the equivalent of those in man. Finally, microangiopathy has been found without demonstrable insulin failure at the time.

Many treatment approaches with dietary control and insulin adjustment have been developed in the hope of ameliorating and even correcting some of the long-term complications in diabetes. But nothing has so far been successfully applied in this country. Even if it is established that insulin failure and uncontrolled diabetes lies at the base of complications, it is highly unlikely that satisfactory correction of the intermediary derangements can be brought about with present-day methods.

Current studies at the animal level indicate that diabetes can be relieved in the experimentally induced diabetic model. Investigations on isolation, preservation, and proper site of implantation of islets in primates would be the next logical step, followed by observations on the course of the recipient in regard to carcinogenic and rejection risks. If these studies would be accomplished, a prospective clinical trial could be mounted in man to determine the effects on the course of vascular disease.

Diabetes mellitus is one of the most complex of medical states. Insulin deficiency is the major known abnormality, but other hormonal impairment undefined as yet may be concerned. Indeed, genetic input may be more influential than realized. Accordingly, it is difficult to judge just what areas would be most profitable to pursue at this stage, since many are hindered by limitations in methodology. Nevertheless, investigations in the mechanisms of insulin failure, the application of implanted pancreases, and clarification of altered tissue metabolism would seem profitable. The impact on society of diabetes and its disease consequences is sufficiently great to warrant a marked increase in efforts to alleviate its public health problem.

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3

ECONOMIC IMPACT OF DIABETES

Paul S. Entmacher

It is extremely difficult to estimate the full economic impact of diabetes, and the data that follow must be considered as significantly underestimating the situation. In developing the cost estimates relating to both morbidity and mortality, only statistics reported for diabetes as a primary cause were considered. Due to the nature of the disease, however, many of the complications, such as blindness and vascular disease, may lead to disability (and in the case of vascular disease to death), and the complication will be listed as causative with no reference to diabetes. Also, the impact that diabetes has as a contributing factor leading to disability or death from unrelated diseases cannot be measured. In addition, it must be remembered that the economic costs are derived from imprecise estimates. Despite these limitations, the cost estimates are of value in depicting the enormous impact that diabetes has on our economy.

The methodology used to develop the cost estimates has been described by the National Heart and Lung Institute Task Force on Arteriosclerosis and their Task Force on Respiratory Diseases. In aggregate, the total economic cost in 1973 due to diabetes was approximately four billion dollars. This figure is arrived at by combining the following:

1. *Direct costs due to illness:* Expenditures for prevention, detection, treatment, rehabilitation, research, training and capital investment in medical facilities. In terms of types of services or object of medical expenditure, direct costs include amounts spent for hospital and nursing home care, physicians' and other medical professional services, drugs, medical supplies, research, training and other nonpersonal services, according to D. P. Rice in Health Economics Series No. 6, 1966.
2. *Indirect costs due to morbidity:* Lost earnings associated with man-years lost to productivity due to illness and disability.
3. *Indirect costs due to mortality:* Discounted present value of lifetime earnings of persons who died.

As shown in Table 1, the total estimated cost resulting from illness and mortality due to diabetes in 1973 was 4.015 billion dollars. The total cost attributed to morbidity was 2.630 billion dollars with about 1.7 billion dollars due to direct costs and almost one billion dollars due to indirect costs representing 112,000 man-years lost from work. The costs due to mortality were 1.385 billion dollars, representing the present value of the remaining earnings of the 38,208 persons who died from diabetes in 1973.

Statistics dating back to 1973 are used in this presentation because that is the latest year for which data are available. It is apparent that this is another cause for understatement of the costs because there has been significant inflation in the intervening two years. An adjustment can be made for the inflationary factor in the following manner. From May 1973 to May 1975, there was a 22.1 percent increase in the medical care component of the Consumer Price Index. This increases the direct morbidity costs from 1.65 billion dollars to 2.015 billion dollars. During the same period the average hourly earnings of production workers and nonsupervisory employees in

the private economy rose 15.5 percent. This increases the indirect morbidity costs from 980 million dollars to 1.132 billion dollars, and the costs attributed to mortality from 1.385 billion dollars to 1.6 billion dollars. Therefore, when inflation is taken into account, the total estimated costs of morbidity and mortality due to diabetes increases from four billion dollars in 1973 to 4.747 billion dollars in 1975.

TABLE 1. Estimated Economic Costs of Morbidity and Mortality, Number of Deaths, and Man-Years Lost Due to Diabetes, United States, 1973.

Costs (millions of dollars)	
Total	$ 4,015
Morbidity	2,630
Direct	1,650
Indirect	980
Mortality	1,385
Number of Deaths	38,208
Man-Years Lost (thousands)	112

Source: Tables 2, 4, and 6.

MORBIDITY

Direct Costs

Estimated direct costs of morbidity due to diabetes in 1973 by type of expenditure are shown in Table 2. The expenditures due to diabetes for 1) hospital care, 2) physicians' services, 3) drugs, 4) nursing home care, and 5) medical professional services other than by physicians and dentists, are derived from the aggregate national health expenditure for each of the five categories by allocating a certain percentage of the total cost as being due to diabetes. The aggregate national health expenditures by type of expenditure are prepared by the Social Security Administration, and those for 1973 are shown in Table 3.

TABLE 2. Estimated Direct Costs of Morbidity Due to Diabetes by Type of Expenditure, United States, 1973.[1]

Type of Expenditure	Amount (in millions)
Total	$1,650
Hospital Care	800[2]
Physicians' Services	400[3]
Drugs	225[4]
Nursing Home Care	185
Other Medical Professional Services	40

[1]Excludes expenditures for dentists' services, eyeglasses and appliances, prepayment and administration, government and other health services, research and medical facilities construction.

[2]Based on days of care in short-stay hospitals.

[3]Cost of patient visits to physicians.

[4]Cost of patient visits to physicians for which drugs were prescribed.

Source: Estimated by the Statistical Bureau of the Metropolitan Life Insurance Company.

TABLE 3. Aggregate National Health Expenditure by Type of Expenditure, United States, 1973.

Type of Expenditure	Amount (in millions)
Total	$99,069
Health Services and Supplies	92,327
Hospital care	38,270
Physicians' services	18,200
Drugs and drug sundries	9,300
Nursing home care	7,050
Other professional services	1,900
Dentists' services	5,970
Eyeglasses and appliances	2,091
Prepayment and administration	3,998
Government public health	1,905
Other health services	3,643
Research and Medical Facilities Construction	6,742
Research	2,484
Construction	4,258

Source: Social Security Administration: Research and Statistics Note No. 1-75, Table 2, National Health Expenditures, Calendar Years 1929-73, February 19, 1975.

Hospital Care: Care of diabetic patients for short-term hospitalizations cost 800 million dollars in 1973, representing over 5.2 million days of hospitalization. The total number of days of inpatient hospital care was obtained from the Hospital Discharge Survey which was published in Monthly Vital Statistics Report, and the number due to diabetes was estimated from data supplied by the Hospital Discharge Survey Branch of the National Center for Health Statistics. The Hospital Discharge Survey is a continuing probability sample of all short-term hospitals in the nation excluding military and Veterans Administration hospitals and hospital units of institutions. By definition, short-term means under 30 days average stay per discharge. Patients were tabulated according to the diagnosis listed first on the summary sheet of the patients' records. The percentage of total days of care provided to diabetic patients was applied to the amount of hospital care expenditures for all illnesses (from Table 3) to yield the estimated amount of hospital care expenditures due to diabetes. This method assumes that the estimated expenditures for hospital care are distributed by diagnosis similar to the distribution of hospital days of care by first-listed diagnosis.

Physicians' Services: The estimated cost of physicians' treatment of diabetic patients in 1973 was 400 million dollars. This represents about 34 million visits by physicians. The National Disease and Therapeutic Index estimated the total number of patient visits to physicians in private practice and the number due to diabetes. Its estimate is based on a continuing study of private medical practice in the United States in which data are obtained from a representative sample of physicians who report case history information on private patients seen over a period of time. The percentage of the estimated total patient visits to physicians due to diabetes was applied to the total amount of expenditures for physicians' services (from Table 3) to yield the estimated amount of expenditures due to diabetes. The assumption is that the estimated expenditures for physicians' services are distributed by diagnosis similar to the distribution of patient visits to physicians by diagnosis.

Drugs: In 1973 the expenditure for drugs by diabetic patients was 225 million dollars. Estimates of patient visits to physicians in which medication was prescribed were reported by the National Disease and Therapeutic Index for the year 1970. The percentage of total visits in which medication was prescribed that was due to diabetes was then applied to the total amount of expenditures for drugs in 1973 to yield the estimated amount of expenditures due to diabetes.

Nursing Home Care: Nursing home expenditures in 1973 for the care of diabetic patients was 185 million dollars. A survey by the National Center for Health Statistics showed the prevalence and distribution of chronic conditions among residents of nursing and personal care homes. The estimated percentage of diabetes among these residents was applied to the total expenditures for nursing home care in 1973, and a crude total expenditure for nursing home care for diabetics was computed.

Other Medical Professional Services: Expenditures for other medical professional services rendered to diabetics in 1973 were 40 million dollars. These expenses include the cost of services provided by other than physicians and dentists. The portion of costs in this category that was due to diabetes had to be estimated by applying the percentage of the combined expenditures for hospital care, physicians' services, drugs and nursing home care to the total expenditures for other medical professional services.

Indirect Costs

Productivity loss because of illness and disability from diabetes in 1973 cost 980 million dollars. This is shown in Table 4. The productivity loss is measured in terms of man-years of work lost, and the economic value of the associated lost earnings. In 1973 diabetes caused 111,600 man-years lost from work. The estimated losses were computed for three population groups.

TABLE 4. Estimated Man-Years Lost from Work and Keeping House and Indirect Costs of Morbidity Due to Diabetes, United States, 1973.

	Man-Years Lost (in thousands)	Amount (in millions)
Total	111.6	980
Currently employed persons	16.1	160
Women not in labor force who keep house	40.0	170
Persons unable to work	55.5	650

Source: Computed by the Statistical Bureau of the Metropolitan Life Insurance Company.

Currently Employed Population: Among currently employed persons with diabetes there were 16,100 man-years lost from work with an associated loss of earnings of 160 million dollars. For the calendar year 1973 the estimated number of days lost from work associated with diabetes was furnished by the Division of Health Interview Statistics of the National Center for Health Statistics. This is a continuing survey interviewing a representative sample of the nation's civilian noninstitutionalized population to determine the presence of acute and chronic conditions and the number of days of disability from these conditions. The estimated work loss days were divided by 245, the approximate number of working days in a year, to obtain the number of man-years lost. Mean annual earnings were applied to the man-years lost to obtain the indirect costs for the currently employed population prevented from working due

to diabetes. The mean earnings for 1973 were estimated by increasing the estimates by the National Heart and Lung Institute Task Force on Arteriosclerosis for 1964 by the ratio of the median income of men and women in 1973 to the corresponding median income in 1964 reported by the United States Department of Commerce, Bureau of Census. Average earnings for persons 14-44 years of age were assumed to be approximately the same as for persons aged 17-44.

Housewives' Services: The estimated number of man-years lost from housework among diabetic women who keep house but who are not in the labor force was 40,000. This resulted in lost earnings of 170 million dollars. Housewives' services are estimated at the average earnings in 1973 for a domestic worker. This imputed value is clearly on the low side for it makes no allowance for the housewife's longer work week and takes no account of the size of the household cared for. Although the economic contributions of housewives are not included in the national income accounts, according to the President's Committee on Heart Disease, Cancer, and Stroke, omitting the value of their services in the calculation of indirect costs distorts comparisons of costs among illnesses with different distributions by sex. Data from the National Health Interview Survey provided the estimated number of days of disability in bed due to diabetes for women, a figure which combines homemaker disability and the disability of women in the labor force. The number of work-loss days was estimated by applying the proportion of all labor force members who are women to the total number of work-loss days for men and women combined obtained from the National Health Interview Survey. The number of female work-loss days was deducted from the bed disability days, and the resulting figure (adjusted bed disability days) was divided by 365 to obtain the estimated number of bed disability years attributed to diabetes. Multiplying this figure by the percentage of women not in the labor force who keep house yields an estimate of the man-years lost for women who keep house. The imputed value of these man-years lost is obtained by multiplying the man-years lost by the average wage of a domestic worker in 1973 which was $4,190 per year, according to the United States Department of Commerce in a Survey of Current Business, 1974.

Persons Unable To Work: Among persons unable to work there were 55,500 man-years of work loss attributed to diabetes. This resulted in a production loss of 650 million dollars. The basic data were obtained from the Social Security Office of Research and Statistics. The proportion of total benefit awards during 1972 that were granted to applicants with diabetes was applied to the total number of disability beneficiaries at the end of 1973 to provide a conservative estimate of the man-years lost by those unable to work at all due to diabetes. Mean annual earnings were then applied to man-years lost to obtain the indirect costs.

MORTALITY

The indirect economic cost of mortality for diabetes to the nation is measured in terms of the discounted present value of lifetime earnings of persons who died. In 1973 this amounted to 1.385 billion dollars. The estimate was obtained by multiplying the 38,208 deaths from diabetes which occurred in 1973 by the expected value of future earnings with age and sex taken into account. Future earnings were discounted at 6 percent to take into account interest that could be earned in the interim with an adjustment made for increased productivity. The estimated present value of lifetime earnings by age and sex are shown in Table 5. These earnings were computed from discounted earnings available for 1964 from the National Heart and Lung Institute Task Force

on Arteriosclerosis which were increased by the ratio of the median income of men and women in 1973 to the median income in 1964, as reported by the United States Department of Commerce, Bureau of Census.

TABLE 5. Estimated Present Value of Lifetime Earnings by Age and Sex, United States, 1973.

Age	Male	Female
Under 1	$55,208	$33,152
1- 4	63,054	38,046
5- 9	84,667	50,618
10-14	113,526	67,842
15-19	146,759	84,398
20-24	174,753	92,081
25-29	186,391	92,141
30-34	183,246	90,677
35-39	171,424	87,460
40-44	152,541	82,974
45-49	127,602	76,232
50-54	100,337	68,017
55-59	73,281	59,415
60-64	43,097	49,308
65-69	21,926	39,993
70-74	18,263	32,580
75-79	13,981	23,958
80-84	8,119	13,004
85 and over	1,332	2,109

Source: Estimated by the Statistical Bureau of the Metropolitan Life Insurance Company

The number of deaths from diabetes in 1973 is shown in Table 6. Also shown in this table are the indirect costs by age and sex which were derived by multiplying the number of deaths by the discounted earnings shown in Table 5. The total indirect costs due to mortality were 1.385 billion dollars with approximately 643 million dollars due to mortality among males and approximately 741 million dollars due to female mortality.

TABLE 6. Number of Deaths and Estimated Indirect Costs Due to Diabetes, United States, 1973

Age	Number of Deaths			Indirect Costs (in thousands)		
	Total	Male	Female	Total	Male	Female
Total	38,208	15,669	22,539	$1,384,639	$643,143	$741,496
Under 1	16	11	5	773	607	166
1- 4	14	9	5	757	567	190
5- 9	22	8	14	1,386	677	709
10-14	52	19	33	4,396	2,157	2,239
15-19	66	33	33	7,628	4,843	2,785
20-24	126	64	62	16,893	11,184	5,709
25-29	243	143	100	35,868	26,654	9,214
30-34	320	171	149	44,846	31,335	13,511
35-39	427	247	180	58,085	42,342	15,743
40-44	616	330	286	74,070	50,339	23,731
45-49	1,046	533	513	107,119	68,012	39,107
50-54	1,752	892	860	147,996	89,501	58,495
55-59	2,715	1,316	1,399	179,560	96,438	83,122
60-64	3,847	1,749	2,098	178,825	75,377	103,448
65-69	5,139	2,256	2,883	164,765	49,465	115,300
70-74	6,146	2,422	3,724	165,561	44,233	121,328
75-79	6,347	2,342	4,005	128,696	32,744	95,952
80-84	5,303	1,843	3,460	59,957	14,963	44,994
85 and over	4,008	1,280	2,728	7,458	1,705	5,753

Source: Deaths from the National Center for Health Statistics, Division of Vital Statistics, and Table 5.

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4

IMPROVING THE ORGANIZATION OF CARE FOR THE CHRONICALLY ILL

Leona V. Miller, Jack Goldstein, and John W. Runyan, Jr.

BACKGROUND

Nearly half the population of the United States suffers from one or more chronic disease (8). This segment of the population is estimated to account for 80 percent of all health care services delivered. If health care costs are to be reduced in any meaningful way, the major stress will have to be in improving health care delivery to the chronically ill. New methods of health care delivery will have to be developed. Pilot programs with representative chronically ill groups will need to be evaluated before widespread changes can be carried out with the total chronically ill population.

Perhaps one of the most representative chronically ill groups are diabetics, as there is hardly an organ system which diabetes may not affect. Organizing systems to deliver care to diabetic patients incorporate, in microcosm, all the problems found in developing systems to care for chronically ill patients in general. For example, the vast majority of admissions to the Diabetes Service at the Los Angeles County University of Southern California Medical Center are not primarily for problems of diabetes management, but rather for other chronic conditions often found among diabetics, such as heart disease, renal disease, pulmonary disease, and neurological problems among others.

The probability that a diabetic will be hospitalized in any one year is 67 percent. The diabetic spends an average of 5.4 days in the hospital each year and loses an average of 15.4 work days per year. Contrast these statistics with that of the nondiabetic. The likelihood that he will be hospitalized is but 12 percent annually. He spends an average of one day in the hospital each year and loses an average of only 5.7 work days (U.S. National Center for Health Statistics). The inability of the health system to respond fully to the diabetic patient's needs affects his life in a variety of ways. Employment may present a challenge to him. Insurance companies, although more liberal in recent years, do exclude health benefits to diabetics (and a variety of other chronic disease groups) under a "preexisting condition" clause which reduces benefits to diabetic subscribers for the first 2 years of a health insurance policy and in many cases provides inadequate benefits thereafter.

The result is that diabetes is not merely a medical diagnosis, but it also inhibits the diabetic in his ability to obtain a better job and often jeopardizes his financial position. The basic problem is to find a way of delivering care to the chronically ill population so that quality of continuing care is more accessible, which in turn may reduce both morbidity and the costs of providing their care. Once a solution is designed for this basic problem, the effect of the collateral problems will be minimized.

CURRENT STATE OF KNOWLEDGE

A number of investigators have acknowledged that the prime utilizers of health care services in this country and elsewhere, are the chronically ill (1). Little, however, has been accomplished in designing health systems to deal specifically with this group. Because of the

economic and political orientation, health delivery systems have developed in a fragmented manner which often do not necessarily meet the needs of the chronically ill. However, there are two models which attempt to deliver care to the chronically ill and the diabetic patient in an organized manner.

The first of these was initiated over 11 years ago in Memphis and Shelby counties (7,6). From the start in 1963, this program has been a joint effort of the local Health Department, the City of Memphis Hospital, and the University of Tennessee College of Medicine.

This program's concept of the care of patients with chronic diseases includes two essential ingredients: (a) geographic decentralization of the health care facilities for ready accessibility to the patients, and (b) the use of specially trained nurses guided by protocols with physician and medical center back-up for patient management to these facilities.

There are currently 18 decentralized clinics. Ten are in urban areas and eight in the semi-rural areas; these serve a population of 225,000 out of the county's population of 750,000. Four of these are multipurpose neighborhood health centers, while the remainder are small installations, called "satellites," located in community centers, public schools, and housing developments. A mobile bus extends medical care to more inaccessible places and remains for the entire day in each of five locations. In 1973 over 40,000 visits were made to these facilities by the more than 9,000 chronic disease patients who are in the program. This includes approximately 3,500 diabetic patients. The equivalent of 20 full-time, specially trained nurses provided the maintenance care. The low missed-appointment rate of 5 to 8 percent is a reflection of the easy accessibility of the facilities, the devotion of the nurses administering the care, the excellence of the follow-up system, and the reduced costs to the patients. It contrasts to the 25-50 percent missed-appointment rate in outpatient clinics of the City Hospital.

The flow of the patients from the decentralized locations to the secondary care facilities and back again is expedited by a liaison service. This service is composed of two nurses, one representing the hospital and the other the Health Department. The consulting physicians in the University Medical Center serve as the medical back-up for the liaison nurses and the nurses in the neighborhood clinics and home. These consulting physicians have developed the medical protocols and policies for the program with the cooperation of the nurses delivering the direct care.

Some observations have been made on a subset of the chronic disease population involving 1,006 patients transferred from the city hospital to the decentralized facilities for maintenance care (6). In this subset, 555 patients had diabetes mellitus. Ambulant care before transfer was primarily in the hospital clinics and in the hospital emergency ward with a combined total of 8,124 visits per 1000 patients per year to these facilities. After transfer there was a shift, as expected, to the decentralized clinics. Combined outpatient department and emergency ward visits fell to 2,585. Some 166 home visits were also made on this subset, an important index of the continuum of care provided and the increased patient contact with health professionals after transfer.

The cost of ambulant care received in the decentralized locations was one-fifth the cost of comparable care rendered in medical center facilities. A visit to a neighborhood clinic cost $4 compared to $20 for a hospital outpatient visit and $35 for an emergency ward visit.

When the pattern of hospitalization for the 2-year period before and after transfer was

examined for those with diabetes, a 49 percent reduction in hospital days and a 42 percent reduction in hospital discharges were found (Table 1). The decrease in the hospital days for diabetic acidosis, serious infections, and problems related to peripheral vascular disease, the preventable complications of diabetes, accounted for the larger share of these reductions. The hospital days required for the renal and vascular complications of diabetes showed, on the other hand, a slight increase indicating the limitations of continuing care in altering the course of these progressive, degenerative complications. The increase of 20 percent in this group was due primarily to readmission to patients with advanced and progressive renal insufficiency frequently with multiple complications.

TABLE 1. Memphis and Shelby County Continuing Care Program.

Hospital Utilization[1]
Diabetic Patients

	Per Thousand Patients Per Year				
		Hospital Days			
	Hospital Discharges	Medical	Surgical	Total	Average Per Admission
Before Transfer[2]	211	1297	2022	3319	15.7
After Transfer[2]	123	746	934	1680	13.6
Reduction	88 (42%)	551	1088	1639 (49%)	

[1]Data based on a survey of 555 diabetic patients transferred to decentralized facilities for continuing care between September 1, 1969 and August 31, 1970.

[2]Data based upon hospitalizations two years before transfer and two years after transfer.

The 49 percent decrease in the total costs of the medical care provided in this program as compared to the costs before transfer is due to the use of the less expensive ambulant services and the decrease in hospitalizations (Table 2).

TABLE 2. Memphis and Shelby County Continuing Care Program.

Medical Care Costs Estimates

	Per 1000 patients per year	
	Before Transfer	After Transfer
Ambulant and Home Care[2]		
Decentralized Clinic Visits @ $4		$ 24,732
Hospital Out-patient Visits @ $20	$ 157,380	50,820
Emergency Ward Visit @ $35	9,025	1,540
Home Visit @ $12	-	1,992
Laboratory @ $24.96	24,960[1]	24,960
Medications @ $54.75	54,750[1]	54,750
	$ 246,115	$158,794
Hospital Care[3]	$ 331,900	$168,000
Total Costs	$ 578,015	$326,794

[1]Exact figures unobtainable.

[2]Based upon data from 1006 chronic disease patients including 555 diabetic patients before and after transfer to decentralized facilities.

[3]Based upon data from 555 diabetics before and after transfer to decentralized facilities.

Past studies using health aides, medical students, and nurses, both in the clinic and in the home, indicate favorable acceptance by the overwhelming majority of the patients of this kind of personnel acting in a professional role. In this study, 98 percent of the 662 patients interviewed preferred this care to other choices. Sixty-four percent considered their general state of health had improved since transfer; only 5 percent felt that their health status had worsened.

The effectiveness of the program for continuing care of patients with diabetes mellitus and chronic diseases is reflected in (a) the highly significant reduction in visits to the clinics and emergency room, (b) the reduction of admissions to the wards of the City Hospital for the preventable complications of diabetes, (c) the approximately 44 percent reduction in cost of continuing care as compared to the cost by conventional methods in use in the same community, and (d) the enthusiastic acceptance of the system by the patients themselves.

While the access to health care has been improved and costs reduced in the Memphis program, financial resources are insufficient to make this system more responsive to patients' needs. A patient needing medical advice or care when the neighborhood health clinic is closed can satisfy his need only by appearing at the emergency room. This tends to disrupt the continuity of care so necessary in caring for the chronically ill. The system in use on the Diabetes Service at the Los Angeles County--University of Southern California Medical Center (LAC-USC) has attempted to solve this problem in another manner. Here too, Nurse Clinical Practitioners are employed to provide care to patients. However, this care is provided within the confines of the Medical Center. This tends to make the care somewhat less accessible than that afforded by the Memphis group. However it tries to improve continuity of care by:

1. furnishing a 7-day-a-week, 24-hour hotline telephone service for its patients;
2. offering medical service at all times either on a scheduled basis in the outpatient department or on an unscheduled basis in a special walk-in clinic maintained by the Diabetes Service. Patients coming to this clinic are evaluated and either cared for on an ambulatory basis, or if diagnostic tests warrent, admitted. Unnecessary admissions have been virtually eliminated by the patients being screened in this Diabetic Walk-In Clinic.
3. maintaining up-to-date records of all 8,000 patients followed by the Diabetes Service in specially formulated, condensed ledgers retained on the Diabetes Service. These ledgers are part of an overall information system that not only permits the rapid information retrieval necessary for providing patient care but also permits computerized review of each physician's performance in health care delivery, the performance of the entire Diabetes Service, as well as epidemiological studies of the inpatient population.

A more complete description of the details of operation of this system is contained in the literature (4). The effect of this system on reducing costs has been extremely impressive (Table 3). Since this system has been in operation, the average number of days spent by a clinic patient in the hospital annually has decreased from 5.6 to 1.7. This figure is not significantly different from the 1-day average of a randomly ill population. It has meant that the probability of a clinic patient being hospitalized in any one year has decreased from .67 to .21. Total costs of hospitalization during the system's first full year of operation of the Diabetes Service have been reduced by over $3 million. This reduction of hospitalization has resulted in annual savings of $588 per clinic patient.

TABLE 3. Los Angeles County Hospital.[2]

Diabetic Hospital Care	Per Year		
	Hospital Discharges per 1000 pts.	Hospital Days Average per pt.	Hospital Cost per pt.
1968	670	5.60 (5.4)[1]	$ 840
1970[2]	208	1.74	$ 261
Reduction	462 (70%)	3.86 (70%)	$ 579

[1]National average.

[2]After introduction of communication and information system to improve patient education.

Unfortunately, health systems for the chronically ill, such as that in Memphis and Shelby counties and the Los Angeles County--University of Southern California Medical Center, are not common. Incentives to increase the efficiency and reduce the cost of health care delivery are lacking.

Some argue that a system of prepaid health care would change these incentives and would stress efficiency and cost control. However, it does not necessarily follow that the health status of the subscribers would also be properly maintained, so any such system would need appropriate surveillance. No single change in the financing of health care can by itself improve health care delivery, control costs, or help in the redistribution of health professionals and facilities. It is the health care organization that is deficient, and this is where research is needed if the system is to become more appropriate and responsive to the needs of our population.

Research is required on how best to focus resources on needs and on how to provide the appropriate financial and other incentives necessary. If incentives are appropriate, income to the health system would be based not on the output of the system, but on the effectiveness of the system. It would be judged on how well a system maintained the health of its population relative to standards established for that population based on disease distribution, age, sex, education, financial status, and other variables that may affect health status.

For this to be accomplished, a major development effort has to be initiated in improving access to information about the health system. Research into understanding the complexity of the present health system and improving it is impeded by an obsolete information system relying principally on the medical record. Numerous articles have been printed on the deficiencies of the medical record (2,3,5,9), but insufficient stress has been placed on the inimical effect this type of documentation has on: delivering care to the patient; monitoring professional performance; maintaining organizational efficiency; and controlling costs.

The use of medical records as the only source of information is effective primarily for the solo practitioner and small groups. It was never designed to be used by large organizations such as hospitals where the need for information contained in the medical record is needed by many diverse users for different purposes, often at the same time. A more automated, better organized system is needed that can deal with large data bases and deliver information more readily to the physician, his chief, the hospital administrator, the planner and the researcher.

To achieve this, research is needed in design and development of improved information systems to capture, store, retrieve, and manipulate data. A new type of health professional with strong technical background will have to be developed who can not only communicate with other health professionals but who can also use mature judgment in designing information systems much as an architect does in building homes.

With the development of new information systems, research into introducing new systems of health care delivery and evaluating them objectively can be expedited.

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THE COMPUTER IN THE MANAGEMENT OF DIABETES

Robert E. Bolinger

The rapid progress in the application of the computer to hospital financial accounting systems contrasts sharply with the relatively slow progress made in application of the computer to health care management. Many of the earlier attempts to apply the computer to health care management failed because of a lack of understanding between the data processing professionals and the health care professionals. A present more cautious attitude exists (3) which takes cognizance of three central problems. (a) A thorough analysis and understanding of the health care system must be feasible and occur before any attempt is made to apply the computer. (b) Facilities for on-line data storage must be extensive. Technology in this respect is advancing and promises relatively cheap on-line storage facilities. (c) Logical systems or software must be developed which readily handle clinical information. A number of systems of this type are currently being developed, such as HIPS and SETRAN (34), MUMPS (14), DATAFLOW (8), and others.

The characteristics of the computer which render it uniquely useful in health care management include its capacity to store large amounts of data; to perform mathematical computations on the data which reveal trends, means, and variability; to carry out reproducible logical sequences based on either discrete or continuous data; and to handle multifactorial problems which are common in the clinical setting. A major stricture often encountered in any computer application to health care has to do with the method of entering data, since a given clinical situation often presents with a large number of variables. Thus, the time required for a human to enter the condition of each clinical variable into a machine may nullify some of the advantages listed above.

Diabetes presents problems of management both as an acute disorder and as a chronic disorder, and it is in the latter situation where the potential of the computer to store large amounts of data and derive trends is particularly useful. Computer applications in the management of diabetes may be considered in three categories: record processing, simulation, and decision making at both the diagnostic and therapeutic levels.

RECORD PROCESSING

A familiar sight, particularly in a diabetic clinic, is a very thick chart written in a multitude of formats in which significant information is often buried in a myriad of apparently irrelevant data. A major thrust in health care research is directed at the development of technics to reduce these data to a form which may be stored in a computer system, retrieved therefrom in a usable format, and statistically processed. Four types of clinical data that are essential are the updated historical and physical examination information, laboratory data, and an outline of problems and treatment. Such information may be stored as purely text information in a form not unlike that found on a regular hospital chart or as coded information. The latter has obvious advantages since it requires far less storage than text information does and can be readily retrieved on the basis of code. It is possible by the use of some extensions of any of the standard medical terminology coding systems (ICDA, H-ICDA, CMT, SNOPS) to handle most of the information of the history, physical examination, and the problem list as coded information. Once

information becomes encoded, it becomes not only readily retrievable but also amenable to various forms of data processing for means, variabilities, and trends, so at the same time a data base for statistical and research purposes can be created. It is not surprising, in view of the many problems which are encountered in the long-term management of the diabetic patient, that there exists such a paucity of reliable statistical confirmation of supposedly accepted modes of practice.

Certain aspects of the medical history, particularly system review, past history, and family history are readily adaptable to computerized systems. Slack et al. (29) have initiated the work on computer-based histories, and a number of others have followed (7,16,22,25) The mode of entry of historical data may vary from a fixed set of questions which are asked the patient to a complicated set of branchings which, through direction from the computer carry the interrogative thread into the areas where emphasis is needed. Extensively branched histories have been developed by Weed et al. (34), by Gottlieb (13), and Grossman et al. (16). From the standpoint of the diabetic patient, an extensively branched history would yield the largest amount of information, but as a practical compromise, certainly, a detailed system review screen with the addition of specific questions highlighting the development of the diabetic process would suffice. An effective input technique for this procedure is some type of optical scanning method whereby the marks indicated by the patient on a questionnaire can be directly read into a machine system. This minimizes the use of the computer time and allows the patient to pace himself according to his own ability. The entry of physical examination data requires some form of physician input and may require provision for branching. This would be true in the case of the diabetic where particular emphasis would be needed on details about the skin, the retina and other features of the eye, the peripheral vascular system, the nervous system particularly related to diabetic neuropathy, etc. Although it might be argued that these features might be part of any physical examination, experience teaches us that they are frequently omitted. Therefore, the inclusion of these in some type of a structured physical examination, slanted particularly toward the diabetic patient, should be of particular advantage in obtaining a reliable data base with respect to these features.

The technology of the processing of laboratory data is much better developed and has indeed become a standard part of many health care systems throughout the country. The features of laboratory data processing which are of particular interest in the management of the diabetic patient, in addition to the simple storage and retrieval of the raw data itself, are the reduction of data and the derivation and computation of trends which can serve as essential guidelines (19) in the management of the patient either by the physician or by other computer programs. The storage and retrieval of problem lists (21,35)can be of particular importance in the management of long-term chronic illness such as diabetes, since it highlights the areas which need attention. The use of the computer in the storage of problem lists is particularly convenient because the lists can be much more easily updated and kept current. Furthermore, the problem list data can be coded according to standard coding procedures and therefore become readily retrievable for statistical purposes in the study of a diabetic population.

The storage and processing of treatment and other management procedures carried out in the patient are particularly important. The patient may be receiving treatment for several different problems besides the diabetes, and often the total management picture of the patient tends to be forgotten by a preoccupation with insulin dosage and medications and other procedures which may

actually be influencing the diabetic status. A readily retrievable display of all current treatment should be a particular advantage in the management of this type of patient as, also, should be a record of results of past medications. Trends in the slow panorama of events representing the development of diabetic complications, such as retinopathy (27), can be successfully followed by the computer.

Disease registry systems are particularly important in the management of chronic illnesses and many diabetic clinics have registry information over a long period of time. Such registry systems particularly permit the storage of prospective data, and in such notable instances as the UGDP (1970), the interdigitation of the computer facilities with a registry system provided a tool for the integration of a mass of information. In particular, registry information becomes a data bank which can be processed and reduced to computable forms and used in the on-line management of the diabetic patient by providing the current updated statistics and probabilities necessary to make decisions as described below. At the same time, if the registry system is interlocked with the on-line management system, it can be continually and regularly updated by the introduction of the coded information at each point in the patient's course.

The effectiveness of the computer-based record in the management of diabetes has been demonstrated by Thomas and Dobson (32) at the Hermann Hospital in Houston, Texas. A system of initial data base entry by punched cards is followed by updating the computer record at each subsequent visit. Summary printouts of the data in problem-oriented format are made available at each visit and included in the permanent record.

SIMULATION

A second area in which the computer has contributed to diabetic management is in the process of simulation. The complex system involved in the regulation of blood glucose can be simulated in the computer (6,23), thus providing a model for research on the development of automatic insulin release devices. Srinivasan et al. (31) developed, by the use of the computer, a model of differential equations to fit the experimental data derived from glucose changes. Grodsky et al. (15) published data using the perfused pancreas and studied insulin secretion, thereby isolating data from a single component of the system. Gatewood et al. (9) and Gatewood et al. (10) proposed a simplified model for the regulation of glucose in diabetics. They were able to show with the computer that most of the variations in the blood sugar could be explained by the insulin level alone.

DECISION MAKING

Clinical decision making has been studied in both the diagnostic and the therapeutic setting. From the diagnostic standpoint, the computer may be valuable first in examining the data from a large patient population and establishing the criteria for the detection and diagnosis of diabetes. This has been extensively used in multiphasic health screening. Hecker (17) noted that 10 percent of the population have 50 percent of the disease and emphasized the importance of setting up specific markers for the detection of these individuals in a screen. Gleser and Collen (11) have developed a model for selecting the significant variable to be tested for in multiphasic health screening and diabetes detection. This is particularly important in diabetes detection where historical factors, nutritional factors, and certain laboratory data may be a clue before specific symptoms of the disease appear.

Karp et al. (24) and Silcock et al. (28) used a computer to analyze the glucose insulin curves during the glucose tolerance test to identify the parameters that could differentiate accurately between normal and abnormal groups. In connection with the multiphasic health screening, Himbert et al. (18) and Bastenie et al. (2) used the computer to study the importance of diabetes among the risk factors encountered in the screening of cardiovascular disease.

The use of the computer as a component of the medical management process is even less developed. Isolated efforts in various fields have applied the computer in part to management decisions in respiratory disease (26) and acid base balance (12). The process of medical decision broadly encompasses--first, the collection of data; second, the processing of the data to arrive at a diagnosis; and finally, the prescription of a plan of action designed to correct the problem. In practice these steps are often telescoped and tend more to approximate a guidance system in which data collection serves as a continuous feedback for modification of diagnosis and for the evolution of a plan of action based on the outcome of the previous action. The central controlling or stopping function for this process is the solution of the patient's problems. Often in clinical practice there may be among the alternatives decisions which are potentially hazardous or even fatal to the patient. Therefore, in each step of a decision process, the logic must be so structured that hazardous outcomes are given a probablility of zero. A large part of the education of a physician is spent in developing skill in this process and, in the case of diabetes, his activities often assume a repetitive pattern, some aspects of which can be easily assumed by a computer. In this clinic Bolinger et al. (4), Bolinger et al. (5) have tested the feasibility of computerizing some of the aspects of the management of diabetes. In this study it was assumed that the diagnosis of diabetes had been established. In the diabetic clinic of the Kansas University Medical Center, the variables which are usually queried in the day-to-day management of the diabetic patient were isolated and a system devised whereby these variables could be entered into the system through a teleterminal and submitted to a series of Boolian operators, leading to the prescription of insulin and diet, as well as to other warning flags in patient management. Provision was made for prescription of insulin dosage in short acting, intermediate acting, and long acting, and for insulin administration at morning, noon, evening, and midnight. Sox et al. (30) have emphasized the possibility of broadening the use of physician assistants in the management of disease by the use of computerized algorithms. It had been possible in his studies to essentially relieve the physician's assistant of any complicated decision process and utilize him strictly as the data-gathering device and as an instrument in prescribing, after the logical work was accomplished by the computer. In Phase II of the diabetes management project at the Kansas University Medical Center, a nurse clinician was used in this capacity, supported by the logical processing of the computer as an on-line decision-making resource, utilizing information which she gathered at that time. The output of the computer was compared with the recommendations of a diabetologist, and the correlation between the computer recommendations and those of the diabetologist for insulin dosage are shown in Table 1. The three phases of the study indicated in the table are based upon versions of the program which in Phase I was overly simplified and therefore not very flexible. Phase II was characterized by the intervention of the nurse clinician, and Phase III represented a final revision of the program, including inpatients. The correlations between the computer recommendations and those of the physician are generally satisfactory, and it is thought at the present that hazardous-type decisions which might lead to

serious hypoglycemia or a diabetic ketoacidosis have been eliminated. The complexity of the decision process as monitored by a trace program was reflected in the number of times the program had to branch and was for insulin-taking diabetics and noninsulin requiring diabetics, 14 and 7 times respectively, indicating the greater simplicity of the logical process in the noninsulin-taking diabetic. With more data of this type, it should be possible to decide upon the level of complexity of a decision which paramedical personnel might be able to handle without the support of a computerized algorithm. This type of analysis of a computerized system which tends to quantitate the complexity of a decision has broad implications in the allocation of responsibility in the clinical process.

TABLE 1. Correlation of Insulin Recommended by Computer vs. Physician

Insulin	r	P	
	Phase I		(N = 14)
AM Lente	.949	<.001	
PM Lente	.000	>.05	
AM Regular	.686	<.01	
PM Regular	.000	>.05	
Total	.983	<.001	
	Phase II		(N = 49)
AM Lente	.918	<.001	
PM Lente	.790	<.001	
AM Regular	.955	<.001	
PM Regular	.649	<.001	
Total	.864	<.001	
	Phase III		(N = 99)
AM Lente	.906	<.001	
PM Lente	.831	<.001	
AM Regular	.878	<.001	
PM Regular	.777	<.001	
Total	.928	<.001	

r = Coefficient of correction
P = Statistical probability using Students t test
N = Number of cases

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The use of a logical model as described by Bartholomay (1) does not appear necessary at the level of resolution ordinarily encountered in diabetic management. Instead, a system of sorting, as shown in Fig. 1, was used with a system of filters which assigned patients either to one group or another at each logical decision point. The model also provided for transfer of patients from the insulin-taking to the noninsulin-taking group and vice versa, depending upon changing conditions.

It is concluded that this type of computer program did demonstrate the feasibility of relatively complicated algorithms in the management of some phases of diabetes. It is also concluded that such a system could stand alone without fairly extensive systems of a similar type handling many of the common intercurrent diseases which occur in connection with diabetes, such as hypertension, heart failure, infections, and some of the other endocrinopathies.

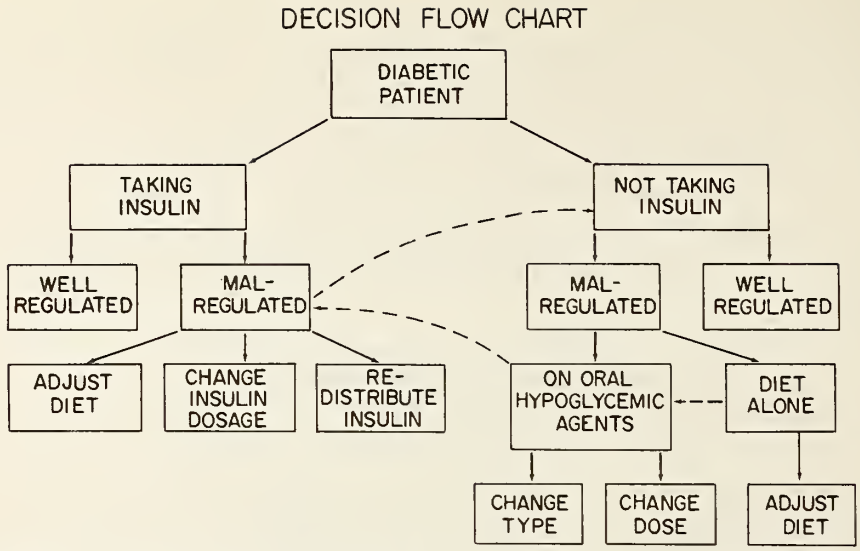

FIGURE 1. The process of grouping which is carried out through the algorithm of the program is shown. At the top, a heterogeneous group of diabetics is postulated and, at the bottom, the sorting of these patients into treatment groups results from the sorting.

RELATION TO THE HEALTH CARE SYSTEMS

An idealized summary of the possibilities for computer assistance in the management of diabetes is shown in Fig. 2. The proposed allocation of functions consigns activities to five areas; i.e., the physician, the paramedic, the man-machine interface, the computer, and its

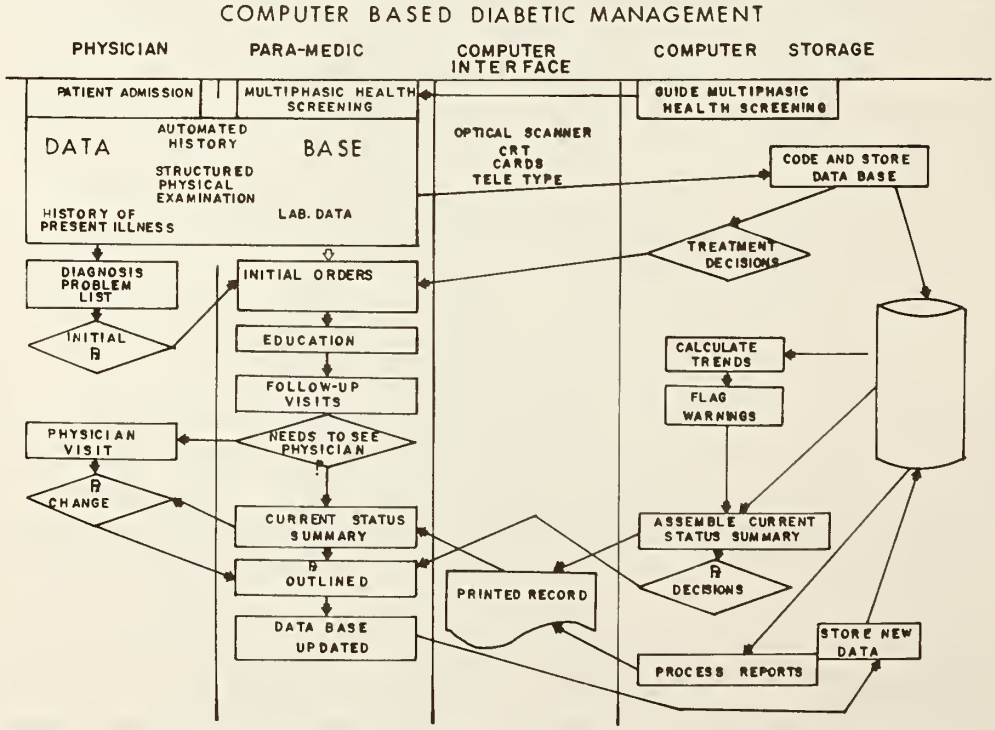

FIGURE 2. A proposed allocation of the informational work is shown for various phases of the management of the diabetic patient. Areas of allocation include physician, paramedical personnel, computer-man interface, computer and information storage.

slow and fast storage capabilities. The frequent exchange of information between these different components emphasizes the necessity of an integrated plan of development and design. The feasibility of the application of each of the components of this scheme have been demonstrated, but in no place is it totally operative. The greatest progress in terms of a functioning system is described by Thomas and Dobson (32). An important area for further research is indicated to determine the functioning specifications of each of these components needed to obtain optimal integrated performance. Any such testing should be undertaken only with a clear definition of the response variables, such that a clear distinction between present and proposed systems can be demonstrated. The response variables to be evaluated should include utilization of physician time vs. paramedic time, convenience to patients, impact on the patients' attitudes regarding the patient-physician relationship, effect on long-term cooperation by the patient, effect on patient education about his disease, long-term morbidity and mortality statistics particularly emphasizing the role of the computer in possible prevention of diabetic complications, costs and convenience of various options available at the man-machine interface, computer hardware and mass data storage systems. With respect to data storage, agreement is needed among authorities in the field of diabetes as to exactly what data should be available as on-line storage for diabetic patients. The rapid advances in the technology of data storage (20) will, however, allow greater flexibility as to the content of the stored data and, at the same time, render an estimate of future storage costs more difficult. With present use of commercial time-sharing facilities, cost estimates of the management system are as follows:

Program Storage	$600/year
Patient Data Storage	$20/year/patient
CPU and Terminal Time	$30/year/patient

The costs of program development involve personnel, and in the case of the system reported by Bolinger et al. (1973) were approximately:

Programmer-analyst	1/2 man-year
Physician	1/8 man-year
Nurse-clinician	1/8 man-year

In addition to this, computer time for testing amounted to about $600.

It would appear that in general the development of the use of the computer in the management of diabetes is relatively dependent upon similar developments in the field of health care in general. Computerized systems for the management of diabetes should be developed at a national level in parallel with development of management systems for other chronic disease states and should thus materially decrease both developmental and operational costs. A very urgent need now is some type of coordination of the developmental efforts going on at several different, presently independently, operating sites in the country.

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ETIOLOGY

6

ISLET CELL DYSFUNCTION

A. H. Rubenstein and D. F. Steiner

BACKGROUND

Despite the contribution of more than a half century of intensive investigation, diabetes mellitus continues to be a poorly understood and highly destructive disease. As is not unusual in such a situation, there is a voluminous literature on the subject and an abundance of speculation about possible etiologies. Although there is convincing evidence that the disorder is genetically determined, no altered protein or gene product has been identified which might account for the predisposition of certain individuals to develop the disease or provide an accurate marker to aid in its early detection. Thus there is still room today for even the most general theories as to the origin of diabetes.

A large body of evidence suggests that the inherited alteration may be confined largely, if not entirely, to the islet, or beta cell organ. Thus the tendency to diabetes is presumably expressed in the form of an abnormal protein, or as an excess or deficiency of some normal constituent in the beta cells of the islets of Langerhans. This alteration manifests itself at some time during the life of the predisposed individual as an impaired ability to produce sufficient insulin to maintain normal metabolic homeostasis in a given genotypic or environmental situation, and clinical diabetes then appears. In accord with this hypothesis, to understand diabetes we must familiarize ourselves with detailed mechanisms of differentiation, function, and regeneration of the beta cell organ. At present, little precise information is available regarding the origin and mechanisms of differentiation of the islet tissue, or the regulation of the total beta cell mass. On the other hand, considerable progress has been made in understanding the functional activities of the beta cell and in examining these for abnormalities which might be causally related to diabetes.

As the unique function of the beta cell is the biosynthesis, storage, and secretion of insulin under the influence of various physiological stimuli, these processes will be examined in some detail in an attempt to develop an understanding of the biochemistry of this cell and lay the framework for interpreting the various hypotheses that have been advanced to explain the defect(s) in diabetes mellitus.

CURRENT STATE OF KNOWLEDGE

a. Insulin Biosynthesis

Prior to 1967 the view was widely held that insulin synthesis in vivo was accomplished by combination of separately synthesized A and B chains. This hypothesis was supported by the initial observations of Dixon and Wardlaw (5) that small amounts of insulin could be reconstituted from mixtures of reduced insulin A and B chains in the presence of cysteine. The first direct evidence of the existence of a single chain precursor form of insulin came from studies of insulin biosynthesis using a human islet cell tumor (32). These studies showed that tritium-labeled leucine

or phenylalanine was incorporated into a higher molecular weight insulin-like protein during incubation of slices from the tumor in vitro. The higher molecular weight protein was characterized in terms of its molecular weight, immunoreactivity with insulin antisera, and structure. Advantage was taken of the differences in distribution of phenylalanine and leucine in human insulin to show that after digestion with small amount of trypsin, the precursor gave rise to an insulin-like component containing A and B chains. It was noted that reduction of the higher molecular weight component prior to tryptic digestion did not release free A or B chains, nor did it significantly alter its molecular size as judged by gel filtration, as would have been expected for a protein consisting of a single polypeptide chain. Phenylalanine, the amino terminus of the B chain, was found to be amino terminal in the precursor as well. This observation suggested that the most likely structure of the precursor (which was named proinsulin) was: NH_2-B chain-connecting peptide- A chain-COOH; this postulate was subsequently confirmed (2, 28).

Although some further characterization of the labeled material from this tumor as well as other sources was possible, it was clear that larger amounts of the precursor form would be required for full chemical characterization. Since the precursor behaved very much like insulin in a number of respects, the possibility that it might occur as a minor component in insulin preparations was considered. Gel filtration was chosen as the initial means of purification, since this procedure has been used to separate labeled proinsulin from insulin. In fact, only 2-3 percent of the total protein in commercial crystalline insulin preparations eluted at the position of proinsulin (30).

Proinsulin consists of a single polypeptide chain ranging in size from 78 (dog) to 86 (human, horse, rat) amino acid residues (Figure 1). The B chain amino acid sequence of proinsulin comprises the amino-terminal portion of the protein and the A chain sequence comprises the carboxyl-terminal portion of the chain (2, 19). Joining the chains is a connecting segment of approximately 30 residues. The amino acid sequences of the connecting peptides of various species are compared in Figure 2. There are numerous differences in their amino acids in contrast with the insulins in these species. At each end of the connecting segments two basic amino acids form connections to the amino-terminal residue of the A chain and carboxyl-terminal residue of the B chain. The remainder of the segment, aside from these residues, has been designated the C-peptide. These junctional regions, which presumably represent the sites of cleavage by an enzyme or encymes in the beta cell to liberate insulin, are identical in all species of proinsulin which have been examined. Two chain intermediate forms of bovine proinsulin have been isolated in which the polypeptide chain has been cleaved either at the junction with the amino-terminus of the A chain, with loss of the lysine and arginine from positions 59 and 60, or at the junction with the carboxyl-terminus of the B chain with loss of the two arginines from positions 31 and 32 (see Figure 3).

Significant progress has been made in the elucidation of the ultrastructural and biochemical organization of the process of insulin biosynthesis and secretion. Studies on a wide variety of secretory cells suggests that all such cells are organized along closely similar lines. The biosynthesis of the secretory proteins occurs in the rough endoplasmic reticulum on membrane bound ribosomes. The newly formed secretory products are then transferred through a transitional zone of the endoplasmic reticulum, possibly in small vesicles termed microvesicles, to the tubular

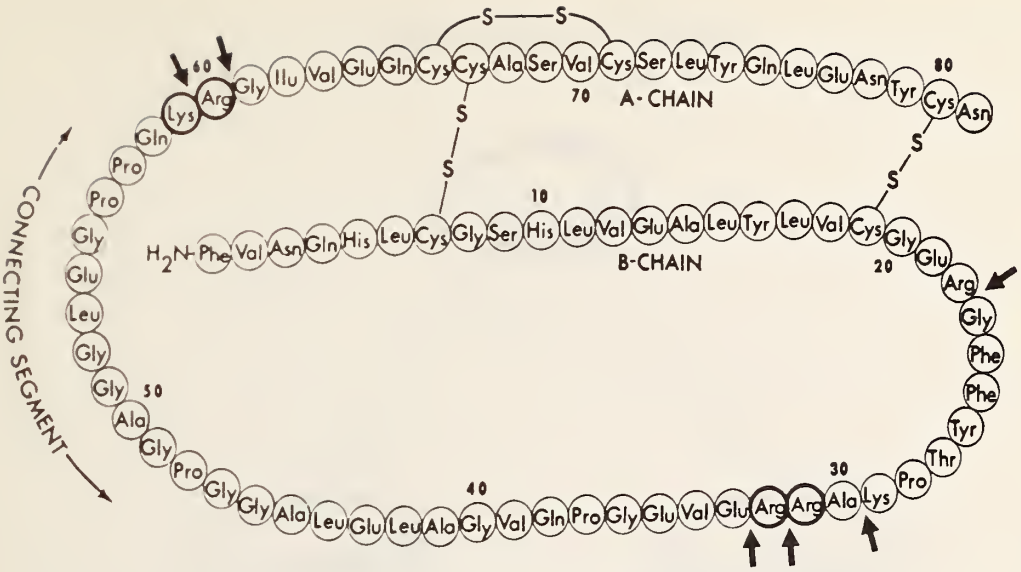

FIGURE 1. Structure of bovine proinsulin, showing sites of cleavage by trypsin.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
NH_3^+ - Glu	- Ala	- Glu	- Asp	- Leu	- Gln	- Val	- Gly	- Gln	- Val	- Glu	- Leu	- Gly	- Gly	- Gly -	MAN
NH_3^+ - Glu	- Ala	- Glu	- Asp	- Pro	- Gln	- Val	- Gly	- Gln	- Val	- Glu	- Leu	- Gly	- Gly	- Gly -	MONKEY
NH_3^+ - Glu	- Ala	- Glu	- Asp	- Pro	- Gln	- Val	- Gly	- Glu	- Val	- Glu	- Leu	- Gly	- Gly	- Gly -	HORSE
NH_3^+ - Glu	- Val	- Glu	- Asp	- Pro	- Gln	- Val	- Pro	- Gln	- Leu	- Glu	- Leu	- Gly	- Gly	- Gly -	RAT I
NH_3^+ - Glu	- Val	- Glu	- Asp	- Pro	- Gln	- Val	- Ala	- Gln	- Leu	- Glu	- Leu	- Gly	- Gly	- Gly -	RAT II
NH_3^+ - Glu	- Ala	- Glu	- Asn	- Pro	- Gln	- Ala	- Gly	- Ala	- Val	- Glu	- Leu	- Gly	- Gly	- Gly -	PIG
NH_3^+ - Glu	- Val	- Glu	- Gly	- Pro	- Gln	- Val	- Gly	- Ala	- Leu	- Glu	- Leu	- Ala	- Gly	- Gly -	COW, LAMB
NH_3^+ - Asp	- Val	- Glu -									- Leu	- Ala	- Gly	- Ala -	DOG

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
- Pro	- Gly	- Ala	- Gly	- Ser	- Leu	- Gln	- Pro	- Leu	- Ala	- Leu	- Glu	- Gly	- Ser	- Leu	- Gln - CO_2^-	MAN
- Pro	- Gly	- Ala	- Gly	- Ser	- Leu	- Gln	- Pro	- Leu	- Ala	- Leu	- Glu	- Gly	- Ser	- Leu	- Gln - CO_2^-	MONKEY
- Pro	- Gly	- Leu	- Gly	- Gly	- Leu	- Gln	- Pro	- Leu	- Ala	- Leu	- Ala	- Gly	- Pro	- Gln	- Gln - CO_2^-	HORSE
- Pro	- Glu	- Ala	- Gly	- Asp	- Leu	- Gln	- Thr	- Leu	- Ala	- Leu	- Glu	- Val	- Ala	- Arg	- Gln - CO_2^-	RAT I
- Pro	- Gly	- Ala	- Gly	- Asp	- Leu	- Gln	- Thr	- Leu	- Ala	- Leu	- Glu	- Val	- Ala	- Arg	- Gln - CO_2^-	RAT II
- Leu	- Gly -			- Gly	- Leu	- Gln	- Ala	- Leu	- Ala	- Leu	- Glu	- Gly	- Pro	- Pro	- Gln - CO_2^-	PIG
- Pro	- Gly	- Ala	- Gly	- Gly	- Leu -						- Glu	- Gly	- Pro	- Pro	- Gln - CO_2^-	COW, LAMB
- Pro	- Gly	- Glu	- Gly	- Gly	- Leu	- Gln	- Pro	- Leu	- Ala	- Leu	- Glu	- Gly	- Ala	- Leu	- Gln - CO_2^-	DOG

FIGURE 2. Amino acid sequences of several mammalian proinsulin C-peptides. These sequences do not include the basic residues at either end which link the C-peptide to the insulin chains in the proinsulin of these species.

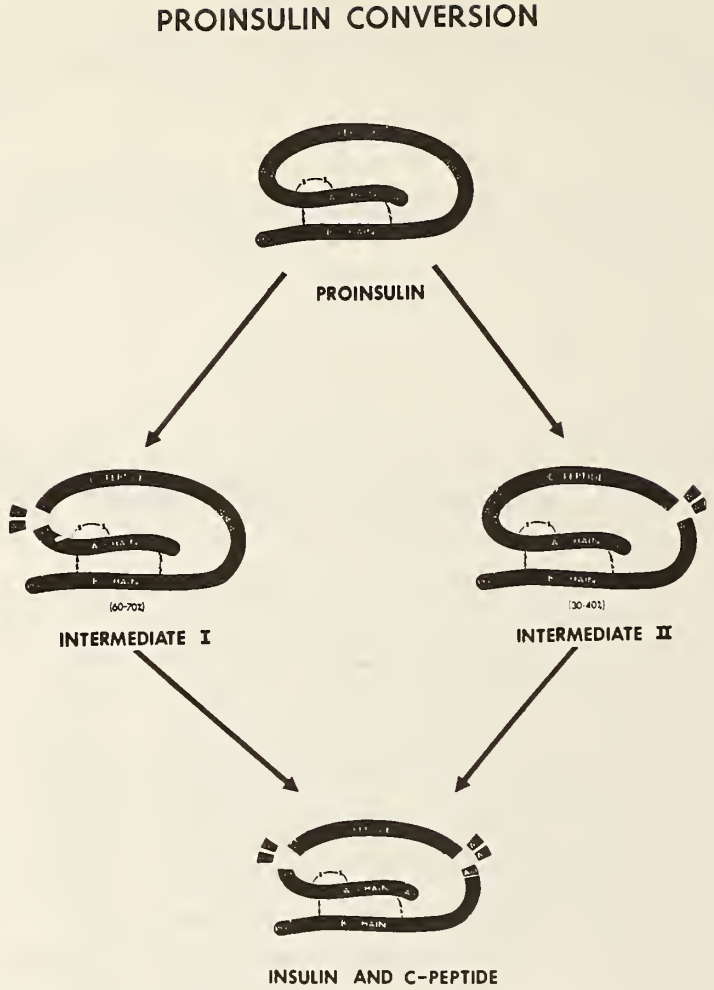

FIGURE 3. Structure of the two principal intermediate forms of bovine proinsulin. The products of the conversion of proinsulin to insulin in the beta cell are demonstrated.

elements in the periphery of the Golgi apparatus (11). The Golgi apparatus performs the function of packaging the newly formed secretory products and is also known to be the site of some biochemical transformations such as the addition of carbohydrate side chains to certain proteins. Secretory products leaving the Golgi region in newly formed granules or "condensing vacuoles," undergo changes in morphology which indicate a continuing biochemical reorganization of their secretory contents. After 40 minutes to one hour, the newly formed proteins begin to be secreted from the cell. By means of electron microscopic radioautography a similar movement of newly labeled protein in beta cells has been observed by Howell et al. (10). Their results indicate that newly synthesized proinsulin is first transported to the Golgi region and is maximally concentrated in that region about 30 minutes after biosynthesis. At later times radioactivity is found predominantly in granules.

From biosynthetic studies with isolated islets from rat pancreas as well as several human pancreatic adenomas, it is clear that the transformation of proinsulin to insulin is a slow process that starts about 10 to 20 minutes after the beginning of biosynthesis and continues for a period

of hours, exhibiting a half time of about one hour (27). The initial delay can be interpreted as an indication of the transport of the newly synthesized proinsulin from the rough endoplasmic reticulum to the Golgi region of the cell. Further evidence to strengthen this supposition derives from studies on the energy requirements for the transformation of proinsulin to insulin. Addition of the potent inhibitor of mitochondrial oxidative phosphorylation, antimycin-A, strongly inhibits the transformation of proinsulin to insulin, but only when added within the first 30 minutes after biosynthesis of the proinsulin has commenced. When added at later times, antimycin has no effect on the transformation. Experiments with other inhibitors of cellular energy metabolism also indicate the existence of this critical early energy requirement, presumably representing a process by which the newly synthesized proinsulin is made available to the proteolytic system that will convert it to insulin (9, 29). In view of the close correlation between the time of onset of conversion, its associated initial energy dependence, and the radio-autographic data mentioned earlier indicating that the initial period after labeling is a time of transfer of the protein from the rough endoplasmic recticulum to the Golgi apparatus, we can tentatively conclude that the transformation of proinsulin to insulin begins in the Golgi apparatus. In addition, because of its long half-life, it is probable that the conversion process continues for a period of many hours after new secretory granules have been formed from the Golgi apparatus. As a consequence of the sequestration of the biosynthetic products within the relatively impermeable membranes of the secretion granules, the C-peptide is retained after the conversion of proinsulin and stored on an equimolar basis with the insulin. Rubenstein et al. (24) have shown that stimulation of insulin secretion is accompanied by the liberation into the circulation of equimolar amounts of the C-peptide in several animal species (Figure 4).

On treatment of bovine or porcine proinsulin with trypsin, cleavages occur rapidly at the sites of attachment of the C-peptide to the chains of insulin and subsequently at position B-29 (lysine), liberating fully active dealanated insulin and the C-peptide with or without lysine remaining at the carboxyl-terminus. In order for an enzyme having the chemical specificity characteristic of trypsin to liberate native insulin from proinsulin in vivo, a second enzymatic activity similar to that of carboxypeptidase-B would be required. Such an enzyme could remove carboxyl-terminal basis residues which would originate through the action of a trypsin-like enzyme. Recently it has been shown that trypsin and carboxypeptidase-B together can carry out the cleavages found in the intermediate components in vitro and thus give rise to intact insulin and C-peptide (12). Nevertheless, it has been difficult to demonstrate conclusively that similar enzymes are involved in the conversion process in vivo (13).

Although it is well established that glucose is an important stimulus to insulin synthesis, the mechanism by which it exerts this effect is not yet understood (31). It is of particular interest that the stimulating effect of glucose is strongly selective for insulin biosynthesis: the synthesis of other cellular proteins being enhanced to a far smaller degree (22). Moreover, the glucose stimulus is not dependent on new RNA synthesis, but rather appears to be due to a selective translational enhancement. Thus actinomycin D initially does not inhibit the stimulation of biosynthesis due to glucose, although it does appear to inhibit a subsequent phase of further enhancement of the biosynthetic rate, that may depend in some way on additional RNA synthesis. Furthermore, the stimulatory effect of glucose on biosynthesis is inhibited by mannoheptulose (15), suggesting a requirement for glucose metabolism in the generation of the response.

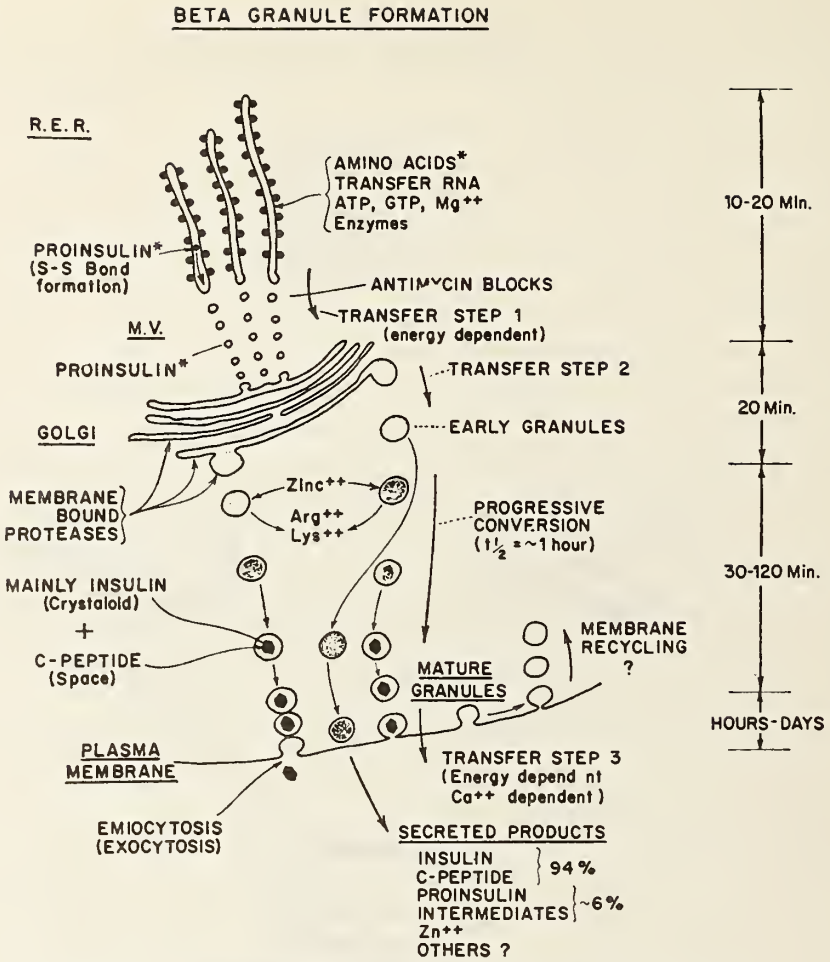

FIGURE 4. Diagrammatic representation of the insulin biosynthetic and secretory mechanism of the beta cell (R.E.R. = rough endoplasmic reticulum: M.V. = microvesicles).

b. Insulin secretion

At present most investigators believe that the defect in diabetes will be found in the intricate mechanism concerned with the secretion of insulin. The system has an afferent component which is involved in monitoring the ambient glucose concentration and an efferent component which is concerned with the secretion of insulin from the beta cell. Although a number of critical steps in these processes are still unknown, a great deal of progress has recently been made in unravelling its molecular basis.

There is now a great deal of evidence that the major path way of insulin secretion is by way of exocytosis (14). In this process, the granule membrane fuses with the plasma membrane. The insulin granules are subsequently extruded into the extracellular space where they undergo dissolution. When insulin release is markedly stimulated, an increased number of cytoplasmic projections, or microvilli, can be seen on the beta cell surface, presumably as a result of the addition to it of many granule membranes. Orci (21) has demonstrated that these microvilli are subsequently reincorporated into the cytoplasm as vesicles.

During the past 6 years, increasing application of improved electron microscopic techniques to islet morphology have led to an appreciation of the intracellular structures involved in the secretory

process. Lacy et al. (14) first suggested that a microtubular-microfilamentous system was involved in the movement of insulin granules towards the plasma membrane of the beta cell. These organelles are composed of actin-like material and are found in close association with mature insulin granules (Figure 5). It is believed that the microtubules may direct the granules towards the cell surface while the microfilamentous web might act as a barrier which controls the access of granules to the cell membrane (16). The use of agents which selectively disrupt these structures has lent support to their involvement in the secretory process. Thus colchicine and vincristine, which interact with microtubular protein and lead to its disappearance or precipitation as crystalline-like material, inhibit insulin secretion in response to glucose or glucose and theophylline. Deuterium oxide, a stabilizer of microtubules, reversibly inhibits secretion stimulated by glucose, leucine, and tolbutamide. Exposure of islets to cytochalasin B, on the other hand, enhances insulin release, presumably by causing a spatial reorganization of the microfilamentous material and margination of granules.

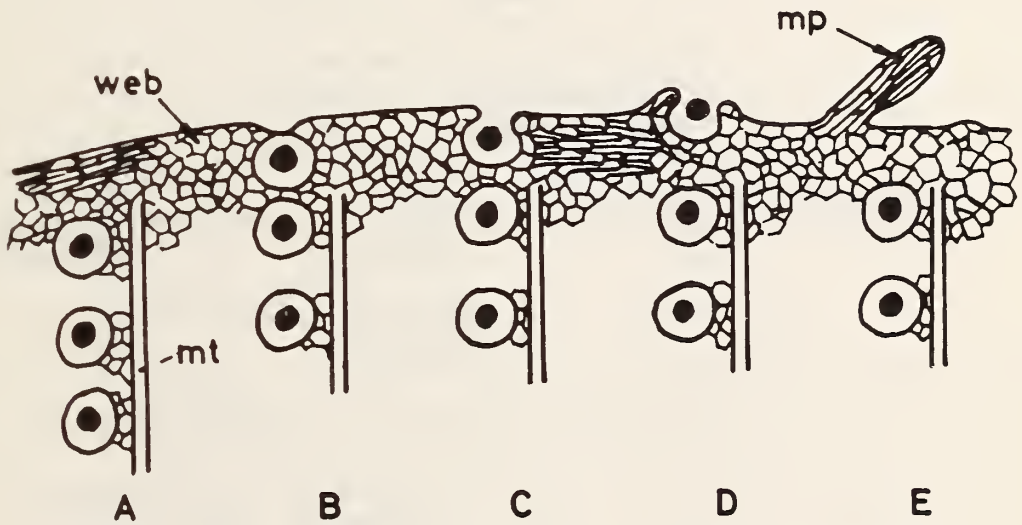

FIGURE 5. Schematic representation of the relationship between secretory granules, microtubules (mt), and the microfilamentous cell web. In the unstimulated beta cell, the secretory granules are kept away from the plasma membrane by the cell web (A). Under stimulation, granules are transported along the microtubules, and the web might participate in their access to the cell membrane (B). After fusion between the granule membrane and the plasma membrane, an emiocytotic aperture occurs (C), and the granule core is extruded into the extracellular space (D). The incorporation of the membranous sacs encasing the granules into the plasma membrane apparently results in the formation of microvillous processes (mp;E). (From Malaisse 1973.) Reprinted with premission from Springer-Verlag (Diabetologia 9:167, 1973).

The concept that insulin release is triggered by activation of the microtubular-microfilamentous system has been strengthened by the demonstration that extracellular calcium is required for this process (16). Thus exposure of islets to glucose in the presence of 45-calcium results in a net accumulation of this ion. If the islets are prelabeled with radioactive calcium, enhancement of secretion is associated with an immediate reduction in calcium efflux. Thereafter, a marked increase in calcium extrusion, associated with insulin release, occurs. It is postulated that the accumulation of calcium from the extracellular fluid, or the redistribution of the ion from intracellular storage sites within beta cells is a necessary prerequisite for activating

insulin secretion. The movement of calcium appears to be coupled to that of another ion, namely sodium (Figure 6). Studies in other systems have shown that calcium uptake is inhibited by high extracellular sodium concentrations, but is enhanced when the intracellular sodium level is raised. It seems probable that two intracellular sodium ions are exchanged for one extracellular calcium ion. The inhibiting effect of diphenylhydantoin, a drug known to reduce intracellular sodium concentrations in brain and muscle, on insulin release, as well as the stimulating action of ouabain, which has the opposite effect, suggest that the sodium dependent calcium uptake also exists in beta cells (23). Consideration of the above findings suggested that depolarization of the beta cell membrane might be an important early event in insulin release. The elegant experiments of Dean and Matthews (4) using ultra microelectrodes to record transmembrane potentials in mouse beta cells under basal and stimulated conditions has confirmed and extended these hypotheses.

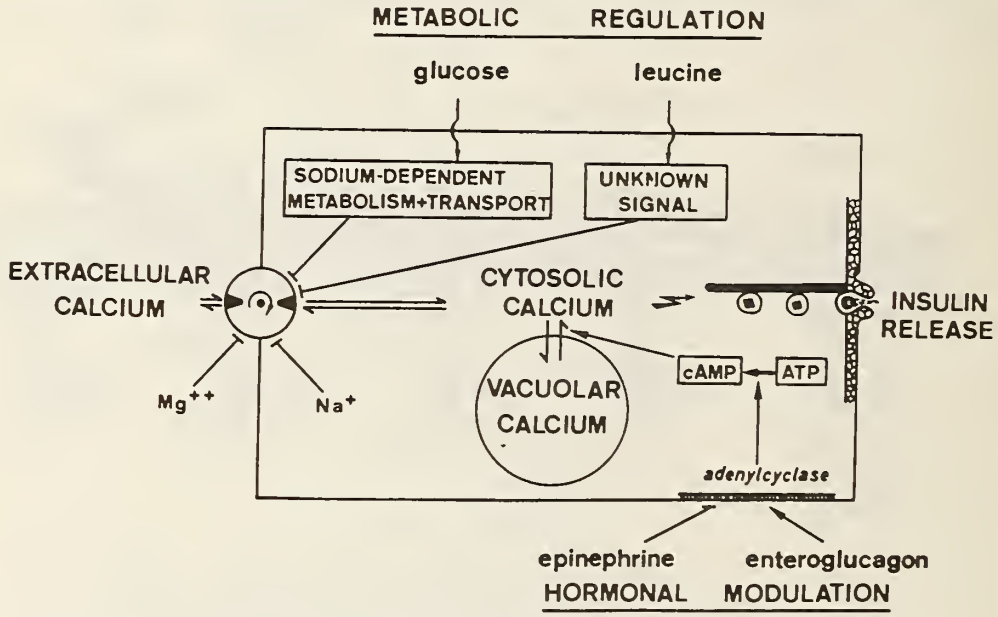

FIGURE 6. An integrated model for the multifactorial regulation of insulin secretion by metabolic and hormonal agents. On the left side, the T-shapes bars represent the hypothetical sites of competition or inhibition of calcium transport across the cell membrane. (From Malaisse 1973). Reprinted with permission from Springer Verlag (Diabetologia 9:167, 1973).

Beta cell cyclic 3', 5'-AMP appears to play a role in regulating insulin release (Figure 6), similarly to its effects in modulating hormone secretion in other endocrine glands. Agents which increase its concentration either by stimulating adenyl cyclase (such as glucagon, gastrointestinal hormones or beta adrenergic stimulators) or by inhibiting cyclic 3', 5'-AMP phosphodiesterase (such as caffeine and theophylline) lead to an enhancement of insulin release (17). The precise mechanism whereby cyclic 3', 5'-AMP affects insulin secretion is controversial. Three possibilities which have received attention involve (1) activation of an enzyme which may phosphorylate the microtubular protein (2) alteration of the intracellular distribution of calcium so as to increase the calcium concentration in the vicinity of the microtubules (3) modulation of a rate limiting step in glucose metabolism. However, it should be realized, that whichever mechanism turns out to be correct, the available evidence does not support a direct role for cyclic AMP in glucose induced insulin secretion. This conclusion is based on the inability of dibutyryl-cyclic-AMP to enhance insulin release in the absence of glucose and the finding that glucose

induced insulin secretion is not associated with detectable changes in islet cyclic-AMP levels.

It has been known for many years that the level of glucose in arterial blood reaching the pancreas is the most important factor regulating insulin secretion. However, the mechanism whereby glucose exerts its effect is still uncertain. Two main theories have been considered (18). The first involves the concept of glucose combining with a specific membrane receptor, presumably a protein, which activates release, either directly or by stimulating a messenger molecule, while the second allows for the metabolism of glucose to a substrate which would be the trigger for insulin release. Part of the evidence favoring the first hypothesis has been derived from analysis of the sigmoid relationship of glucose concentration to the rate of insulin release; the rapidity of insulin release after exposure to glucose; measurements of intracellular metabolite levels (which may show no changes at times when insulin release is stimulated), as well as alterations in the sensitivity of the insulin release mechanism to glucose after fasting, and the inhibition of the restoring effect of refeeding by actinomycin D (8). In addition, the finding that certain non-metabolisable amino acids stimulate insulin release has added weight to this concept (6), as has the recent demonstration that the two anomers of D-glucose, which are metabolized similarly, have different potencies in stimulating insulin release. The major arguments for the metabolism hypothesis are based on experiments showing that mannoheptulose, which inhibits the phosphorylation of glucose, blocks its effect on insulin secretion. In addition, non-metabolizable sugars, or those that can only be phosphorylated, do not release insulin (34). It is obvious that further information will be required to resolve these theories.

c. Beta cell dysfunction in diabetic patients

With the development of an immunoassay for insulin, it has become increasingly accepted that diabetes usually results from some degree of secretory failure of the pancreatic beta cells. In juvenile diabetics the extent of this failure is severe and is reflected in gross destruction of islet tissue. In adults diabetics, secretory failure is less pronounced, but when patients are carefully classified so that variables, such as obesity, are controlled, some degree of impairment of insulin secretion is almost always observed. In the glucose tolerance test there is both a quantitative decrease in total insulin secretion, as well as a sluggish early response with a tendency for the peak level to occur later than normal.

Whether the time course of the insulin response provides a significant clue to the nature of this defect is a controversial question, which has received much attention (1), especially in terms of the concept of two separate phases of insulin secretion, an early rapid burst followed by a later, more prolonged phase (3). On the other hand, the initial delay in secretion generally correlates well with the tendency in these tests for the blood sugar level to rise to higher levels and to peak at later times. It has been claimed on the basis of these results that islet cells of diabetics may have an inherent or acquired alteration in their sensitivity, or ability to respond to a glucose stimulus. This concept is also supported by the observations that other stimuli to insulin secretion, such as tolbutamide and glucagon, elicit normal secretory responses in mild diabetics, at a time when the response to glucose is already impaired (25).

Studies of the pathologic changes in the pancreatic islets in adult onset diabetes support the concept of a primary failure of islet responsiveness. Gepts (7) has pointed out that there is almost invariably a reduction in total islet tissue mass in the diabetic pancreas, amounting to approximately 50 percent in many cases. Moreover, there is a reduction of insulin stores

in the surviving pancreatic beta cells of these individuals as evidenced by partial degranulation of the islet tissue and by a decrease in the total insulin that can be extracted from the pancreas. These pathological data suggest that the defect may involve the production of insulin and the regeneration of islet cells, as well as the secretion of the hormone. It is interesting to hypothesize that these defects may have a common and interdependent origin, in the context of an altered glucose receptor mechanism in the diabetic's beta cells. Such an alteration could possibly contribute to the impaired renewal of islet tissue through failure to adequately stimulate cell division, for there is a suggestion that hyperglycemia may play a role in stimulating mitotic activity in the islets. In addition, this failure to respond normally to glucose could lead to a decrease in insulin biosynthesis and storage, because the glucose concentration is known to be a potent stimulus for this process. Finally, the failure of an adequate mechanism to monitor the extracellular glucose concentration would, of course, result in impairment of insulin secretion.

Although it is possible that the beta cell defect may affect only the efferent component of the insulin release mechanism, one would anticipate that the cellular stores of insulin would not only be preserved, but might be even greater than normal under these conditions. One might anticipate also that islet cell regenerative activity would lead to islet cell hyperplasia and cell proliferation. This situation has been found in the diabetic spiny mouse (26), where there is a great increase in total islet tissue mass and the beta cells contain numerous secretion granules and large quantities of insulin. Recent evidence has indicated that these animals have an intrinsic defect in their secretory mechanism, probably involving the microtubular-microfilamentous system.

While the concept of a genetically determined intrinsic defect in the beta cells of most diabetics is an attractive working hypothesis, other factors, of largely environmental origin, merit further consideration. Recent studies of the inheritance pattern of diabetics and its incidence, particularly in identical twins (33) strongly suggest that other causes for diabetes may exist and account for a significant fraction of the total number of patients. Studies of pancreatic pathology, particularly in juvenile diabetics, indicate the occurrence of a complex destructive lesion in the islets of Langerhans that may be due to extrinsic causes acting in a genetically favorable situation. Among such causes are two of particular interest and concern: autoimmunity and viral infection. These subjects will be dealt with in other chapters in this monograph.

Although there is little evidence at present to support the idea of a defect of the receptor for insulin in diabetes, recent studies with lymphocytes have indicated that abnormalities in membrane-binding of insulin may occur under certain conditions (20). Elucidation of the important structural features of insulin necessary for its biological action, as well as the molecular events associated with its biological effects, are both of obvious importance to the complete understanding and successful treatment of diabetes.

APPRAISAL OF INFORMATION WHICH NEEDS TO BE ACQUIRED THROUGH RESEARCH

The information in this chapter is concerned with the fundamental defect(s) which give rise to the disease diabetes mellitus. Although there is considerable evidence that abnormalities in beta cell structure or function may be etiologically involved in the expression of diabetes, there is not, as yet, absolute certainty on this point. Clearly, therefore, any experimental attack on

the problem of human diabetes must be carried out on a very broad front. It is important to appreciate that the development of more basic knowledge concerning the regulatory mechanisms of the beta cells, their growth, replication, and biosynthetic processes, as well as their secretory mechanisms, are of utmost importance to the ultimate elucidation of the diabetic defect. Moreover, it is impossible to predict how new information derived from other areas of biological investigation may change our concepts regarding the causes of diabetes. Nevertheless, it seems safe to suggest that as our information of the normal structure and function of beta cells expands, the possibility of pin-pointing abnormalities in diabetes becomes more likely.

The implication in the above comments is that it is possible that no one single defect underlies every case of diabetes. Many diverse abnormalities in beta cell integrity may result in a clinical syndrome characterized by carbohydrate intolerance. Moreover, one must also bear in mind that many insults, both genetic and environmental, may affect a specific process. Until all these ramifications are dissected out, it would be as well to pursue both basic and clinical research into many areas of beta cell function.

Among the many approaches that may be profitable, one might mention experiments designed:

a. To elucidate the natural history of beta cells. Their mitotic potential and factors which may influence or initiate this process need to be determined. Information about the length of time beta cells survive should be sought. The capacity to maintain islets and the beta cells in organ and monolayer culture respectively will undoubtedly become one of the most important methods to pursue these questions.

b. To determine the progenetor cells of the pancreatic islets. Do beta cells arise from proliferating pancreatic ductules which are derived from the original doudenal diverticula? Do diabetic patients have a normal complement of beta cells before their disease manifests? These questions will require quantitative morphological and functional studies on beta cells at various stages of gestation in both animals and humans.

c. To determine the factors regulating insulin biosynthesis and the mechanism of their effect. Isolation and characterization of the converting enzymes which transform proinsulin to insulin is also necessary for the complete understanding of insulin formation. The existence of a precursor form of insulin has provided an enzyme mediated step in biosynthesis where inherited defects could occur. Although initial studies in diabetic patients have failed to support the hypothesis of a defect in conversion of proinsulin to insulin, further investigations on a more sophisticated level are required.

d. To study the amino acid sequence of proinsulin and insulin in diabetics. The genetic defect of diabetes may express itself through point mutations in the structural gene for proinsulin. Amino acid substitutions in the connecting peptide region of proinsulin could distort the folding of the peptide chain so as to reduce the effectiveness of disulphide bond formation, while alterations in the sequence in the regions of proinsulin cleavage could also lead to abnormal products. Of course, mutations within the insulin chains themselves could result in biologically ineffective insulin molecules. Refinements in techniques for extracting these proteins from single human pancreata, and the ability to determine their amino acid sequences using very small quantities of material will be needed to accomplish these aims.

e. From knowledge of the amino acid sequences of proinsulins from several mammalian species,

certain predictions can be made regarding the structure of the gene(s) in the nuclear DNA that encode the prohormone. Recent advances in techniques for the isolation of genes make it technically feasible to now undertake the isolation of the genes for proinsulin. To do this will first require the isolation of the proinsulin messenger RNA from the polyribosomes of beta cells actively engaged in the synthesis of insulin. This mRNA can then be used to transcribe copies of one of the DNA strands of the proinsulin gene for use as a probe in identifying the proinsulin gene in the chromosomal DNA. The ultimate availability of "proinsulin-DNA" will also enable measurements to be made of the amount of proinsulin mRNA per beta cell, and it should then be possible to learn more about how insulin production is regulated by glucose, cAMP, and other factors at the levels of genetic transcription and of translation into protein.

f. To resolve the issue of whether a structural gluco-receptor is present in beta cells, and to determine its structure if it is found. This problem will require extensive studies on the morphology and chemistry of beta cell membranes and the correlation of these results with its biochemical properties. A long-term goal would be directed towards determining whether a defect in the "gluco-receptor" may be present in diabetic patients. This would require new and innovative clinical and basic research initiatives.

g. To determine whether insulin receptors are abnormal in diabetic patients.

HOW THIS INFORMATION WOULD LEAD TO IMPROVEMENT IN PREVENTIVE MEDICINE

It is obvious that a necessary first step in the train of events leading to the prevention of diabetes mellitus is an understanding of the etiology of this disorder. For this reason, it is at present impossible to predict with certainty what these approaches may be. However, experience gained from other diseases has suggested that the most effective preventative measures will require this information. While it is true that this is a long-term endeavor, it would be a mistake to sacrifice support for these basic projects for shortterm, but less fundamental management of the disease.

COST

I do not know of any way to accurately estimate the cost of the experimental plans that are needed. Perhaps one could arrive at a reasonable figure by obtaining information from the National Institutes of Health regarding all approved diabetes-related research grant applications dealing with this aspect of the disease. The total budgets of these grants, both those that have been funded and those that have not been funded because of lack of funds, may indicate a reasonable dollar estimate.

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7

INFECTIOUS AND IMMUNE MECHANISMS IN THE ETIOLOGY AND/OR PATHOGENESIS OF DIABETES MELLITUS[1]

Bryce L. Munger

Diabetes mellitus in man and animals has been the subject of innumerable books, monographs, and scholarly works. Yet, in the middle of the twentieth century, we do not know in what way the β cell in the islets of Langerhans are abnormal, or if indeed they are abnormal (77). This is an amazing revelation in an era of subcellular and molecular pathology. The disease entity, diabetes mellitus, has survived the era of tissue pathology prior to the 1850's and the era of cellular pathology from the time of Virchow (circa 1856) to the mid-twentieth century, and the recent era of biochemical and molecular pathology without a satisfactory explanation as to the basic etiology and/or pathogenesis of the disease. This admission of basic lack of knowledge may be hard for scholars to accept, but it is a fact.

Several factors have contributed to this present state of knowledge, or rather the lack of it. Gepts (22, 23) has provided the most recent summary of the pathologic changes in the pancreas in acute juvenile diabetes. He collected 22 specimens of acute juvenile diabetes from various hospitals, and as one might expect, the quality of preservation varied considerably. Despite this limitation, Gepts' study is still one of few thorough light microscopic studies on the acute cytopathology of juvenile diabetes in man reported in the recent literature. This study documented the degranulation and vacuolation of β cell cytoplasm as well as a constant lymphocytic infiltrate and scarring in the vascular stoma of the islets. This study has strengthened the suggestion that infectious and/or immune mechanisms might be involved in the etiology and/or pathogenesis of diabetes in man, based in part on the omnipresent mild inflammatory reaction in the islets as reported by Gepts. We shall return to this point subsequently.

In contrast to a paucity of studies concerning the acute cytopathology of the islets in human diabetes, our understanding of the pathogenesis of glomerular lesions has been facilitated by electron microscopic study of renal biopsies from living patients. The same can be said for various diseases of liver, lung, intestinal tract, skin and muscle--all amenable to biopsy and study by modern methods of cytochemistry, biochemistry, and especially electron microscopy. Electron microscopic study of the human endocrine pancreas has been based on isolated cases of surgical biopsies in cases of tumor (56). We dare not biopsy the pancreas in an acute case of juvenile diabetes, e.g., a patient age 14, and perform these kinds of studies. We have thus been unable to study acute human diabetes mellitus and have instead searched for adequate animal models, including the use of chemical poisons, such as alloxan, which kill β cells in the pancreatic islets but does not result in the vascular, neural, optic, and renal problems encountered in human diabetes.

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In the ensuing discussion, juvenile diabetes will be emphasized, since the pathology of the islets is more pronounced, and the possibility of infectious and/or immune mechanisms in the pathogenesis of the disease more plausible, as contrasted with typical maturity onset diabetes. The classification of types of diabetes (i.e., clinical, asymptomatic, latent, and potential) will follow that of Cerasi and Luft (9).

The above comments clearly indicate that our understanding of human islet pathology is severely restricted. This restriction also limits our understanding of the pathogenesis of the disease, especially as it relates to the islets. These limitations in terms of understanding of pathogenesis in terms of an organ are further complicated by a lack of understanding of metabolic and/or molecular changes in islet β cells as it relates to juvenile diabetes mellitus.

These restrictions have led scholars to search for appropriate animal models of the disease. Our knowledge of animal models has been summarized in two Brook Lodge Workshops on Spontaneous Diabetes in Laboratory Animals (63, 64). The models to date are all clearly genetic or dietary in etiology. This brings us to another problem. Human diabetes mellitus has a genetic component, but it is definitely not solely a genetic disease, as is cystic fibrosis of the pancreas or sickle cell anemia. If a genetic etiology is to be used, partial expression, partial penetrance, multiple alleles, and other "weasel terms" must be invoked to explain the disease. So we now couple inadequate explanation of cellular pathogenesis with multiple forms and compound the issue by involing only a "genetic component." Since a genetic etiology is not adequate, the need clearly exists to examine other models for the etiology, as well as the pathogenesis of the disease. These terms will be used in the ensuing discussion as follows: etiology is a specific causative agent, e.g., polio virus causes poliomyelitis, a gene abnormality causes sickle cell disease, pneumococcus causes pneumococcal pneumonia, etc. The pathogenesis of a disease is its cellular and tissue life history or progression. Our concepts of the pathogenesis of tuberculosis as expressed by Arnold Rich (71) are an adequate example of this concept.

One major area of interest other than genetic as to the pathogenesis of diabetes mellitus invokes infectious agents or immune mechanisms in the etiology and pathogenesis of the disease (9). One factor missing from this approach has been the absence of an animal model in which the pathogenesis of the disease resembles that seen in man. An animal model of viral (infectious) etiology with a genetic component has recently been described by Craighead (12, 13). A second animal model of contagious etiology also possessing a genetic component has recently been studied in our laboratory (59). The ensuing discussion will first present human and animal models of infectious and immune mechanisms and close with a brief discussion of our guinea pig model of contagious diabetes mellitus and its possible implications for future study. Where possible, potential fertile research areas will be pointed out.

INFECTIONS AND HUMAN DIABETES

Most studies or reports of the association of diabetes mellitus with various infections are temporal associations of two clinical events. This subject has been recently reviewed by Levy and Notkins (48). A prime example is mumps and diabetes. From the early description of Harris (28), repeated examples of diabetes following mumps can be found. These isolated reports on the development of diabetes following mumps usually consist of two or three cases added to a small literature search (26,31,51,52,67). Two very interesting cases, described by

King (35) and by Messaritakis et al. (53), report the development of diabetes in two siblings each following mumps. As evident from this literature, interest has only been sporadic with a couple dozen cases reported. The existence of this association in sibs is perhaps another matter. Certainly the criticism of Levy and Notkins (48), that neither mumps nor diabetes had been proven in those cases prior to 1940, would not hold in the case of the two siblings described by Messaritakis et al. (53). Certainly the pathogenesis of mumps-associated diabetes is a subject deserving careful attention. An association of mumps and diabetes is possible since mumps virus can involve the pancreas. The pathology of these accumulated cases has yet to be described. Thus, a few cases have been described wherein mumps and diabetes are temporally related. As an etiologic agent, mumps virus can only be suspect. These comments say nothing as to the pathogenesis of changes in the pancreatic islets or other organs.

A more recent addition to the list of potential suspects are the Coxsackie viruses, specifically type B4. Gamble and coworkers (21, 20) have found positive correlation in seasonal incidence, as well as elevated titers of B4 antibodies, in recently acquired (less than three months' duration) diabetes. A seasonal variation has been noted since the pioneering study of Adams (1) who agrees basically with Gamble and Taylor (21). Furthermore, Coxsackie virus has been isolated from human pancreas in five cases reported by Fechner, Smith, and Middlekamp (17). To complicate matters thoroughly, Hadden et al. (27) failed to find elevated B4 titers in 58 newly diagnosed diabetics; 34 required insulin. An animal model involving Coxsackie virus will be discussed shortly.

Other agents associated with diabetes are much more tenuous in their relationship as a possible causative or etiologic role. White (78), John (33), Grishaw et al. (24), and more recently Brown (6) have all argued for a role of infection in the onset of overt clinical diabetes. Brown has been most persuasive in his arguments as to an infectious origin for juvenile diabetes mellitus. The list of isolated agents associated with diabetes or pancreatitis includes hepatitis, poliomyelitis, influenza, tick-borne encephalitis, rubella, and cytomegalic inclusion disease (reviewed by Levy and Notkins, 48). A relationship between brucellosis and diabetes has been reported by Leon and Aguirre (46) and Harris (29); however, firm evidence is lacking. Harris (29, and personal communication) is still convinced he has seen numerous examples in his practice, and I personally have also encountered a unique case with a relationship between chronic brucellosis and diabetes.

The case for maternal rubella associated with the onset diabetes is also puzzling in that the evidence is recent and adequate (18,19).

Forrest, Menser, and Burgess encountered two cases in their own clinical experience following former rubella cases. They sent letters to a local newspaper in Sydney, Australia, as well as a medical journal, seeking additional cases. Their search located three additional cases. The authors state that the association of diabetes and congenital rubella could be mere chance, as all five cases had a family history of diabetes. Subsequently Forrest, Menser, and Burgess (18) studied 50 young adults with congenital rubella by means of a standard two-hour glucose tolerance test in 44 of the original group of 50. Five cases of overt diabetes were detected (11 percent) and four cases (9 percent) of asymptomatic diabetes (slightly abnormal glucose tolerance tests with delayed insulin elevation). These statistics are difficult to argue

against. A total of 20 percent diabetics in a group of 44 cases of congenital rubella is astounding, and in the words of Forrest, Menser, and Burgess (18) "strongly suggests a causative relationship between the two conditions."

We thus have questionable suspects as etiologic factors, mumps, Coxsackie viruses, and congenital rubella, as well as several more distant possibilities. If we consider the long list of associated events (upper respiratory infections in general plus numerous specific disease entities) described as related to the onset of diabetes, the situation can be considered confusing at best. While any of these could be an etiologic factor, what is the pathogenesis? Certainly we have no evidence clearly pinning down a single infectious agent associated with the onset of human diabetes mellitus. In terms of needed research, more clinical data, including antibody titers and detailed histories, are badly needed on newly diagnosed acute diabetics. The results of Forrest, Menser, and Burgess (18) could never have been predicted from any single personal clinical experience. By looking carefully at 50 cases of congenital rubella, a 20 percent incidence of diabetes (overt and asymptomatic) was uncovered. Studies of this sort, i.e., looking carefully at groups of patients with mumps, Coxsackie infections in detail with glucose tolerance tests, as well as plasma insulin, need to be done. Specific suggestions as to how we ought to be handling newly diagnosed diabetics will be covered later.

INFECTIOUS AGENTS AND ANIMAL MODELS

The pathology and pathogenesis of viral-induced pancreatic lesions date to the studies of Robertson (72) on pleurodynia virus-induced pancreatitis in mice. Due to the fact that Coxsackie viruses could be passed through adult mice and result in pancreatic lesions (66, 14), Burch et al. (7) studied the pancreatic lesions resulting from type B4 viruses (the strain reported to have elevated antibody titers in human acute diabetes) (20). Necrosis of acinar tissue and inflammation around islets did result, but not diabetes.

A more specific islet lesion has been described by Craighead and co-workers (11,12,13). This model involves a strain of the encephalomyocarditis virus that produces selective necrosis of the islets. The lesion is indeed inflammatory involving necrosis of β cells and release of immunoreactive insulin into the circulation. In a small number of animals (5 to 10 percent), a chronic diabetic condition persisted; and in some of these animals, renal glomerular lesions were described. In addition, genetic factors seemed to be involved since the DBA strain of mice are susceptible, whereas only infrequently are C_3H mice affected. Unfortunately, the lesions in the pancreas do not mimic the pathology of human diabetes mellitus (22, 23, 77). The validity of this animal model must await further studies on both the pancreatic islets and other systems (renal, vascular, occular) usually involved in diabetes.

These studies by Craighead et al. provided some logic for an editorial in Lancet (16) which discussed the fact that Burch's work might be relevant to Craighead's, since their viruses were all from the picornavirus group (RNA viruses) (poliomyelitis, ECHO, Coxsackie, encephalomyocarditis, and foot-and-mouth disease). What is even more remarkable is that in a spontaneous outbreak of foot-and-mouth disease in Italy, some cattle developed diabetes (4). This group subsequently produced the disease experimentally in other cattle (5). The lesions consisted of inflammatory cell infiltrates in the islets and a reduced granulation and number of β cells. Quite coincidentally, Platt (69) described exocrine pancreatic lesions in guinea pigs infected

with foot-and-mouth disease.

Certainly the above is sufficient to deem additional study as badly needed, in terms of both human and animal models. Any such studies in animals must include the spectrum of biochemical, physiologic, and anatomic methods available for the study of disease processes. In most cases our knowledge of pathogenesis is fragmentary. Detailed anatomic studies (histological and ultrastructural) are the only way to carefully document the pathogenesis of the disease. Hopefully some animal model will be found in which the pathogenesis resembles that of human diabetes mellitus.

IMMUNE MECHANISMS IN THE ETIOLOGY AND/OR PATHOGENESIS OF HUMAN AND EXPERIMENTAL DIABETES MELLITUS

Inflammatory changes in the islets in acute juvenile diabetics has been documented by Gepts (22, 23) and LeCompte (42). LeCompte and Legg (43) have documented recently two cases of "insulitis" in maturity onset diabetes. Reports of inflammatory change dating to the early studies of Opie (65) and Heiberg (30) are even more convincing in the work of Gepts (22) and Steiner (73), and are reviewed by Warren, LeCompte, and Legg (77) and Gepts (23). To summarize Gepts' opinion (22, 23) the characteristic pathologic changes in the islets of 22 cases of recent onset (acute) juvenile diabetes mellitus consist of degranulation of β cells (100 percent); hydropic change (the cytoplasmic inclusion described on p. 79 (53 percent); fibrosis (63 percent); mild insulitis (68 percent), and cytoplasmic basophilic bodies (Körnchen) are constantly encountered. Insulitis is defined as the presence of inflammatory cells, mainly lymphocytes in the islet proper. Even though the inflammatory cell infiltrate is mild in most instances, the presence of a mild inflammatory reaction has led numerous individuals to speculate on allergic, immune, or autoimmune mechanisms in the etiology and/or pathogenesis of diabetes.

Two converging lines of evidence have provided support for the concept of a possible immune mechanism in diabetes. First, the development of a form of experimental diabetes in animals using techniques of immunology; and secondly, the discovery of tissue specific antibodies in acute human clinical diabetes.

Animal models of so-called "immunodiabetes" were explored as a consequence of finding antibodies to insulin in sera of diabetic patients. Dating from the work of Moloney and Coval (54), numerous studies have been done on the influence of antibodies on pancreatic islet function. Diabetic syndromes and/or islet pathology (insulitis) can develop as a direct response to the repeated injection of heterologous insulin (25,37,44,75), as well as by injecting one species with insulin antibodies made in a different species (2,3,39,49,50,79). The pathology in both situations, i.e., direct immunization and transfer of antibodies, is comparable. The animals (mice, rats, rabbits, guinea pigs, cows) evidence β cell granulation, β cell destruction, and lymphocytic infiltration in the islets. In most instances, hyperglycemia persists (i.e., chronic diabetes develops). As pointed out by numerous workers (9,39) the pathologic changes do resemble those seen in the islets of babies born to diabetic mothers. However, severe insulitis and β cell necrosis are *not* part of the pathology of acute human juvenile diabetes (23).

The severity of the insulitis is variable in immunodiabetes. Korcáková, Titlbach, and

Lomský (36) in their study of immunodiabetes in guinea pigs have described minimal, if not negligible, inflammatory changes in the islets. They also have demonstrated precipitating (circulating) antibodies in these guinea pigs injected with beef insulin. The absence of a cellular infiltrate in guinea pig islets is in direct contrast to all other reports of the islet pathology in immunodiabetes. The ultrastructural characteristics of this type of immunodiabetes has been described by Titlbach and Korcáková (74). Eleven animals were studied, and the most marked change was degranulation of β cells with masses of granular ER present in the cytoplasm. Small amounts of glycogen were seen in one animal. This particular case had β cells which by electron microscopy resembled those seen in the infectious model of guinea pig diabetes (58).

In a subsequent study, Korcáková, Titlback, and Nouza (37) reported cyclophosphamide administration after and/or before onset of immunization inhibited the formation of insulin antibodies and a reduced severity of islet pathology. This study was used to confirm the concept that immune mechanisms are operable in the pathogenesis of diabetes since this cytotoxin had been used to reduce the severity of experimental thyroiditis and allergic encephalomyelitis. This type of study needs to be expanded to cover other animal models, and other antigens possibly capable of producing immunodiabetes need to be explored in addition to beef insulin.

With a variable degree of insulitis now demonstrable in immunodiabetes, research in this area could be extremely profitable. Considering our present state of knowledge of immunology, and a potential future role for transplants as a possible cure for diabetes (47), our need for knowledge concerning the immunology of the endocrine pancreas should be a priority area for research. What is needed is a thorough study of immune mechanisms of all endocrines, especially the pancreatic islets. What components of normal islets are potentially immunogenic, as well as capable of producing lesions in pancreatic islets? What are the possible roles of antibodies in disturbing islet function? The list of potential specific research projects is endless, and present technology is available and adequate to answer the questions.

The second factor implicating immune mechanisms in the etiology and/or pathogenesis of diabetes is the recent discovery of various tissue specific antibodies in sera of acute diabetics. Antibodies to insulin have been found in the absence of insulin therapy in numerous studies on acute diabetic subjects (10,68). Chetty and Watson tested 167 diabetic individuals not treated with insulin and 58 percent had a positive complement consumption test (antibodies to insulin) as opposed to 28 percent of controls.

A second recent development is the recognition of the association of pernicious anemia, thyroiditis, and diabetes. Moore and Neilson (55), as well as Landing et al. (40), have observed an association of chronic thyroiditis and diabetes. Moore and Neilson reported 83 diabetic subjects with approximately 20 percent having complement fixing antibodies to thyroid and gastric mucosa. Ungar et al. (76) reported in 400 diabetics (200 insulin-dependent and 200 not insulin-dependent) an incident rate for pernicious anemia of 4 percent in insulin-dependent diabetics, whereas the incident rate was 0 percent in non-insulin-dependent diabetics. In this same study, 28 percent of insulin-dependent diabetics were found to have antibodies to gastric parietal cells. Irvine et al. (32) studied 1054 diabetics and 871 controls. They found an increased incidence of antibodies to thyroid cell cytoplasm and gastric parietal cell cytoplasm in insulin dependent diabetics.

Perhaps the most exciting recent development is that described by Nerup et al. (62) in demonstrating an "organ-specific, species-specific, antipancreatic hypersensitivity of the cellular type" in diabetics of short duration in the absence on insulin therapy. These studies were done on pig pancreas where the pancreatic ducts had been ligated to produce atrophy of acinar tissue, and the sera of five out of six acute clinical diabetics inhibited leukocyte migration into the pancreas. A similar concept was used by Nerup (60, 61) to prove that hypersensitivity may play a role in the pathogenesis of idiopathic Addison's disease. The existence of Schmidt's syndrome (thyroid and adrenal insufficiency) with coexistent diabetes in 10 out of 15 cases (8) would certainly strengthen the argument that these endocrine deficiencies can be autoimmune in mechanism.

If idiopathic Addison's disease, pernicious anemia, and chronic thyroiditis can be conceptualized as autoimmune diseases, and autoantibodies are present in acute clinical diabetes, then the opinion of Lancet in an editorial (15) is an understatement--more research in this area is badly needed and long overdue. Patients with diseases suspected of being autoimmune in nature should be studied in detail and repeatedly for the possible onset of asymptomatic and/or overt diabetes. These patients should also be screened (as described subsequently) for associated or antecedent infectious events.

CONTAGIOUS DIABETES MELLITUS IN GUINEA PIGS

For the past several years our group at Hershey has been studying a contagious model of diabetes mellitus in guinea pigs (57, 58, 59). Since this model has only been the basis of one scientific report, our findings to date will be briefly summarized.

An original group of 18 Abbyssinian guinea pigs was obtained from a local fancier. Several members of her colony were found to be diabetic (hyperglycemic, glycosuric, and had demonstrable lesions in the pancreatic islets). In periods of time from 6 weeks to 3 months, many (approximately 60 percent) white Hartley guinea pigs purchased from Perfection Breeders and brought into our colony began to have glycosuria, hyperglycemia, and all diabetic animals autopsied in the acute phase of the disease had characteristic lesions in the pancreatic islets. In addition to elevated GTT's, the serum triglycerides were elevated, as was aorta cholesterol, even though serum cholesterol was normal (41).

The acute pancreatic lesion consists of: 1) degranulation of β cells as evidenced by a reduced aldehyde fuchsin stainability in paraffin sections, a reduced number of β granules, and also a reduced electron opacity of individual granule cores as seen in the electron microscope; 2) an increase in cytoplasmic masses of granular ER also seen at the light microscopic level as basophilic bodies (Körnchen); 3) a cytoplasmic inclusion consisting of masses of material resembling glycogen and admixed with β granule cores recognized in paraffin sections as "hydropic degeneration;" 4) a striking fibrosis in the vascular stroma of the islets; and 5) the presence of elongated fibroblast processes between the capillaries and the endocrine cells of the islets. The kidneys, muscle capillaries, and other organs of acutely ill animals are entirely normal. At no time have any infectious agents been observed in the islets, and inflammation or insulitis was never present.

The cytoplasmic inclusion in paraffin sections frequently dropped out of the section giving rise to the appearance of "hydropic degeneration." The inclusion is periodic acid Schiff (PAS) positive and removed by diastase indicating the presence of carbohydrate moieties that stain like

glycogen. These pathologic changes closely resemble those enumerated by Gepts (22) in acute juvenile diabetes in man (cited on p. 73).

Asymptomatic as well as overt diabetics are seen in this animal model (58). All had elevated GTT's at 1 and/or 4 years. At autopsy the pancreas was nearly normal; however, renal glomerular lesions were striking. The glomeruli in PAS-stained sections, as well as in electron micrographs, evidenced marked thickening of the basal lamina, especially prominent around the mesangial cells. Small nodules of PAS-positive material were present, associated with peripheral capillary loops. Although these nodules were not as massive as in those encountered in human Kimmelstiel-Wilson disease of the kidney, they were distinctive enough to deserve further study.

The few (six) chronic animals (diabetes of 1 to 3 years' duration) show a consistent pancreatic lesion and a variable renal and vascular lesion. The pancreatic lesion consists of severe hyalinization and fibrosis of the islets, a variable degre of β cell degranulation, and variable prominence of the cytoplasmic inclusion. The renal lesions are so variable as to deserve further study prior to any scientific report. Capillary basal laminae in striated muscle are quantitatively thickened (70).

This animal model is clearly contagious in nature, but an infectious agent has never been seen in the pancreatic islets. It could well involve immune mechanisms in the pathogenesis of both pancreatic as well as renal/vascular lesions.

SUMMARY

We have presented evidence that diabetes mellitus in man may be associated with infectious events prior to the onset of clinical diabetes. Certain animal models of viral infections can cause necrosis and inflammation of islets. The guinea pig as a contagious model perhaps also involing immune mechanisms still awaits elucidation as to the nature of the transmissible agent. Certainly, the immune state of some diabetics suggests an association that could be part of the pathogenesis of human disease. Immune factors involving renal disease have been well-documented in the case of glomerulonephritis. Since diabetics presently do not usually die from their pancreatic disease, the importance of the pathogenesis of the other organ systems assumes an added importance. One potential spin-off from the current surge of interest in viral oncology will be methods and concepts of studying infectious agents that may not fit our classical concepts of a "viral infection," i.e., poliomyelitis destruction of anterior horn cells. With the current interest in transplantation and cancer, the field of basic immunology has exploded.

The present arguments would suggest several immediate courses of action.

1) Basic research on areas touched in this review needs to be strengthened including more research on the following:

   a. A study of the normal developmental biology of islets (a potential value also for transplantation applications).
   b. Study of altered states of endocrine secretion in animal models using known physiologic parameters to alter islet cell function and follow cellular alterations in the islet. We do not have an adequate knowledge of the repertoire of possible normal cellular activity in pancreatic islets.
   c. Basic pathologic (light and electron microscopic) studies of human endocrine pancreas, in both diabetics and nondiabetics.

d. Application of techniques of modern virology and immunology to study of diseases of the endocrine organs, including diabetes.

e. Research efforts involving animal models in which infectious and/or immune mechanisms have been implicated need to be expanded.

2) A new approach to handling of human diabetes:

a. New cases of overt diabetes should be studied exhaustively, using the resources of academic health centers on a regional basis. Detailed histories of prior infectious events, antibody titers to those agents thought to be associated with the onset of diabetes (mumps, rubella, Coxsackie viruses), the presence of organ specific antibodies, relationships to other clinical entities thought to be immune in nature, all should be studied in detail.

b. Clinical diabetics should be followed with periodic exhaustive clinical, laboratory, and pathologic study on a long-term basis, again with the resources of the academic health center. The pathologic changes in kidney need to be studied temporally by light and electron microscopy. Those cases of justifiable biopsy of the pancreas (cysts, trauma, and cancer) need to be studied by light and electron microscopy, collecting and sharing this relatively rare tissue regionally. Such studies would provide better documentation of the pathogenesis of human diabetes, both in the endocrine pancreas and neurovascular systems.

c. Families of known diabetics should be examined for potential diabetes. These individuals should be exhaustively studied and followed, watching for infections and changes in their immune system.

I would predict a possible explosion in knowledge regarding the etiology, pathogenesis, and management of human diabetes mellitus in the next decade. Certainly the concepts of infection and immune mechanisms provide a new basis for approaching the study of diabetes. Our research efforts of the past 40 years have been, with justification, weighted in the direction of learning about the effects of insulin in physiologic and biochemical terms. With a technology available to answer new types of questions regarding diabetes, the time is ripe for an expanded research effort on the part of the biomedical community.

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FACTORS INFLUENCING DEVELOPMENT OF THE DIABETIC STATE

8

ENVIRONMENTAL FACTORS INFLUENCING THE DEVELOPMENT OF THE DIABETIC STATE

Ronald A. Arky

Controversy persists regarding the relative importance of hereditary and environmental factors in the development of the diabetic state. Consensus acknowledges that both factors are relevant, that in certain populations one factor may predominate, while in other populations the other factor predominates. This brief review will address itself to specific environmental factors that are known to influence the appearance of the diabetic state. Among these are: 1) nutritional factors, 2)infection, 3) pregnancy, and 4) pharmacological agents.

NUTRITIONAL FACTORS

Since the original description of diabetes mellitus, nutritional principles have been considered as factors in both the pathogenesis and treatment of the disease. Not until 1788 did Rollo (10) describe a sound foundation for dietary treatment of diabetes. Over the last century, it has become apparent that a paradox exists regarding diabetes mellitus and the status of an individual's nutrition. States of excessive caloric intake (obesity) and states of caloric deprivation (starvation) are frequently associated with abnormal carbohydrate tolerance.

a) Starvation

i. Acute starvation.

Claude Bernard (9) described "starvation diabetes" when he noted: "It is possible to make an animal diabetic if carbohydrate is given under certain circumstances namely if sugar is given to it after 24 to 36 hours of fast." Lehmann (64) corroborated these findings when he observed glucosuria in dogs that renewed feeding after prolonged fast. Bange (4) made similar observations in starved human subjects and coined the term "pseudodiabetes." DuVigneud and Karr (28) demonstrated that the severity and duration of the carbohydrate intolerance that followed a period of starvation correlated with the length of starvation. Ingle (49) induced starvation diabetes in rodents fasted for 10 days and observed that insulin administered to such animals only slightly improved the defect. He concluded that "starvation diabetes" is not caused by hypoinsulinism.

This conclusion was corroborated by Unger et al. (105) using a radioimmunoassay to measure insulin and to demonstrate that although hyperinsulinism accompanies the glucose intolerance in healthy subjects starved for three days, it was associated with an initial delay in insulin release. Yalow and her associates (112) observed that in nonobese subjects fasted for two and one-half days, the glucose intolerance (after an oral glucose load) occurred in the setting of "diabetic-type hyperinsulinism;" however when subjects of similar body build were fasted for five and one-half days the "peak insulin concentration never significantly exceeded" the peak value observed in the pre-fast study.

Cahill and his colleagues (17) fasted six healthy subjects for eight days and observed that an intravenous load of glucose was removed from the circulation at a greatly diminished rate when contrasted to the rate observed before fasting. These studies showed that the insulin response to glucose after fasting was prompt but of lesser magnitude than that prior to the deprivation of calories. These workers also demonstrated that the effectiveness of endogenous insulin was markedly decreased after starvation.

Obese, nondiabetics respond to acute total starvation in a manner similar to normal weight subjects. Several studies (Table 1) indicate that in overweight subjects with normal carbohydrate tolerance prior to fasting, starvation impairs the ability to handle an oral or intravenous challenge with glucose. Such impairment generally occurs without discernible alterations in the magnitude of the insulin response, although frequently the peak insulin levels observed after starvation occur later in time than in the pre-fast test. (Figure 1).

TABLE 1. Effects of Acute Starvation on Glucose Tolerance and Insulin Response in Obese Nondiabetics

Author	Number of Patients	Duration of Fast	Glucose Tolerance Type/Result vs. Prefast	Insulin Response
Beck et al.	4	4-14 days	Oral - 100 Gm Impaired	Delayed peak. Mean insulin response unchanged from pre-fast value.
Genuth, S. (1966)	6	Minimum 6 days	I.V. - 25 Gm Disappearance rate fell. K from 1.30 to .84 %/min.	Peak response slightly delayed. Integrated area unchanged.
Sussman, K. (1966)	12	5-14 days	Oral - 100 Gm Impaired	Delayed peak.
Jackson, I.M.D. et al. (1968)	5	2 1/2-18 weeks	Oral - 50 Gm Deteriorate	Peak generally delayed. 0 and 30 minute levels lower than prefast.
Tzagournis, M. et al. (1970)	7	14 days	Oral - 100 Gm Deteriorate	Delayed peak. Diminished 1/G ratio at one hour.
Jackson, R. A. et al. (1972)	7	14 days	Oral - 100 Gm Levels at 90 and 180 minutes higher after starvation	Delayed peak.

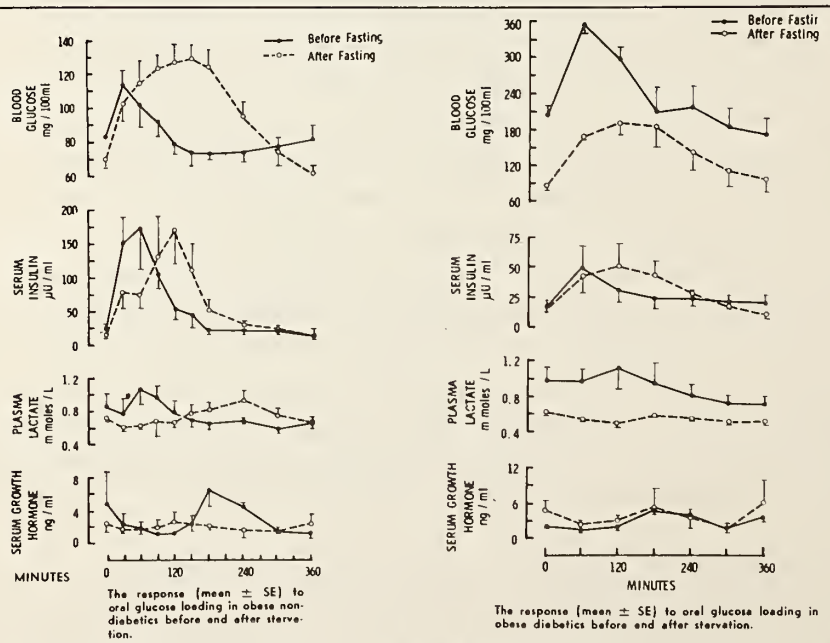

FIGURE 1. Effects of starvation on glucose tolerance in obese normals and diabetics. Results of the normal (nondiabetic) subjects are on the left. Fasts lasted for 14 days and oral glucose tolerance was tested with 100 gram loads (52). Reprinted with permission from Diabetes 20:214-227, The Journal of the American Diabetes Assn.

Prolonged starvation produces variable changes in the glucose tolerance of obese, mild diabetics. Reports of improvement (Figure 1) (5,51,90), of improvement and deterioration (112), and no change (38) have been made. These studies point up the heterogeneity of obese maturity onset diabetics as a group.

Sweeney (101) emphasized that it is the deficiency of dietary carbohydrate that leads to "starvation diabetes." Feeding medical students a "pure fat" diet causes an alteration in glucose tolerance analogous to that induced by total starvation. A number of studies (Table 2) have attempted to define the minimal dietary carbohydrate required to inhibit deterioration in glucose tolerance. While Wilkerson et al. (110) suggest that a 150 gram carbohydrate intake is as effective as Conn's (23) 300 gram carbohydrate diet in preventing alterations in glucose tolerance, some questions remain as to the minimum dietary carbohydrate needed tc prevent alterations in glucose tolerance.

TABLE 2. Studies Comparing the Effect of Dietary Carbohydrate on Glucose Tolerance

Investigator	Period of Preparation	Carbohydrate Content (Grams) of Trial Diets	Comments
Himsworth (1933)	5 days	55 vs 600+	Use 50 Gm oral load. Low carbohydrate diet impairs tolerance. High diet impractical.
Conn (1940)	3 days-300 Gm 5 days- 20 Gm	20 vs 300	Used 1.75 Gm glucose per Kg, Felt 300 Gm carbohydrate needed in preparatory stage. Showed misdiagnosis on low carbohydrate intake.
Irving and Wang (1954)	4 days	100 vs 300	Used 100 Gm glucose. Lower carbohydrate caused earlier high levels. Two hour levels were equivalent.
Wilkerson et al. (1960)	4-5 days	Usual vs 20 vs 150	Deterioration on 20 Gm intake returns to "usual" after four days on 150 Gm.
Hales and Randle (1963)	5 days	Usual (>200) vs <50	Performed both 50 and 100 Gm oral tolerance tests. Deterioration on <50 Gm diet.

ii. Chronic starvation.

That acute starvation induces an abnormal tolerance to glucose in man and other species is well established; however the effects of prolonged chronic starvation and malnutrition are not completely defined. Kwashiorkor is a chronic form of starvation that develops in youngsters ingesting diets poor in protein yet containing calories from nonprotein sources(30). This form of starvation is accompanied by glucose intolerance and a diminished insulin response after a glucose challenge (3,13,96). Marasmus develops in children whose diets are deficient in both protein and total calorie content. These children have relatively normal low fasting glucoses and lower normal plasma free fatty acids. However, carbohydrate tolerance has been reported as normal (13, 40). Chronic starvation is noted in adult populations in the form of anorexia nervosa, a condition reported to be accompanied by normal glucose tolerance (26,87).

b) Overfeeding - obesity.

i. Induced obesity.

In contrast to starvation hyperphagia or forced overfeeding also induces alterations in carbohydrate tolerance. Hofmeister (46) observed that overfeeding dogs with carbohydrate caused glucosuria. Lesions in the ventromedial nucleus of the rat's hypothalmus caused hyperphagia and

consequently an impaired carbohydrate tolerance (14). Similarly hyperphagic monkeys with hypothalamic lesions demonstrate a high incidence of postprandial hyperglycemia and glucosuria (42). Keys et al. (61), Mann et al. (69), and most recently Sims et al. (94) have overfed subjects and assessed metabolic changes. The last group of investigators overfed two groups of subjects, four University of Vermont students and then later a group of prisoner volunteers at the Vermont State Prison. Each subject ingested two to three times his normal intake of calories; the prisoners reached an average of 26 percent above their initial lean weight and their adipose tissue mass increased by 70-100 percent. Significant reduction in the oral and intravenous glucose tolerance followed weight gain although the oral tolerance curve and disappearncce curve after intravenous glucose remained within the "accepted range" (93). After the gain in weight the pancreatic secretion of insulin was greater than that observed prior to overeating, even though the glucose challenge was comparable in both instances.

Mahler (67) performed a similar experiment with healthy male students who ingested an additional 50 percent of their normal caloric intake either at one sitting (guzzling) or at hourly intervals throughout the day. All subjects gained weight: the "guzzlers" gained more than the "nibblers." Fasting blood glucose increased significantly at the end of the second week of "guzzling" but did not change in the "nibblers." However, glucose tolerance (50 grams) as measured by the area above the baseline value did not change significantly during either type of feeding. During overeating mean fasting insulin levels increased from 11.2 $\pm$ 2.8 microunits/ml to 32.5 $\pm$ 5 microunits/ml after one week of "guzzling" but were unchanged after "nibbling."

Further studies (48) showed that although basal insulin increased nearly 50 percent in five volunteers after overfeeding had induced a 15-25 percent gain in weight, the fasting arterial glucose levels were unchanged. However, those circulating amino acids that are usually governed by insulin, e.g., leucine, isoleucine, tyrosine, phenylalanine, and valine increased by 18 to 25 percent. This "peripheral resistance" to insulin after overfeeding was further substantiated by studies in which exogenous insulin injected interarterially after weight gain had a diminished effectiveness when contrasted to the effectiveness prior to the weight gain.

ii. Spontaneous obesity.

These observations on "overfeeding" or induced obesity emphasize several relationships between overweightness and disordered carbohydrate metabolism. Three salient facts that underlie current concepts are:

a. *The incidence of diabetes mellitus is high among the obese population.*
Among 4596 diabetics 78.5 percent of the males and 83.3 percent of the females were overweight at the time of their maximum weight (70). A study of 100 obese men and women who were at least 25 percent or heavier than ideal body weight showed that 22 percent had elevated fasting blood glucoses and 58 percent had impaired glucose tolerance tests (99). Yet not all longstanding obese subjects develop diabetes and the interplay of obesity with the multiplicity of other pathogenic factors requires clarification.

b. *Obesity is accompanied by insensitivity to insulin.*
Insensitivity of muscle and adipose tissue to insulin (82), hypertrophy of the pancreatic islets (73), higher basal insulin levels (2), and exaggerated insulin to glucose and other insulin secretogogues (16,60,77) all characterize obesity and theoretically can be linked into a cycle that eventuates into diabetes mellitus. In obese mice high insulin

response progresses to an insulin deficient type of diabetes (78). The "messenger" that transmits to the beta cell that "obesity is present and more insulin is needed" defies definition at this time. Whether the elevated plasma levels of branch chain amino acids (valine, leucine, isoleucine, etc.) in obese, hyperinsulinemic subjects (34) provide this signal remains speculative. Moreover, whether the basic insulin resistance of tissues of the obese can be attributed solely to the insulin insensitivity of oversized adipose cells (88) is debatable.

c. *Weight loss may reverse some of the metabolic derangements of obesity.*

Newburgh and Conn (72) showed that weight loss corrected the abnormal glucose tolerance in 90 percent of a group of middle-aged obese patients. Weight loss induces improvement in glucose and tolbutamide tolerance in spite of lower basal insulin levels and reduced insulin response to various stimuli (32,59,95).

c) Nature of dietary carbohydrate.

Does the nature of dietary carbohydrate play a role in the pathogenesis of diabetes mellitus? Arguments pro and con can be mustered, so that it is apparent that a dogmatic answer is unavailable. Since the recognition of diabetes mellitus as a "disorder of sugar metabolism," there has always been a popular notion that "too much sugar might induce the problem." While this proposition has been in part reputed, a number of epidemiological studies reignite general interest in the problem. Each of these studies considers the generic term "carbohydrate" to consist of two general classes: a) the "natural, unconcentrated carbohydrate as exists in grain, potatoes and fruit and b) the "unnatural, concentrated carbohydrates" as exemplified by refined sugar and flour. A brief summary of these epidemiological studies and the populations involved:

i. Yemenites.

Cohen (20, 21) cites the prevalence of diabetes mellitus among new Yemenite immigrants to Israel as 0.06 percent, while that in Yemenite settlers who have been in Israel for 25 years as 2.9 percent. He attributes this marked increase in prevalence to the high sucrose content (25-30 percent of total carbohydrates in the Israeli diet is sucrose) of the old settlers' diet as contrasted with the high starch, low to absent sucrose content of the diet eaten in Yemen. While "old settlers" are heavier and report a slight increase in caloric consumption compared to immigrants, Cohen feels that the substantial appearance of diabetes after acclamation to Israeli dietary habits is related to diminished consumption of bread and dietary polysaccharides which are replaced with increased consumption of disaccharides as sucrose. Cohen and his associates (22) attempt to demonstrate "better" glucose tolerance after preparation with a high polysaccharide diet than after a disaccharide diet.

ii. Indians.

Cleave and Campbell (19) note that the overall prevalence of diabetes in India is less than one percent, yet among Indians in Natal the prevalence ranges from 2.3 percent ("barrack dwellers") to 5.2 percent ("village dwellers"). They attribute this "veritable explosion of diabetes..." among peoples of the same origin to the higher consumption of "unnatural refined sugars" in Natal. Evidence of a similar trend is observed among urbanized Zulu as contrasted with tribal Zulus who have a low prevalence of diabetes.

iii. Great Britain.

Concerned with the increasing frequency of fatal coronary attacks and the increasing prevalence of atherosclerosis and diabetes in Britain, Yudkin (114, 115) cites the massive consumption of refined sugar in present-day England (120 pounds per head per year). This he contrasts to the English diet of the 1800's that abounded in fruits and "natural carbohydrates." He implicates the high disaccharide content of the diet as a major factor in the pathogenesis of atherosclerosis and diabetes.

A number of animal studies have sought to differentiate the effects of the "two classes" of carbohydrate on glucose tolerance. Uram et al. (106) detected only minor differences in post-glucose blood sugars in rats maintained on casein-sucrose diets as compared to animals on cereal ration. Early in the century von Noorden (106) showed marked benefits from an oatmeal diet to diabetics with profound glucosuria. As emphasized by West and Kalbfleisch (108), it is extremely difficult to evaluate precisely the sugar intake of a large population; moreover, "refined sugar" intake is associated with affluence and with an increased total caloric intake.

d) Frequency of meals.

The temporal intervals between the ingestion of calories influences the metabolic perturbations induced by feeding and possibly the disposition of the nutrients. Gwinup et al. (39) demonstrated in four subjects an impaired ability to handle an oral glucose load after the individuals had been on isocaloric diets fed once daily (between 4:00 and 5:00 p.m.). These workers distributed evenly in ten feedings at two-hour intervals and after three-meal-a-day pattern and found that "guzzling" (one meal per day) produced the worse glucose tolerance. Young et al. (113) observed similar results when college students on reducing diets were studied after periods of "nibbling" and "guzzling".

Broader implications of the effects of meal frequency upon several metabolic parameters were apparent in the study of Fabry et al. (29). A survey of 379 men revealed an inverse correlation between the frequency of meals and the degree of carbohydrate intolerance, as well as the incidence of obesity and hypercholesterolemia; i.e., the fewer the meals eaten per day, the greater the tendency for carbohydrate intolerance. These survey data are compatible with "stuff and starve" feeding patterns in rodents (47).

INFECTION FACTORS

Among the several "stress" factors that affect carbohybrate tolerance infection must be given a prominent place. While it is universally acknowledged that diabetes mellitus is aggravated in the presence of infection, the role of infectious processes in the etiology of permanent diabetes mellitus has not been established. Inflammatory lesions exert their effects upon intermediate metabolism by either involving specific tissues (e.g., pancreas, liver) or indirectly by altering the body's hormonal milieu and thus influencing the availability and utilization of substrate.

Little evidence for infection as an etiological factor in diabetes has been offered in the last twenty years. John (53) and Broun (15) felt that "heredity and infection" were the two prime factors in the etiology of diabetes. In 500 cases of juvenile diabetes (53) there was a recent history of infection in 164, and in 87 diabetes appeared within 60 days of an acute febrile illness. The seasonal occurrence of diabetes (36), geographic distribution, and coincidence of upper respiratory tract infections in newly diagnosed diabetics have been used as arguments for an etiological role of viral and bacterial infections in diabetes mellitus.

The mumps virus may directly affect the endocrine pancreas, although the number of reported cases of diabetes mellitus immediately following mumps is small (45, 71); less than 20 appear in the English literature since the first suggestion of this relationship (43). Members of the picornavirus group have been implicated as etiological agents in diabetes mellitus. One of these viruses, the encephalomyocarditis virus, induces histiological changes in the islets of Langerhan four days after inoculation. The islets become shrunken and beta cells degranulate. Simultaneously with these changes, glucose intolerance is apparent and insulin secretion diminishes (25). Among other viruses that affect the pancreatic beta cell are foot and mouth disease virus, Coxsakie B virus (36, 37), Reo virus, and hepatitis virus.

An infectious etiology of acute pancreatitis is questionable, but whatever the cause, acute pancreatitis is commonly accompanied by hyperglycemia and glucosuria. This "transient diabetes" infrequently persists, and if the pancreatitis is severe enough to destroy the bulk of islet tissue it is usually fatal (107). In the United States and Europe chronic pancreatitis is an uncommon cause of diabetes, but in such regions as East Africa (62), it may account for 13 percent of the newly diagnosed cases. Diabetes developing secondary to known bacterial infections of the pancreas or pancreatic abscesses is rare (107).

In the early 1900's a number of clinical studies noted that hyperglycemia and glucosuria were frequently found accompanying acute infections. Labbe and Boulin (63) observed glucosuria in 75 percent of nondiabetics with febrile illnesses and could not relate the severity of the illness with the magnitude of the glucosuria. They speculated: "It is possible that recurrence of this transient disturbance of glucose balance creates a true diabetes more frequently than believed." Williams and Dick (111) demonstrated decreased dextrose tolerance in nondiabetics with respiratory and renal infections. In recent years radioimmunoassay techniques have permitted the measurement of peptide hormone fluxes in both naturally occurring and induced infections. Within 24 hours of induced infection with tularemia in man, the rate of glucose disappearance as measured by intravenous glucose tolerance is diminished. Simultaneously the rise in insulin after glucose is greater, and the fall in insulin slower than in corresponding studies performed in the pre-infection state (92). Similar observations have been made following the induction of a self-limited febrile illness, Sandfly fever, in man (84).

An intricate alteration in the circulating levels of humoral factors and metabolic substrate accompanies infection (8) and effects the hyperglycemia and glucosuria that is so frequently seen. Bagdade (2) has summarized current concepts on the modes of action of these hormonal components that are increased with infection and relates these actions to the resultant "transient diabetes." Recently elevated levels of glucagon have been detected in human and experimental infections (86); the diabetogenic influence of this peptide may augment the actions of increased glucocorticoids and catecholamines that accompany the "stress" of infection.

PREGNANCY

Pregnancy represents a major diabetogenic stress: a) it places a progressive demand on the maternal beta cell and the insulin secretory mechanism; b) it is a state of relative insulin resistance (55). Normal women tolerate pregnancy without significant alteration in carbohydrate tolerance; women with marginal pancreatic reserve develop an abnormal glucose tolerance or gestational diabetes. As many as one pregnant woman out of 116 falls into this category (74).

While the diabetes frequently clears after delivery, 28 percent of such women have frank diabetes five years later, and by 16 years 52 percent of gestational diabetics have permanent disease (O'Sullivan, personal communciation). The risk of impaired carbohydrate tolerance increases with successive pregnancies--a woman who has had five pregnancies has three times the chance of developing diabetes as a nulliparous individual (81).

During the course of normal pregnancy, basal levels of insulin increase progressively and the insulin response to serious secretogogues also is greater in the pregnant than nonpregnant state (12,89,97) (Figure 2). The need for "extra" insulin in both the basal and stimulated state is proposed to stem from:

a) Maternal insulin has an abbreviated biological life due to the ability of the placenta to degrade insulin (35, 80).

b) Maternal tissues are resistant to the hypoglycemic effects of insulin because of circulating "antagonists" produced in part by the placenta.

GLUCOSE TOLERANCE IN NORMAL PREGNANCY

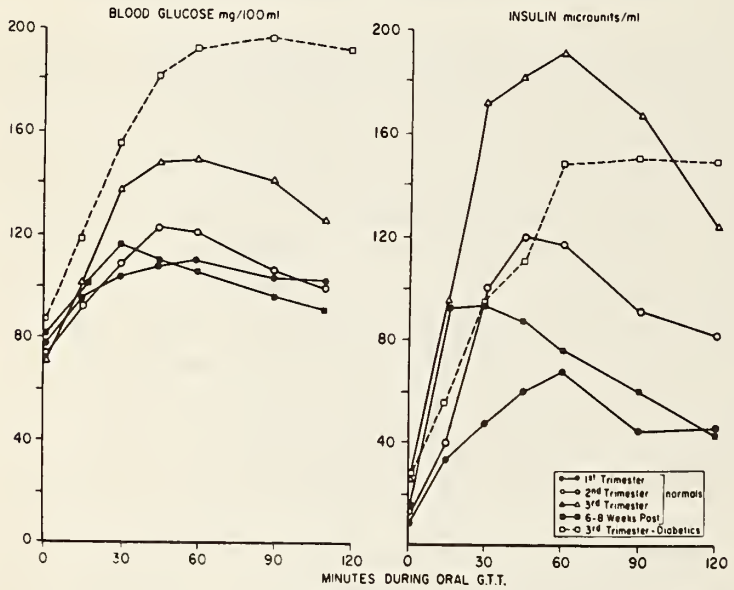

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FIG. 2. Blood glucose and insulin levels during an oral GTT in seven normal pregnant patients in each of the three trimesters and 7-8 weeks postpartum, compared with the levels in ten gestational diabetics in the third trimester.

Among the placental hormones that contribute to the diabetogenic effects of pregnancy are:

a) *Placental lactogen (HPL).* A polypeptide immunologically similar to growth hormone with contrainsulin action when given parenterally (7), HPL causes impaired glucose tolerance in subclinical and overtly diabetic patients (56). The precise site of the contrainsulin action of HPL is undetermined; the aggravation of mild diabetes caused by infusions of HPL is not accompanied by hyperinsulinism.

b) *Progesterone.* When this steroid is administered to males and hysterectomized females in quantities that cause 24 hour urinary pregnanediol excretions that simulate those observed in late gestation, fasting levels of insulin and the insulin responses to glucose and tolbutamide are increased. Yet the oral and intravenous glucose tolerance tests are not altered (5, 57). Isolated islets of rats treated with 5.0 mgm of progesterone for 21 days were hypertrophied and had an insulin output similar to the islets from pregnant animals (24).

c) *Estrogens.* Estradiol benzoate causes islet cell hypertrophy, increased insulin output of isolated islet cells and an increased insulin response to glucose in rodents treated for three weeks (24). The specific effects of natural and synthetic estrogens upon glucose and insulin metabolism in normal and diabetic humans are debatable (98,102), with contentions that diabetes is ameliorated or not affected by estrogens.

Fluctuations in other hormones may in part account for some of the diabetogenic influences of pregnancy. Most of the elevation in serum cortisol during pregnancy is attributed to increments in the corticosteroid binding globulin and the increment in free cortisol is minimal and not felt to be an important factor in the altered maternal carbohydrate metabolism in pregnancy. Serum growth hormone levels do not increase in pregnancy and thus play no role in this insulin resistant state (58). Consensus is that the placental hormones are the chief offenders in altering substrate balance and causing the minor deviation of glucose tolerance in normal pregnancy (75).

As with diabetes in general, gestational diabetes may be accompanied by anatomic as well as biochemical abnormalities. Perinatal infant mortality is higher in insulin-requiring diabetic mothers than in nondiabetics; infants of diabetic mothers are longer, heavier, and fatter than controls (76). There is substantial evidence that "control" of the metabolic derangement in the pregnant diabetic is the most important factor in improving the fetal salvage rate. Even among gestational diabetes the fetal salvage rate was improved and weights of infants reduced when mothers were treated with small doses of insulin during pregnancy (O'Sullivan, personal communication).

PHARMACOLOGIC AGENTS

During the last two decades knowledge of the mechanisms involved in the synthesis, storage, and release of insulin from the pancreatic beta cell has burgeoned. Simultaneously the use of pharmacologic agents by the public has increased markedly. Agents that impede any step in the process of insulin formation or secretion will induce diabetes. An understanding of the structure and mode of action of therapeutic compounds that affect carbohydrate metabolism has become a major responsibility of practicing physicians.

Historically alloxan (mesoxalyurea) is recognized as the prototype of a beta-cytotoxic agent. Although synthesized in 1818, alloxan was not discovered to possess diabetogenic action until 1943 (27), when it was shown that the agent causes necrosis of the pancreatic islets. In normal animals the sequence of alloxan's actions are: a) a marked early hyperglycemia (1-4 hours); b) hypoglycemia lasting up to 48 hours after administration; c) chronic hypoglycemia. Although alloxan has a structure similar to uric acid (Figure 3), there is no evidence that abnormalities in uric acid metabolism cause the accumulation of alloxan.

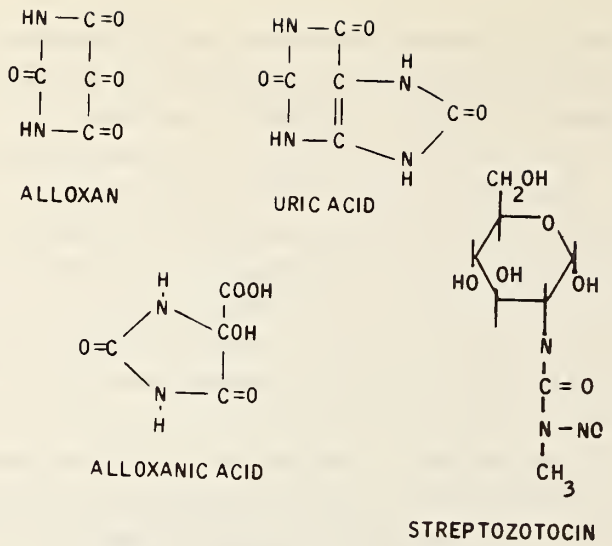

FIGURE 3. Diabetogenic agents. Uric acid is not diabetogenic, but presented to show similarity in chemical structure to alloxan.

Ascorbic acid augments the diabetogenic action of alloxan, but is not itself diabetogenic. Experimentally the quinoline derivatives, 8-hydroxyquinaldine and 8-hydroxy-6 aminoquinoline are diabetogenic. These compounds have organic metal-binding properties and Kadota and Abe (54) postulate that the diabetogenic effects of these compounds stem primarily from the binding of zinc within the beta cell and subsequent interference with synthetic processes.

A specific beta-cytotoxic action analogous to alloxan is observed in the broad-spectrum antibiotic streptozotocin. This substance causes degranulation of the beta cell and disruption of the islets. The triphasic pattern in glucose levels observed after the administration of alloxan is also noted after streptozotocin. Nicotinamide, pyrazinamide and 2-deoxyglucose protect against the diabetogenic effects of streptozotocin; nicotinic acid and glutathione are ineffective in this role (85). Streptozotocin is used to produce experimental diabetes in animals and in the treatment of insulin-secreting tumors. The mode by which streptozotocin selectively destroys the beta cell is not fully understood, but it is possible that the glucose moiety of the compound (Figure 3) binds specifically to cells with glucose receptors and that the nitrosurea group causes the actual cell damage.

The autonomic nervous system participates in the control of insulin secretion. Epinephrine and norepinephrine inhibit the release of insulin from glucose-stimulated beta cells (79); the diabetogenic action of these catecholamines is mediated via alpha receptors in the beta cell. The benzothiadiazines tend to suppress insulin release. Diazoxide is the most powerful of these substances (31, 91) and its hyperglucemic action is potentiated by trichlormethiazide in normal subjects and patients with insulin secreting tumors. Diazoxide, a benzothiadiazine that causes retention of sodium and no significant loss of potassium probably exerts its hyperglycemic effects directly by inhibiting release of insulin. The benzothiadiazine diuretics that induce significant negative potassium balance may exert their diabetogenic effects through the latter effect (83). Non-thiazide diuretics as chlorthalidone also induce hyperglycemia (18).

Diphenylhydantoin in amounts that are frequently prescribed for neurological disorders inhibits the secretion of insulin in normal subjects (68). This agent has also been utilized to treat insulin-secreting tumors. In the hamster serotonin inhibits insulin secretion (33), but stimulates release of insulin in the rabbit (103). L-asparaginase, an agent used to treat acute leukemia diminishes insulin secretion and causes glucose intolerance (109).

Glucocorticoids administered in excess may induce diabetes by increasing the breakdown of protein, increasing gluconeogenesis, interfering with glucose utilization and insulin's effectiveness.

FUTURE INVESTIGATION AND EVALUATION

From this brief review of several environmental factors that influence the development of the diabetic state, it is evident that many questions persist. An abbreviated list of these questions is offered as the basis for future investigation and evaluation.

1. Nutritional factors.
   a. Obesity
      1) Are there differences in physical or metabolic characteristics between the longstanding obese subjects who develop diabetes and those who do not?
      2) Is a specific "humoral factor" responsible for signaling the beta cell that obesity is present? Does the signal (if present) disappear with minimal weight reduction?
      3) Is it possible to define a population that has altered a specific dietary foodstuff over the course of several years and does not exhibit weight change? All of the populations that have shifted from "natural" to "unnatural" carbohydrate have gained weight.
      4) Can the relative hyperinsulinemia of obesity be altered solely by modifying the diet yet maintain isocaloric status? (i.e., substantiation of the work of Grey, N. and Kipnis, D.M., New Engl. J. Med. 211:811-916, 1969, in a large population).
   b. Starvation
      1) At what point during starvation in normal man is the "diabetes" secondary to reduced insulin production as opposed to increased peripheral resistance?
      2) What characteristics differentiate the maturity onset diabetics whose carbohydrate tolerance improves with starvation and those who show no improvement?
      3) What factors account for variation in carbohydrate intolerance in the several "chronic starvation" states, i.e., kwashikor va. anorexia nervosa?
2. Infection
   a. Does a current updating substantiate the relationship between acute infections and the onset of diabetes in North American populations?
   b. Is carbohydrate homeostasis affected more by mild viral infections in persons with a hereditary predisposition to diabetes vs. no hereditary predisposition?
3. Pregnancy
   a. Does periodic evaluation of individuals who demonstrate gestational diabetes help reduce the incidence of overt disease in later years?
   b. What factors determine the onset of overt diabetes in women who have had abnormal carbohydrate tolerance during pregnancy?

Undoubtedly many other questions can be raised by the data presented in the Review. The above list is presented as a starter.

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9

THE ROLE OF THE NEUROENDOCRINE SYSTEM IN THE DEVELOPMENT OF DIABETES MELLITUS

Daniel Porte, Jr.

INTRODUCTION

Diabetes mellitus is a syndrome in which carbohydrate and lipid fuel transport is grossly disordered. The nature of the primary defect is not understood. However, the resulting hyperglycemia has been used as an index of a presumed underlying genetic defect. The lack of a more specific marker for the presence or absence of the disease has led to the question of whether all hyperglycemia is due to "diabetes" or not. To answer this question one must consider the role of the neuroendocrine system as an important controller of body glucose metabolism and its role in the development of the diabetic state. A malfunction of this system can produce hyperglycemia. The question then is whether hyperglycemia is due to a neuroendocrine abnormality, to an interaction of the neuroendocrine system with some other primary factor ("diabetes"), or to "diabetes," independent of the neuroendocrine system.

Although hormones have at times been considered to be independent of the central nervous system, it is now apparent that hormonal signals and neural signals are two parts of one integrated system for regulation of body metabolism. These factors are inter-organ controllers which are superimposed upon the primary substrate controllers. For example, the concentration of glucose is a primary regulator of its rate of uptake and metabolism by tissues, but this rate is modulated by the neuroendocrine system via several hormones and neural transmitters. To complete the feedback loop, the output of these neuroendocrine controllers is regulated by the primary substrates, particularly glucose. Finally, these neuro-humoral factors are regulated by each other. The net result of these multiple interactions is to keep the blood glucose within a relatively narrow range. This neuroendocrine control system is no different from any other control system in that the stability of the product (glucose), depends on the multiplicity of the interactions. Therefore a malfunction of any component part can cause problems in glucose regulation.

The purpose of this section is to: 1) describe in simplified terms the complex neuroendocrine regulation of fuels; 2) indicate those areas in which disordered control mechanisms may lead to hyperglycemia; and 3) consider the question of how the hyperglycemia observed clinically may be related to "diabetes" and/or a neuroendocrine abnormality.

PHYSIOLOGY OF THE NEUROENDOCRINE SYSTEM

The presumed purpose of such a control system is to provide for messages to be given between the organs which have to do with the provision, utilization, and storage of fuels (9). The organs involved include the *gastrointestinal tract*, which acts as an input source for glucose, amino acids, and fats, and is a source of gut hormones which signal the pancreas, liver, and adipose tissue of the presence of nutrients. Absorbed water soluble nutrients pass first to the *liver* which acts as an ultimate source for glucose between meals and which serves as a short-term storage system for recently absorbed carbohydrate. The uptake of these nutrients by the liver is regulated as a primary function of their concentration and is secondarily controlled by the hormones insulin, glucagon,

cortisol, and epinephrine, and the autonomic nervous system. The other major fuel storage and release site, *adipose tissue* is also regulated by the same hormones and nervous factors to provide for a balance between storage of excess calories and provision of fuels between periods of nutrient absorption. *Muscle tissue* is the primary utilizer of fuels, mostly fatty acids, but does require glucose either as stored glycogen or from plasma glucose for the initiation of exercise. This tissue also acts as a major storehouse of the amino acids which provide for long-term maintenance of blood glucose during starvation after conversion to glucose in the liver and kidney. Thus the liver primarily and the *kidney* secondarily are the only sources for glucose production during starvation. All other tissues provide for a constant glucose utilization which is insulin independent. Of these, the *central nervous system* is the prime user of glucose, metabolizing approximately 80 percent of glucose provided by the liver in the overnight fasted individual. It is the absolute need of the central nervous system for large quantities of glucose which demands the precise and complex regulatory system for maintaining blood glucose levels within a relatively narrow range. The central nervous system therefore is both the major utilizer of glucose and the prime regulatory control center. Although insulin is clearly the major controller of blood glucose concentration, such that any excess or deficiency is associated with major abnormalities in blood glucose regulation, this regulation occurs in concert with glucagon, growth hormone, cortisol, epinephrine, sex steroids, and the gut hormones, secretin, pancreozymin, and gastrin. Therefore any change in the output or sensitivity to the effects of any of these other hormones may also be expected to be associated with either hyper- or hypoglycemia. In fact, as discussed later, the concentrations of many of these hormones are abnormal in the diabetic syndrome. The question as to whether or not these hormonal abnormalities are cause or effect will be discussed later. The next section will briefly describe the major metabolic effects of each of these gluco-regulatory hormones.

SPECIFIC METABOLIC REGULATORY HORMONES

Glucagon. Glucagon is the other pancreatic endocrine hormone. It is an activator of the enzyme adenyl cyclase in many tissues. In the liver this leads to an increase in cyclic AMP which tends to increase glucose output and its administration is therefore followed by hyperglycemia. In adipose tissue glucagon is lipolytic, that is it promotes mobilization of fatty acids. In the pancreas, insulin release is increased, thus modifying the degree of hyperglycemia resulting from glycogen breakdown in the liver. The primary substrate controllers for glucagon are glucose and amino acids. In general, the higher the glucose concentration the lower the glucagon secretion rate, and conversely, the lower the glucose concentration the higher the glucagon secretion rate. Amino acids, on the other hand, stimulate glucagon release and insulin secretion. This bihormonal stimulation maximizes storage of these nutrients in muscle during absorption while preventing simultaneous hypoglycemia. Secondary hormonal controllers for glucagon secretion are epinephrine (27) and pancreozymin, both of which stimulate glucagon release. Neural control of glucagon is via the sympathetic nervous system which stimulates glucagon release when activated (38). A parasympathetic control has been recently described (7,27). In general terms, therefore, glucagon increases blood glucose and is secreted in response to ingestion of amino acids or activation of the autonomic nervous system by stress or hypoglycemia.

Growth hormone. This hormone is secreted by the anterior pituitary gland. Although essential for childhood growth, its primary function in adult man remains to be elucidated. Given in large doses, it may acutely mimic the effects of insulin, but in the physiologic range over a longer

period of time, it opposes the action of insulin on a variety of tissues including muscle, adipose tissue, and the liver. Metabolic control of growth hormone is incompletely understood (39), but glucose clearly appears to suppress its release and hypoglycemia to increase it. Amino acids also stimulate the release of growth hormone as does alpha adrenergic stimulation. The physiologic alpha-adrenergic stimulator may be neurally released norepinephrine or dopamine. Growth hormone is also released during certain phases of sleep. The physiologic significance and control of this phenomenon is unknown. The insulin antagonism that is caused by growth hormone is often overcome by an increase in insulin secretion and thus there may be little effect on blood glucose concentration per se. However, should this increase in insulin secretion fail to occur, growth hormone will cause hyperglycemia. Although growth hormone and insulin have been considered antagonistic in glucose homeostasis, this is misleading in the sense that they are synergistic for growth which requires both hormones to be present.

Cortisol. The steroid hormone of the adrenal cortex is regulated by ACTH from the anterior pituitary. Cortisol opposes the action of insulin and allows for the effects of several insulin antagonistic hormones to be expressed. These are lipolysis secondary to growth hormone, glucagon and epinephrine in adipose tissue and glycogenolysis and gluconeogenesis secondary to glucagon and epinephrine in the liver. Cortisol also directly increases gluconeogenesis in the liver and thereby tends to increase glucose output by that organ (2). There are no known substrate controllers for the hormone, regulation being provided by pituitary ACTH. Secretion of ACTH itself is controlled by brain centers in the hypothalamus. These centers release peptides called releasing factors which in turn are regulated by cortisol feedback or nerve impulses from the brain's limbic system during periods of stress. Stresses such as hypoglycemia, surgery, etc., that tend to activate the sympathetic nervous system also activate ACTH and growth hormone release. Therefore cortisol, which is insulin antagonistic, is partly responsible for the hyperglycemia of stress (20).

Gut hormones--secretion, gastrin, and pancreozymin. These have all been shown to stimulate the secretion of insulin directly and to augment the insulin secretion stimulated by a primary substrate such as glucose or amino acids (15,44,47). Pancreozymin has also been shown to stimulate glucagon secretion. It is presumed that these or other related hormones act as signals from the gastrointestinal tract to the pancreas during the process of meal absorption. The presence of food in the stomach is a stimulus for gastrin secretion and the presence of acid in the small bowel is stimulus for secretin, which suggests that these hormones may be relatively nonsubstrate specific. However, one report has claimed that insulin can suppress endogenous secretion levels (10), therefore, it is possible that other endogenous factors control the secretion of these two hormones. Pancreozymin stimulation is believed to be related to a direct stimulatory effect of amino acids in the gastrointestinal tract.

Epinephrine. This catecholamine is released from the adrenal medulla by stress. Epinephrine inhibits the action of insulin in muscle and activates adenyl cyclase in liver and muscle, thereby promoting glycogenolysis in both organs. It also increases gluconeogenesis and from these mechanisms leads to an increased glucose release from the liver. Glycogenolysis in muscle is expressed as increased lactate production which after transport from muscle to liver can be used as a substrate for gluconeogenesis. In addition, epinephrine directly inhibits insulin secretion by activation of pancreatic adrenergic alpha receptors (43, 45). Epinephrine secretion in turn is controlled by pre-ganglionic sympathetic neurons to the adrenal medulla which release acetyl choline

as a neural transmitter. In a sense then, the adrenal medulla is analagous to a ganglion which is activated as part of the general response to stress. Large doses of glucagon stimulate epinephrine release from the adrenal medulla, but a physiological role for this phenomenon has not been described (32). In the pancreas, epinephrine also stimulates the beta cell adrenergic beta receptor to increase beta cell adenyl cyclase. This stimulation of both adrenergic alpha receptors which inhibit insulin secretion, and adrenergic beta receptors which stimulate insulin secretion provides a push-pull control of the islet beta cell. It prevents complete cessation of islet function even during severe stress, and leads to a super-normal compensatory response to glucose after the termination of the stress-related epinephrine effect (45).

Autonomic nervous system. The autonomic nervous system is generally divided into two major, usually antagonistic, sets of neural reflexes which have as effectors either norepinephrine or acetylcholine. Although there may be peripheral dopaminergic and serotinergic neurons, these have not been described well enough to determine whether they play any physiological role as peripheral effectors of the autonomic nervous system. Activation of the sympathetic nervous system produces an integrated response throughout the body leading to changes in all of the organs concerned with fuel metabolism. Stimulation of sympathetic nerves releases norepinephrine which causes glycogenolysis in liver and mobilization of liver glycogen, lipolysis in adipose tissue with release of free fatty acid, inhibition of insulin secretion from beta cells and stimulation of glucagon release from alpha cells. These are augmented by epinephrine released from the adrenal medulla which in addition to all of these effects increases glycogenolysis in muscle with release of lactic acid. Central sympathetic reflexes are also probably involved in the increased secretion of glucosteroids by activation of ACTH release and increased growth hormone output, possibly through central dopaminergic neurons. Most of this system has been well appreciated for many years, but despite a well described neural inervation of the islets of Langerhans by early investigators, the incorporation of the islets into the neuro-endocrine system is very recent (56). Activation of the parasympathetic nervous system releases acetylcholine which produces effects which are opposite to the metabolic effects of sympathetic stimulation. There is an increase in glucose uptake by the liver, and a decreased glucose output from the liver; an increase in insulin secretion and an increase in the release of gut hormones.

Our understanding of substrate feedback to these two systems is really embryonic, but evidence has been presented for central glucose receptors which are insulin dependent (11). These receptors have been related to two hypothalamic nuclei. Electrical stimulation of the ventromedial hypothalamus is associated with a sympathetic-like response with a decrease in plasma insulin and an increase in plasma glucagon (21). This suggests these centers activate nerves to the adrenal, liver, and pancreas. Stimulation of the lateral hypothalamus, on the other hand, has been reported to cause a decrease in blood glucose and an increase in plasma insulin (51). This would suggest this center to activate parasympathetic nerves to the liver and pancreas. The fact that one of the more reliable methods of producing hyperinsulinism and obesity is to destroy the ventromedial hypothalamic nucleus illustrates the importance of these nuclei to body metabolism (3,18,19,55). Such a procedure is reliably followed first by increased insulin secretion and decreased growth hormone, then by increased eating and obesity.

Modulation of physiologic adaptations. The neuroendocrine system acts in a concerted fashion to provide for a smooth interorgan adaptation to several major physiologic states--fasting, exercise, stress, feeding, and growth. The transition from the fed to the fasted state appears to be an integrated sympathetic-like response with a decrease in insulin secretion, and an increase in glucagon, growth hormone, and epinephrine secretion. Exercise appears to be a more powerful stimulus of the same type in which there is a decreased insulin secretion, increased glucagon, growth hormone and epinephrine, and clear-cut activation of the entire cardiovascular related peripheral sympathetic nervous system. Finally, in the most severe form of stress, such as severe exhaustive exercise or a variety of pathophysiologic events, such as burns, myocardial infarction, hypothermia, severe infection, shock, and hypoxia, there is an increased output of ACTH and cortisol as well. In general terms then, activation of the sympathetic system produces physiologically normal but diabetic-like states in non-diabetic persons (25,38,43,44,45).

Feeding appears to be an example of an integrated parasympathetic response, but the overall pattern of response is dependent upon the nutrient absorbed. Glucose ingestion results in an output of gut hormones, particularly secretin, followed by an increase in insulin, a decrease in glucagon, a decrease in growth hormone, and possible a reduction in ACTH (46), although this latter phenomenon is uncertain at the present time. There is also some suspicion that there is a simultaneous suppression of sympathetic nervous system activity, but this has not been documented except for the observation that glucose administered directly into the central nervous system will alter firing of some hypothalamic neurons (1, 56). The pattern of response after amino acid ingestion is a similar parasympathetically oriented response except for an increase in glucagon and an increase in growth hormone which appears to be directly substrate mediated.

Growth, or the long-term adaptation to nutrient ingestion appears to be similar to the feeding response. The nature of the neuroendocrine response has not been well characterized, but growth hormone, thyroid hormone, and insulin are all necessary for growth to occur.

METABOLIC PATHOPHYSIOLOGY OF THE NEUROENDOCRINE SYSTEM

Just as deficient insulin secretion will produce hyperglycemia, so will oversecretion of any of the insulin antagonistic hormones, glucagon, cortisol, growth hormone, and epinephrine, or activation of the sympathetic nervous system. The clinician may be hard pressed at times to differentiate the hyperglycemia produced under these conditions from the genetic diabetic syndrome. In many such instances the patient returns entirely to normal after a period of hyperglycemia. In others some residual disability may be maintained. Which patient has "diabetes"? The situation is further confused by the fact that one patient with a neuroendocrine hormonal abnormality does not have hyperglycemia of any significant degree, whereas another may be severely hyperglycemic and require insulin therapy. Are some patients unusually sensitive to these abnormal hormonal states, or has metabolic stress selected individuals with an inherent genetic diabetic defect which is only expressed when some other pathologic abnormality appears? At the moment there appears to be no simple answer to this question, but to approach it we must first consider the effect of major abnormalities of each of the glucoregulatory hormones.

Excess cortisol secretion. Primary excess cortisol secretion may be due either to a primary adrenal neoplasm with autonomous hormonal release or to a primary pituitary or hypothalamic abnormality resulting in excess ACTH secretion and secondary hypercortisolism. In either case an insulin

resistant state is produced which is coupled with an excessive breakdown of body protein and conversion of mobilized amino acids into glucose through the process of gluconeogenesis in the liver (2). In some individuals compensation occurs by increased secretion of insulin while in others this compensation fails and hyperglycemia ensues (12, 44). Iatrogenic hypercortisolism is now extremely common due to the frequent use of these compounds in various rheumatic diseases. The degree and frequency of hyperglycemia appears to depend upon (a) the dose of cortisol used; (b) the duration for which it is given; and (c) the underlying genetic constitution of the individual (40). Low dose steroids have been used to detect individuals with the presumed diabetic gene. No long-term follow-up of such individuals has been made and therefore it is unknown whether persons developing hyperglycemia after steroid administration have in fact been identified as genetically abnormal people. Since excessive steroid secretion occurs in response to a variety of stresses, it is reasonable to assume that such stress-induced hypercortisolism must contribute to the hyperglycemia observed.

Excess growth hormone. Studies in patients with pituitary tumors secreting excessive amounts of growth hormone (acromegaly) have indicated that there is a general resistance to the hypoglycemic effects of insulin. These patients have increased basal and stimulated insulin levels (44). The mechanism for this resistance is unknown. Many patients with acromegaly will show some deterioration of glucose tolerance and occasionally develop clinically overt diabetes. Some workers believe that overt diabetes will only occur in those subjects with some genetic predisposition (35), but firm evidence for this belief is not yet available. Whenever excess growth hormone is found, an increase in insulin resistance would be expected to contribute to any hyperglycemia observed.

Excessive glucagon secretion. Primary hypersecretion of glucagon has only been reported rarely from tumors derived from islet alpha cells. It is a potent glycogenolytic and gluconeogenic hormone and these properties are evident in such rare patients with a glucagonoma whose diabetic state is difficult to clinically distinguish from other causes of the diabetic syndrome (37). Milder abnormalities of glucagon secretion are common in the diabetic syndrome particularly associated with sympathetic nervous system activation. The etiology of this finding will be discussed later.

Excessive secretion of epinephrine and/or norepinephrine. Tumors of the adrenal medulla are regularly associated with hyperglycemia and glucose intolerance. These hormones are known to be glycogenolytic in both muscle and liver, to inhibit insulin secretion, and to produce antagonism to the peripheral effects of insulin. As circulating hormones they may also activate the sympathetic nervous system and promote further hyperglycemia via neural reflexes (14).

Thyrotoxicosis. Excessive thyroid hormone secretion has been associated with an increased frequency of carbohydrate intolerance. The mechanism of this association is not completely understood but it may be related to increased sensitivity of peripheral tissues to the effects of catecholamines. In some instances there has been improvement of carbohydrate tolerance by the use of catecholamine-depleting drugs (44). Long-term follow-up of patients who develop hyperglycemia during thyrotoxicosis has not been done. Therefore it is again unknown whether this is purely a physiologic or pharmacologic effect of thyroid hormone or whether a genetically susceptible individual has been detected.

Stress states. A variety of stress states have been associated with hyperglycemia and glycosuria. These include trauma, surgery, hypovolemic shock, burns, myocardial infarction, hypothermia, and severe psychologic stress (43, 44, 45). Each of these is characterized by generalized activation of the sympathetic nervous system, with stimulation of epinephrine release from the adrenal medulla as well as high levels of ACTH and cortisol, and growth hormone. More recently hyperglucagonemia has also been reported (6,31,38,49). These all appear to be coordinated effects which can be explained by activation of the sympathetic nervous system. In some cases, alpha adrenergic blocking agents or catecholamine depleting drugs have been used to treat the hyperglycemia observed. In other instances, insulin in combination with glucose, has been administered. In both cases improved mortality and morbidity have been reported to result from this treatment (44, 45). There has been no evidence presented to suggest that some or any of these patients have an underlying genetic defect, but it would seem likely that a person with a genetic predisposition to the diabetic syndrome would be more likely to develop hyperglycemia in the presence of severe stress than a genetically normal subject. Even the mild stress of simple exercise is also associated with inhibition of insulin secretion, increased levels of glucagon, and accelerated glucose production (16). Therefore, there appears to be a continuum from the physiologic response to exercise to the pathophysiologic stress state. The importance of social stress to hyperglycemia remains largely unexplored. Whether such stress related events can be looked upon as beta cell injuries which contribute to the eventual development of permanent islet damage is unknown.

POSSIBLE CONTRIBUTIONS OF THE NEUROENDOCRINE SYSTEM TO ACUTE COMPLICATIONS OF DIABETES

Ketoacidosis. In this form of severe absolute insulin deficiency, growth hormone, glucagon, and catecholamine levels have all been shown to be elevated. These elevations are partly if not completely reversed by administration of insulin. All of these changes are consistent with activation of the sympathetic nervous system and in many ways the whole syndrome of ketoacidosis suggests a caricature of severe stress. Much of what happens in ketoacidosis can be mimicked by administration of these hormones and neuro-transmitters and therefore the question has been raised as to exactly how much of this syndrome is dependent upon them (43). It has been shown that the ketosis is directly dependent upon the mobilization of free fatty acids and can be reversed by inhibitors of fatty acid mobilization, including those that are believed relatively specific for the sympathetic nervous system. It has also been observed that some patients with clinical ketoacidosis may fully recover and not require permanent insulin therapy. This could be explained if the aggravation of the diabetic syndrome were in some way related to reversible elevations of epinephrine, norepinephrine, glucagon, growth hormone, and cortisol. Whether or not these factors are truly etiological, there can be no doubt that the severity of the syndrome must in part depend upon their presence. In some instances patients have been treated with adrenergic blocking agents with a reduction in the frequency of ketoacidosis (4). It would appear that the insulin deficient diabetic is unable to compensate for these counterinsulin hormonal states and in this sense the diabetic state confers an increased susceptibility to the effects of stress on blood sugar. The question then arises as to what role stress and hormonal aberrations play in the day-to-day control of blood sugar in the clinically diagnosed diabetic. Although no well-documented long-term case studies of this effect are available, in short-term controlled studies it has been found that hypnosis and a stressful interview will increase ketone body levels more in a diabetic than in a normal subject (26).

It is also a frequent clinical observation that psycholgical factors play an important role in the day-to-day regulation of glucose level in the diabetic patient. It seems reasonable to hypothesize that it is the neuroendocrine system which is responsible for these effects.

CONTRIBUTIONS OF THE NEUROENDOCRINE SYSTEM TO CHRONIC COMPLICATIONS OR CONCOMITANTS OF THE DIABETIC SYNDROME

The diabetic syndrome consists of four associated abnormalities: 1) microvascular disease, associated with capillary basement membrane thickening; 2) large vessel arterial disease, particularly accelerated atherosclerosis; 3) neuropathy and loss of nerve transmission function; and 4) hyperglycemia and metabolic abnormalities. In general, it is believed that the neuroendocrine system has a role only in the regulation of blood glucose. However, evidence has been produced that this system may play a role in the microvascular disease and the accelerated atherosclerosis.

Microvascular disease. The chance observation that a hypophysectomized patient had a spontaneous remission of diabetic retinopathy has led to considerable effort to determine whether the pituitary produces something which exacerbates diabetic retinopathy. An extensive series of pituitary ablations seems to indicate that there is some amelioration of the progression of the disease and that this occurs despite the replacement of thyroid hormone and cortisol. This has left the gonadotropins, prolactin, and growth hormone as potential candidates for the necessary factor for progression of retinopathy. Most attention has been focused on growth hormone, but the others have not been definitively ruled out. Whether growth hormone secretion is abnormal in diabetes is not totally clear and this will be discussed below. Nevertheless, in the only double-blind series studied, about 50 percent of the patients hypophysectomized have amelioration of the retinopathy (30). Since growth hormone secretion is normally related to a central dopaminergic alpha adrenergic mechanism (39), central adrenergic factors may be related in part to the development of this particular type of vascular degeneration (42).

Accelerated atherosclerosis--role of hormones. Accelerated atherosclerosis occurs as a regular feature of the diabetic syndrome. The mechanism of this association is unknown, but may involve a host of complicating interacting factors such as hormonal factors, lipid abnormalities, and obesity. The best evidence for direct hormonal involvement in atherosclerosis has been related to cortisone, thyroid hormone, insulin, and stress.

Cortisol. Accelerated atherosclerosis has been associated with hypercortisolism whether induced by therapeutic administration of steroids or in patients with pituitary hypersecretion of ACTH (22). Whether this is a direct effect of the steroids on the vessel wall or related to some other induced abnormality, such as the associated hyperinsulinism, or hyperlipidemia, is unclear.

Insulin. Hyperinsulinism has been reported in a variety of atherosclerotic subjects when compared with control groups, although in some cases the controls were not well matched (53). Such an association is of interest because there is now evidence that the arterial smooth muscle cell is insulin sensitive (52). It is well known that insulin is a lipogenic hormone. One hypothesis has been that excessive amounts of insulin or high levels of insulin at inappropriate intervals may predispose this insulin sensitive cell to atherosclerosis (53). Although it is clear that in diabetes there is relative deficiency of insulin secretion in response to challenge, the frequently associated obesity is itself associated with excess insulin secretion in the basal state. Therefore, on an absolute basis, many diabetic subjects have basal hyperinsulinism even if there is simultaneous relative deficiency of the insulin response. The exposure to high levels of insulin

may be exacerbated by the deficient early insulin response which then leads to a subsequent prolongation of the insulin response to meal challenge. In this sense insulin levels are often elevated at inappropriate periods. Even the insulin treated patient may have this problem, since therapy with insulin involves the administration of long-acting preparations in quantities which may be superphysiologic either due to the fact that there are antibodies to insulin or because the normal intermittent secretion pattern of insulin cannot be mimicked by injection therapy, resulting in inappropriately high insulin levels during part of the 24 hour period.

Thyroid. Hypothyroidism has been used as an experimental tool to produce plasma lipid abnormalities and accelerated atherosclerosis for many years (22). In some species this is the only means to induce atherosclerosis, therefore it is not surprising that clinical hypothyroidism in man has been associated with accelerated atherosclerosis. Here again, the clinical studies are not as carefully controlled as the experimental animal. Recently the squirrel monkey has been compared on an atherogenic diet with and without hypothyroidism and there was a striking difference in respect to coronary atherosclerosis (33). Therefore the question has been raised as to whether or not subclinical hypothyroidism may contribute to the atherosclerosis problem.

Catecholamines and stress. Subjects with an increased risk for coronary heart disease have been identified by psychological testing (17). It has been suggested that their biologic response to life stress is the mechanism by which personality relates to atherosclerosis. The pathogenic link between life stress situations and the development of atherosclerosis needs to be documented and explored, but almost certainly involves activation of the sympathetic nervous system, with elevated levels of cortisol, glucagon, catecholamines, growth hormone, and inappropriate secretion of insulin. It is of interest that there is an association between the degree of hyperglycemia during glucose loading and the presence of atherosclerotic complcations (13). Since activation of the sympathetic nervous system would be expected under stress conditions, one could postulate that either the hyperglycemia is associated with coronary artery disease because of stress, or that stress creates changes in metabolic regulation which themselves induce accelerated atherosclerosis. Some have hypothesized that fuels have been mobilized as a response to stress for expected muscular work, but in the presence of a sedentary existence these fuels are rechanneled into lipoprotein production leading to higher lipid levels than expected (54) and accelerated atherosclerosis. Although the diabetic would not be expected to have any unusual propensities for this relationship, diabetes mellitus resembles stress in many ways, and it therefore seems possible that the same series of hormones contribute to atherosclerosis in the diabetic by a mechanism which has much in common with sustained stress. In addition, stress in a diabetic may be expected to be less well counter-regulated than in otherwise metabolically normal persons.

EVIDENCE FOR A ROLE OF THE NEUROENDOCRINE SYSTEM AS A PRIMARY ETIOLOGIC AGENT IN THE DIABETIC SYNDROME

As discussed above, it is not unusual to find elevated concentrations of growth hormone, catecholamines, glucagon, and cortisol in subjects without obvious stress who have been diagnosed as having diabetes. Although in many instances it is possible to relate these abnormalities to either an associated stressful event, or to the stress of one of the complications or concomitants of the diabetic syndrome, some of the same disorders of hormonal regulation have been reported in patients with the mildest abnormalities of carbohydrate tolerance. For example, in a group of

"prediabetic subjects," those with two known diabetic parents and a normal glucose tolerance test, an inappropriately high growth hormone response to glucose was noted during the test which was not present in normal subjects (50). Similarly, plasma glucagon levels have been found to be either abnormally elevated in the basal state or to lack the normal suppression after glucose loading; (8,23,41). There have been few if any studies of catecholamines or cortisol in such subjects, but there is one patient reported by Robertson who was found to have an apparently overactive alpha-adrenergic receptor (48).

Since the central nervous system is known to contain glucose-responsive neurons which are in or near the hypothalamus, one is tempted to integrate these findings and suggest that all of the hormones known to be related to the sympathetic nervous system may be inappropriately activated. Whether this activation represents some primary phenomenon in the diabetic syndrome or is secondary to a basic abnormality of the pancreas is unknown but there is no *a priori* reason for suggesting one possibility over the other. There is a considerable body of evidence that there is a basic problem in the recognition of glucose by the beta cells of the endocrine pancreas. It seems conceivable that this problem may be more generalized than realized and that glucose may not be adequately recognized in other endocrine cells, such as the alpha cell, or in central regulators of fuel metabolism, such as the glucose receptors of the hypothalamus. Certainly the levels of many counter regulatory hormones appear to be inappropriate for the hyperglycemia found in diabetes because they are normal or elevated at a time when they might be expected to be suppressed.

One other potential role for the neuroendocrine system as an etiologic agent in diabetes is in relation to the observation that acute injuries often appear to be in some way related to the onset of diabetes mellitus. Although genetic constitution obviously plays an important part in the underlying etiology of the disease, it has been suggested that the apparently low penetrance may be due to the need for some additional factor to precipatate the clinical illness. Infection, surgical procedures, myocardial infarction, etc. have often seemed to play an important role in this precipitation. The question arises as to whether repeated abnormalities in neuroendocrine control might provide a form of injury which eventually leads to clinical diabetes mellitus. It is of some interest that in a study by Loubatieres (34) of the effects of catecholamines on pancreatic endocrine function in dogs, partial pancreatectomy followed by infusions of catecholamines appeared to result in a permanent diabetic syndrome. Thus the neuroendocrine system, particularly in terms of stress activation, may be one of the injurous factors which precipitates the expression of the clinically overt diabetic state.

RELATION OF THE NEUROENDOCRINE SYSTEM TO INSULIN RESISTANCE

Ever since the demonstration that hypophysectomy or adrenalectomy markedly increase peripheral sensitivity to the effects of an injected dose of insulin, it has become clear that hormones play a major role in determining the sensitivity of many tissues to insulin. Hormones, such as cortisol, growth hormone, glucagon, and epinephrine all induce insulin resistance and have already been discussed. There are several clinical syndromes such as uremia, pregnancy, and chronic liver disease where resistance to the action of insulin has also been demonstrated. In some cases it may be that hormones are playing a role in the nature and degree of this resistance. However in obesity, the most common and important cause of insulin resistance, there is no evidence that this resistance is related to hormonal factors. Growth hormone, in fact, is usually believed to be lower than

normal, and although there is increased secretion rate of corticosteroids, plasma levels are normal (5).

On the other hand, the genesis of the obese state probably involves some disturbance of the hypothalamic recognition of total fat mass. There is a series of studies indicating that in some animal models with spontaneous obesity the weight gain is regulated (55). That is, if additional calories are force-fed, there will be a return to the original overweight state when forced overfeeding is stopped. Similarly, if calories are restricted, weight is lost, only to be regained to the original state (but not beyond) when free feeding is allowed. This phenomenon is common in human obesity. Obese subjects rarely have difficulty in losing weight for a short period of time, but often regain it promptly. This has led to the concept that human obesity may also be a regulated obesity (55). To explain this phenomenon one should consider the ventromedial hypothalamic lesion as one of the striking animal models for obesity with a known pathogenesis (55,56). When obesity is produced by destruction of the ventromedial nucleus of the hypothalamus these animals immediately become hyperinsulinemic which is followed by overeating and obesity. If they are force-fed after reaching a new stable elevated weight, they will immediately lose any excess weight due to the force feeding and return to their original degree of obesity. In this case then, damage to the hypothalamus leads to a regulated obesity. It has usually been assumed that the association between diabetes and obesity in man is due to the stress that the insulin resistance of obesity places upon the islet, requiring increased secretion of insulin, both in the basal and stimulated states. However, in the ventromedial lesioned animals, it is quite clear that there is a central defect which leads to hyperinsulinism prior to the obesity, and the insulin resistance then follows. Although at this time there is no solid evidence for it, it is possible that the clinical associations of these two syndromes may in certain instances be related to some central defect in appetite regulation in the genetically diabetic subject which leads first to hyperinsulinism and obesity and only later to defective insulin release and diabetes.

KNOWLEDGE GAPS

It is obvious from this discussion that the neuroendocrine system and its controls are only understood in an elementary way at the present time. There is a particular lack of knowledge of the central integrating mechanisms for the neuroendocrine system and how communication between the periphery (e.g., the size of the adipose mass) and the appetite regulating centers occurs. Furthermore, the nature of the central receptors and their location, sensitivity, and to which specific hormones they are related are only poorly understood. It should also be clear that the action of any one hormone may be influenced and altered by the simultaneous action of another, and that endocrine actions often include stimulatory as well as repressive activities upon the secretion of the glands. Although these are qualitatively describable at the present time, the quantitative nature of these interactions is very incomplete. Therefore the relative importance of substrate controllers versus hormonal controllers to the overall regulation of fuel metabolism cannot as yet be assigned with any precision. The finding that there are many hormonal aberrations in diabetes mellitus in addition to defective insulin secretion still leaves the question of whether these hormonal aberrations are secondary to the underlying inability to secrete insulin or whether some of these hormonal aberrations produce the diabetic syndrome or influence insulin secretion per se as a primary event. Perhaps one of the most important unknowns is the nature of the controller

for integration of appetite and weight. There seems to be no doubt that the frequency of clinical diabetes mellitus is very definitely increased in populations of excess body weight. If this factor could be understood and treated one might expect a marked amelioration of at least the carbohydrate abnormalities that have been observed. Whether chemical or other modifications of this system in an attempt to restore the abnormal hormonal set of the diabetic to a more normal physiologic function is useful or feasible is totally unknown, and cannot even be approached at the present time without further understanding of the basic system. It would seem that knowledge at the present moment is poised at the point where there is general recognition that hormones, such as insulin, are not solely regulated by primary substrate messages delivered through the circulation, but are also importantly controlled by circulating hormone controllers and by direct neural input as well. The integration of the neuroendocrine system through the autonomic nervous system is at best imperfectly understood and is the major factor preventing the determination of its influence upon the diabetic state.

In order to rectify these gaps, it will be necessary to have an integrated interaction between psychologists, neuroendocrinologists, and clinicians interested in the problem of diabetes mellitus. At the present time, people in each of these disciplines are interested in their own subset of problems relating to the overall system, but there has been very little interaction between any of the three, except for rare collaboration between investigators with neurophysiological and clinical experience, or psychologists with persons experienced in metabolic problems. It would seem at the present time that if major progress were to be realized, it would necessarily require the collaborative efforts of these disciplines.

A major gap in clinical information relates to the long-term follow-up of individuals who have been identified during some period of stress as having an abnormal carbohydrate metabolism during stress. That is, in subjects who develop hyperglycemia in the presence of myocardial infarction, surgery, infection, or administration of estrogens, steroids, etc., there has been almost no information available as to what percentage of these subjects eventually develop clinical diabetes mellitus as compared with appropriate control groups. Although it is commonly assumed that such stresses have in fact identified genetically abnormal persons, only a long-term cooperative study of such individuals could realize this potential. This is of course important if we are to understand the natural history of the diabetic syndrome and would be necessary if we are to identify those individuals in whom preventive measures should be undertaken.

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ACUTE COMPLICATIONS OF DIABETIC STATE

10

DIABETIC COMA

Daniel W. Foster

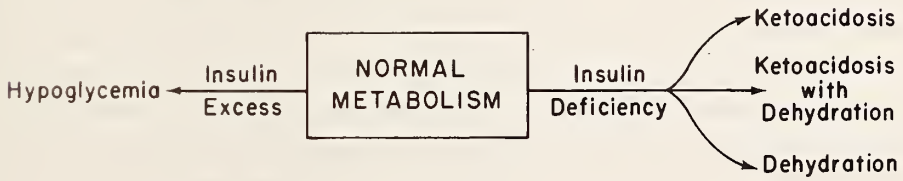
BACKGROUND

The diabetic patient is vulnerable to a series of acute metabolic complications. As indicated in Figure 1, the insulin deficiency which characterizes this disease may result in a spectrum of disorders which tend to cluster in three recognizable (but overlapping) syndromes:

(1) Relatively pure ketoacidosis (minimal or no dehydration),

(2) Ketoacidosis with dehydration,

(3) Relatively pure dehydration (minimal or no ketosis).



The first two syndromes are commonly designated as "diabetic coma" or "diabetic ketoacidosis," while the third is generally defined as hyperosmolar, nonketotic coma. From a physiologic standpoint, the clinical presentations can be understood by considering that uncontrolled diabetes is manifested by severe hyperglycemia, which causes dehydration, and by overproduction of ketone bodies in the liver, which results in a metabolic acidosis. If the former predominates, the clinical picture is that of hyperosmolar coma, to be discussed in a subsequent section, while if the latter takes precedence, the presentation will be that of diabetic coma, the subject of this discussion.

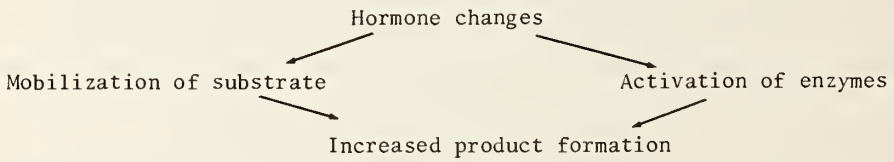
It should be emphasized in the beginning that diabetic coma is not a rare condition, although patients at risk are primarily those with severe enough disease to require insulin therapy. At the Los Angeles County-University of California Medical Center, there were 340 admissions for ketoacidosis (with 32 deaths) in a 3-year period (6). Since each episode may be life threatening to the individual and since each admission requires a significant period of hospitalization, it is clear that advances in the understanding of the ketogenic process, which might lead to prevention or more rapid treatment, would be of substantial benefit to the individual diabetic and to society as a whole in terms of public health and its cost estimate (economic impact).

CURRENT STATE OF KNOWLEDGE

The problem of diabetic ketoacidosis has been reviewed recently (38). A brief summary of the pathophysiology and clinical picture can be constructed as follows. Diabetic coma is initiated by a deficiency of insulin which is more profound than that found in the uncomplicated

diabetic state. Very recent studies (49), utilizing a connecting peptide immunoassay to assess insulin secretion, have shown essentially no insulin secretion during the period of ketoacidosis, while insulin release (at least in some patients) subsequently may return to measurable levels. Concomitant with insulin deficiency, there appear to be changes in other hormones with increased plasma concentrations of cortisol, growth hormone, and glucagon being regularly observed (28, 43). Associated with the hormonal changes there is an activation of gluconeogenesis, which, when coupled with a diminished peripheral utilization of glucose, results in marked hyperglycemia with its consequence of osmotic diuresis, volume depletion of body fluids, osmolar concentration, and ultimately shock and renal shutdown. At the same time ketone body formation begins to increase and progresses to the severe metabolic acidosis which characterizes diabetic coma. Essentially all of the clinical aspects of the ketoacidotic picture are directly traceable to these two processes and their interrelationships. The mechanisms whereby they are regulated thus assume major importance.

a. Gluconeogenesis: The control of gluconeogenesis in starvation and diabetes has been the subject of enormous study but remains a matter of controversy. The problem, as can be seen from Exton's review (22) of the subject, is that control has been postulated for many different sites and evidence can be adduced to support each of these. As emphasized by Srere (52) such data, particularly that obtained in vitro, may have little relation to the situation operative in the intact organism. Doubtless all major pathways of intermediary metabolism are under multiple interlocking controls to assure normal function. It follows that in disease states abnormalities likely exist at multitudinous points. Nevertheless, in the broad sense, it can be stated that gluconeogenesis, under the influence of the hormonal changes mentioned above, is activated both by the provision of increased quantities of substrate and by acceleration of certain key enzymatic reactions of the gluconeogenic pathway in the liver and perhaps the kidney. Thus the following general formulation can be put forth:



In the case of gluconeogenesis the substrates mobilized are amino acids, glycerol, lactate, and pyruvate (17,22,23). The uptake of the amino acids in the liver may also be hormonally controlled, though evidence for such a process with the other substrates is lacking. From the standpoint of enzyme activation, attention has focused on four sites in the pathway from pyruvate to glucose; pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-diphosphatase, and glucose-6-phosphatase. Activities of these enzymes appear to increase in concert when gluconeogenesis is activated. It is also thought possible (or likely) that the counterpart enzymes of the glycolytic pathway (e.g., phosphofructokinase, pyruvate kinase, pyruvate dehydrogenase) are inhibited under these circumstances. These changes can apparently occur very rapidly (4-5 minutes) under certain circumstances (53). No firm insight is available as to the mechanism of activation of gluconeogenic enzymes, though both allosteric regulation and increased enzyme synthesis are presumed to play a role. Whatever the mechanism, the end

result is a marked overproduction of glucose by the liver: in diabetic coma hepatic glucose release may reach 1000 G a day, some 3 times higher than the maximum rate attained in starvation (12, 17).

b. Ketogenesis: The general formulation given for enhanced gluconeogenesis in diabetic coma is also applicable to the increase in ketone body formation. Here again the process is thought to be the result of hormonal changes which cause mobilization of substrate to the liver and which alter the metabolism of that organ by changing enzyme activity. In this case the substrate mobilized is free fatty acid from peripheral fat stores while the products are acetoacetic and β-hydroxybutyric acids. The enzymic sequence primarily involved is the β-oxidation pathway for fatty acids (39). Under normal circumstances free fatty acids taken up by the liver are utilized primarily for triglyceride synthesis, while relatively little is oxidized to CO_2 or ketone bodies. During starvation or diabetes, on the other hand, a very significant fraction of the fatty acids taken up enter the oxidative pathway. In contrast to the situation with gluconeogenesis, control of ketogenesis may be primarily vested in a single enzymatic reaction, the long chain fatty acylcarnitine transferase reaction. Compelling evidence for this conclusion is the demonstration that octanoic acid, which does not require a carnitine mechanism for transfer into the mitochondrion, is oxidized at similar rates by livers from normal, starved, and diabetic animals. Moreover, while reversal of ketosis by insulin in vivo cannot be overcome by infusing long chain fatty acids (8) the infusion of octanoate immediately restores ketone production to the previous rate (36). Such findings have led to the concept that the capacity for β-oxidation in the mitochondrion is fixed and high and that the rate-limiting step for the sequence is the transport of the long chain fatty acid through the mitochondrial membrane. Once this is accomplished, oxidation to acetyl CoA occurs rapidly with the subsequent formation of ketone bodies. When flux through the pathway is rapid, the utilization of acetyl CoA in the Krebs cycle and for fatty acid synthesis is minimal and the bulk of the acetyl CoA is converted to acetoacetate and β-hydroxybutyrate. Accordingly, significant importance has been attached to the possible role of the long chain acylcarnitine transferase reaction, though to the present little information is available regarding its control (44).

It should be noted that the removal (utilization) system for ketone bodies in peripheral tissues appears to be saturable and that the final level of plasma ketones is the consequence of both increased hepatic production and impaired utilization (4,5,35).

c. Clinical picture and treatment: Since the clinical details of diabetic ketoacidosis are well known, it will not be described in detail. The composite picture is one of hyperglycemia, intracellular and extracellular fluid volume deficit with osmolar concentration, total body potassium and phosphate depletion, and severe metabolic acidosis. Therapy, which is generally successful, consists of the administration of large amounts of insulin and saline infusions with careful attention to serum potassium concentrqtions (38).

INFORMATION NEEDED TO BE ACQUIRED THROUGH RESEARCH

No attempt will be made to outline specific and detailed experiments in this section or to exhaustively list unsolved problems. However, certain major questions needing to be addressed will be indicated as representative problems under four major categories:

a. The physiology of insulin and its mechanism of action,
b. The control of gluconeogenesis,
c. The control of ketogenesis,
d. Clinical problems.

a. The physiology of insulin and its mechanism of action: Few hormones have received the extensive study accorded insulin. While a great deal has been learned regarding its effects in man and various animal species, a number of fundamental questions remain that are immediately related to the problem of diabetic coma.

(1) How does insulin work in the liver? This is the most basic of all questions. There is little doubt that insulin has a direct effect on hepatic metabolism in vivo which is powerful and rapid (26.41) yet effects in vitro are extremely difficult or impossible to demonstrate. It is clear that insulin binds to hepatic plasma membranes (19) as it does to other tissues, and it has been possible to show effects of insulin on glycogen synthesis and gluconeogenesis (30) in the perfused rat liver. The changes are of small degree, however, and often restricted to rigidly defined conditions. In regard to ketogenesis the record is even more blank. In many experiments under widely varying conditions, Dr. McGarry and I have never been able to show a depressant effect of insulin on ketone body production in vitro (37). While experiments should continue to be directed at biochemical interrelationships of insulin such as with the cyclic AMP system and membrane enzymes like the oubain inhibitable ATPase (11), it seems clear that another fruitful area for investigation would be hepatic structural changes produced by insulin (47). Finally, consideration should be given to the possibility that the failure to demonstrate insulin effects on the liver in vitro might be related to a marked activation of insulin degrading systems (20). Major effort should be expended on these problems.

(2) What is the nature of insulin resistance in diabetic ketoacidosis? It is a well-known clinical observation that small amounts of insulin normally suppress hepatic ketone body production even under circumstances where hyperglycemia is not controlled. On the other hand, in diabetic acidosis very large quantities of insulin are ineffective in rapidly reversing ketogenesis. It is not known whether this resistance is due to plasma factors (24) or to changes in the liver itself.

(3) Why does insulin release stop in ketoacidosis? As mentioned above, Rubenstein and coworkers have shown that insulin release becomes immeasurable in ketoacidosis. The mechanism is totally unknown. Since a variety of stresses are known to precipitate ketoacidosis, the presumption would be that the cut off is a final common pathway of stress, possibly hormonal in nature. A number of mechanisms are already recognized for the normal control of insulin secretion (46) but to the present careful evaluation of control phenomena in ketoacidosis has not been carried out. The catecholamines would appear to be likely candidates here, but attention should also be given to interaction with other hormones (such as gastrin) which seem to function primarily as facilitators of the normal beta cell response to glucose (48). Similarly, it would be extremely important to know if the synthetic pathway for insulin (54) is altered during the acute episode of ketoacidosis (or immediately preceding it). At the present there is no possibility of carrying out these studies in humans, requiring that an

animal model be used. The latter is complicated by the fact that ketoacidosis does not occur spontaneously and would have to be induced.

(4) Is the insulin:glucagon ratio a unique function? Recently a number of investigators, following the suggestion of Unger (55), have indicated that the insulin:glucagon ratio in plasma may play a distinct role in uncontrolled diabetes; i.e., rather than considering the abnormalities of diabetic coma as the integrated result of multiple hormonal changes, the hypothesis has been put forward that a unique role is played by the combination of insulin deficiency and glucagon excess. This issue, while difficult, can be approached in the experimental animal utilizing antiglucagon antibodies during the induction of diabetic ketoacidosis or through the development of specific inhibitors of glucagon release. The latter compounds could have clinical usefulness. Preliminary studies in this area clearly need to be expanded.

b. The control of gluconeogenesis: Since gluconeogenesis, as mentioned above, contributes in a major way to the hyperglycemia of diabetic coma and other forms of uncontrolled diabetes, broader understanding of its control is needed.

(1) Does glucagon stimulate gluconeogenesis in man? There seems to be little question that glucagon stimulates gluconeogenesis in the rat (9, 10). On the other hand, Madison (42) has been unable to confirm a gluconeogenic effect in the dog. Definitive information on this point is sorely needed. If glucagon enhances gluconeogenesis, the mechanism must be further explored. From studies in rat liver, it has been suggested that glucagon only works in the presence of adrenal corticosteroids (21) but the precise site of action remains uncertain (10).

(2) How is gluconeogenesis controlled? As mentioned above, a great deal of work remains to be done regarding the control of gluconeogenesis. For example, the once quite popular theory of regulation by the redox state of the cell has come to be doubted (29) and current work emphasizes the role of substrate phosphorylation, particularly GTP formation (16). The critical issue is to discern the operating features in vivo, since so many mechanisms appear to be operative in vitro. At the present time no good techniques exist for correlating in vitro and in vivo findings and new methods will obviously have to be developed.

(3) What is the relationship of gluconeogenesis to ketogenesis? Both of these processes are accelerated in diabetic coma, and the general rule is that when flux is accelerated over one pathway, it is also increased in the other. Indeed, Flatt (25) has suggested that ketogenesis is limited by the rate of gluconeogenesis. On the other hand, there are a number of circumstances in which the two can be separated. Preeminent here would be the infusion of lactate to the isolated perfused liver from ketotic animals; this substrate stimulates gluconeogenesis but inhibits ketogenesis (36). Of even greater interest is the observation (McGarry and Foster, unpublished) that alcohol, which is potently antiketogenic in the presence of active gluconeogenesis, is totally ineffective when gluconeogenesis is blocked. Dissection of these interrelationships may provide clues to the fundamental mechanism through which carbohydrate and lipid metabolism are linked. (This relationship is seen in classic fashion when the accelerated fat metabolism and ketosis of starvation are reversed by carbohydrate feeding).

c. The control of ketogenesis: Because of the close interrelationship between the mechanism of insulin action, the control of gluconeogenesis, and the regulation of ketogenesis, several problems relating to ketogenesis have already been mentioned. Other representative

areas needing exploration are identified by the following questions:

(1) How is fatty acid oxidation controlled? As has already been described, ketone body formation is essentially regulated by the rate of fatty acid oxidation, which in turn appears to be controlled directly or indirectly by the presence of glucose and insulin. Since glucose itself is not antiketogenic in vitro, while products or precursors such as lactate and dihydroxyacetone are (37, 58) an attractive possibility is that some glycolytic-gluconeogenic intermediate acts allosterically to activate the beta oxidation sequence. For reasons outlined earlier in the discussion, the prime candidate for the site of such control is the long chain acylcarnitine transferase reaction. While considerable new information regarding this enzyme is available (15,33), basic control mechanisms remain to be elucidated.

(2) What controls hepatic lipase activity? As emphasized earlier, accelerated ketogenesis can only be sustained if the supply of substrate, fatty acid, is maintained high. It is well known that the diabetic liver has an increased triglyceride content and that the isolated perfused liver from diabetic animals continues to make ketone bodies at a high rate in the absence of exogenous substrate (37,56). Since the hormone sensitive lipase of adipose tissue is rapidly inhibited by insulin (32), while lipase activity in the liver is not (at least in the perfused liver), it follows that study of the hepatic enzyme is imperative. Indeed, since there are several hepatic lipases (3), the enzyme coupled with ketosis will have to be identified.

(3) What limits ketogenesis in hyperosmolar coma and generalized or partial lipodystrophy? The absence of ketosis in hyperosmolar coma remains an enigma (27). Similarly, the absence of ketosis in the lipodystrophies needs to be explained (51). The problem is difficult, but in the former state portal vein insulins need to be measured. Since peripheral blood insulin values are equivalent in diabetic ketoacidosis and hyperosmolar coma, both types of patients need to have ketone response measured after ingestion of medium chain triglycerides as a source of octanoic acid. This would indicate whether or not the ketogenic machinery itself is intact.

(4) Is there a role for inhibitors of fatty acid oxidation in human ketoacidosis? McGarry and Foster (40) have reported the rapid reversal of experimental diabetic ketoacidosis in the rat with (+)-decanoylcarnitine. The importance of this observation lies in the fact that recovery times with (+)-decanoylcarnitine may be one-half or less those achievable with insulin alone. More recently we have synthesized a homologue of (+)-decanoylcarnitine which is equally potent in reversing ketoacidosis but which has remarkably diminished surface active properties that should make it much safer for in vivo use. The possibility of utilizing pharmacologic agents of this sort in human diabetic coma has much potential. Studies in this area clearly need to be expanded.

d. Clinical problems: The following are examples of clinical problems that need to be addressed.

(1) What is the cause of central nervous system dysfunction in diabetic coma? It is a remarkable fact that despite many years of study, the nature of the central nervous system depression in ketoacidosis remains unknown. The interrelationships between CSF pH (45), cerebral oxygen uptake (31) and direct ketone toxicity (50) all need to be carefully reexamined.

(2) What is the mechanism of cerebral edema during treatment of diabetic acidosis?

The now well-recognized syndrome of cerebral edema associated with reversal of human ketoacidosis (60) is thought to be associated with osmotic disequilibrium between plasma and brain during the rapid lowering of the blood sugar (18). However, precise mechanisms have not been worked out and the possibility of direct membrane damage is intriguing (2). The area is ripe for further investigation.

(3) Should phosphate solutions be used routinely in diabetic coma? In view of the depletion of red cell 2,3-diphosphoglycerate in diabetic ketoacidosis (7), it has been suggested (38, 1) that phosphate salts might be useful in treatment. While there are sound physiologic arguments to support this suggestion, controlled clinical studies are needed to test the idea.

APPLICATION TO PREVENTIVE MEDICINE

It is obvious that the complications of uncontrolled diabetes, such as diabetic coma, are enormously costly from the standpoint of morbidity, mortality, and economics. Studies of the type outlined should, over the short range, provide new and improved forms of treatment for diabetic ketoacidosis. I believe this will happen within the next 3 years. From a long-range standpoint, basic understanding of the pathophysiology may lead to ways of completely preventing the onset of diabetic coma.

SUMMARY

Diabetic coma is a serious clinical problem about which much remains to be learned. It is not necessary to search for new areas of research, since so many basic questions are recognized and are obviously in need of answers here and now. This chapter is intended to indicate this fact.

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11

HYPEROSMOLAR COMA

James B. Field

Although patients with severe hyperglycemia, hyperosmolarity, profound dehydration, and altered central nervous system function without ketoacidosis had been observed previously, the report by Sament and Schwartz in 1957 (27) focused attention on the entity which is now known as hyperosmolar nonketotic coma. The profound hyperglycemia is primarily responsible for the increased plasma osmolarity, but almost all of the patients also have azotemia which contributes to the hyperosmolarity. In some patients the hyperosmolarity is further aggravated by hypernatremia. Despite the absence of ketoacidosis, many of the patients have significant reductions in plasma pH as a consequence of lactic acidosis, uremia, or increased H^+ concentration of unknown source. The central nervous system manifestations of the syndrome may be quite varied and range from absence of deep tendon reflexes to focal neurologic signs and confusion; coma and convulsions (16). Several recent reviews have examined various aspects of this entity (1,2,11,18,28).

Hyperosmolar coma is approximately one-sixth as frequent a cause of coma in diabetics as ketoacidosis (11). Although the syndrome is much more common in the older age group, Rubin et al. observed six pediatric patients with this entity in less than one year (26). The youngest patient in their series was 6 weeks old. Most of the patients were not known to have diabetes prior to the development of hyperosmolar coma (18). Almost all of the patients who had been previously diagnosed as having diabetes had mild maturity-onset diabetes which was treated with either diet or oral hypoglycemic agents. However, the syndrom has developed in insulin-dependent patients some of whom had previous episodes of ketoacidosis (14). In many patients, a precipitating infection or an underlying disease can be implicated in the genesis of this entity. Only two of the twenty patients observed by Gerich et al. did not have any underlying disease (11). Hyperosmolar coma has occurred in association with hemodialysis (23), peritoneal dialysis (4), severe burns (25), tube feedings (32), and after intravenous hyperalimentation (8). It has been reported in patients who developed severe dehydration from a variety of other causes including infantile diarrhea or central nervous system disease which interferes with the patient's ability to drink. An ever-increasing list of drugs has been implicated including dilantin (13), diuretics (thiazides and furosemide)(22), steroids (28), and immunosuppressive drugs (30). Seventeen of the twenty patients reported by Gerich et al. were receiving diuretics (11). Thirteen of these were taking thiazides and two furosemide.

The syndrome develops more insidiously than does diabetic ketoacidosis, and in one series the average duration of symptoms prior to admission was 12 days (11). The most common symptoms are weakness, polyuria and polydipsia, reflecting the osmotic diuresis induced by the glycosuria and its attendant dehydration. Vomiting, a lack of thirst appropriate to the dehydration, or an inability to obtain adequate fluids can all accentuate the dehydration. Most of the patients manifest profound alterations in their state of consciousness, but occasionally they may be quite alert and well oriented (11). Initially, the symptoms of the precipitating or associated condition may dominate the clinical picture. Dehydration is the most striking finding on physical examination.

The average blood sugar is usually greater than 1000 mg percent and the plasma osmolarity usually exceeds 350 mosm/liter (2,11,18). The serum sodium has varied from 118 meq/liter (2) to as high as 188 meq/liter (18). Generally, the serum K^+ is normal, but patients with both hypokalemia and hyperkalemia have been observed (11). Characteristically, the syndrome is not accompanied by ketoacidosis, but many of the patients have significant reductions in the plasma pH and bicarbonate for other reasons. In one series of 71 patients, 29 had significant metabolic acidosis due to either renal failure or lactic acidosis (18). In another series, the cause of the acidosis in most patients was unknown (2).

Many aspects of the pathogenesis of this entity are unknown or only poorly understood. Although the majority of patients were not known diabetics prior to their illness, it seems most likely that they had some preexisting abnormality in their ability to metabolize carbohydrate. This is substantiated by the observation that the survivors of hypersomolar coma have almost uniformly demonstrated abnormal carbohydrate tolerance. Most of them have been successfully treated with diet or diet and oral hypoglycemic agents but occasional patients, especially in the younger age group, have required insulin (2,11,26). Infections of various types have been the most common precipitating illness (2,11,18). Such stress undoubtedly has contributed to the deterioration of carbohydrate metabolism and the ensuing hyperglycemia and glycosuria. The resultant osmotic diuresis accounts for the dehydration, especially if the patient is unable to replace adequately his fluid loss. This might reflect the associated nausea and vomiting of an underlying disease or an inability to obtain adequate fluids due to some alteration of central nervous system function. In addition, the studies of Singer et al. suggested that diabetic patients might have some abnormality in the regulation of their thirst mechanism (29). These investigators reported that asymptomatic, maturity-onset diabetic patients had hyperosmolarity of the serum which correlated with their degree of hyperglycemia. During the period of polydipsia, patients often consume large quantities of carbohydrate-containing beverages which contribute significantly to the hyperglycemia. As long as normal renal function persists, hyperglycemia is somewhat limited as the excess glucose is excreted in the urine. However, many of the patients have underlying renal or cardiovascular disease which has already compromised their renal function. As a consequence of dehydration and pre-renal, azotemia, glomerular filtration rate is reduced and renal excretion of glucose is diminished. Development of shock in a significant number of patients has been an important factor in the high mortality of this entity (1,2,11,18,26,28).

The reasons for the severe alterations in central nervous system function are poorly understood. During shock, decreased perfusion of the central nervous system is obviously an important factor. This may be especially true in older patients who might already have areas of compromised circulation. Hyperglycemia probably also makes a significant contribution. Geiger observed that the threshold for glucose penetration into the brain is about 400 mg/100 ml (10). Thus, the profound hyperglycemia causes significant dehydration of the cells in the central nervous system. Although the glucose concentration was significantly higher in the plasma than the cerebrospinal fluid, Arieff and Carroll found that the two were in osmotic equilibrium because of elevation of sodium and its anions in the cerebrospinal fluid (2). Alterations in acid-base balance do not appear to be important in the central nervous system involvement. Arieff and Carroll found no significant difference between the pH of arterial blood and cerebrospinal fluid (2). In those instances where arterial pH was less than 7.15, cerebrospinal fluid pH was always higher than the

arterial pH. In this study, the cerebrospinal fluid K^+ was very close to 2.9 meq/liter despite wide variations in plasma K^+, ranging from 2.2 to 7.8 meq/liter. Maccario has contrasted the frequency of neurologic signs in patients with hyperosmolar coma in comparison to those in diabetic coma (16). Although the hyperglycemia is greater in the former patients, it seems unlikely that this is the entire explanation for the observed differences.

One of the main unsolved aspects of this entity is the absence of ketosis. Most of the patients have mild maturity-onset diabetes, which is usually not associated with diabetic ketosis. However, some of the cases have occurred in insulin-dependent diabetic patients (2,11) or patients who had previous episodes of diabetic ketoacidosis (2). DiBendetto et al. suggested that hyperglycemia per se might be an important factor (7) since Mirsky et al. prevented ketosis in pancreatectomized dogs by glucose infusions (2). However, such hyperglycemia does not prevent ketosis in diabetic ketoacidosis indicating that other factors must be of greater importance. Since plasma free fatty acids are the precursors for ketone body production by the liver, alterations in release of free fatty acids from adipose tissue or their conversion to ketone bodies in the liver could account for the absence of ketosis. Gerich et al. reported that plasma FFA levels in patients with hyperosmolar coma (mean 958 $\pm$ 126 μeq/liter) were higher than normal but not nearly as elevated as the values observed in a group of patients in diabetic ketoacidosis (2,256 $\pm$ 250) (11). Arieff and Carroll also noted only moderate elevations of plasma FFA in patients with hyperosmolar coma (1), although the range was up to 1338 μeq/liter, a value which overlaps that found in some patients with ketoacidosis. More significant elevations of FFA were present in the patients observed by Vinik et al. (31). Their patients also had elevated triglyceride levels suggesting that the mobilized FFA were being utilized by the liver. Lipolysis in adipose tissue can be influenced by several hormones, and these have been measured in an attempt to explain the absence of ketosis. Since insulin is a potent antilipolytic agent, it has been suggested that patients in hyperosmolar coma, although insulin-deficient, have sufficient insulin to inhibit lipolysis but not to maintain normal carbohydrate metabolism. Gerich et al. found that plasma insulin values were no different in patients in hyperosmolar coma compared to those with ketoacidosis, suggesting that this was not responsible for the absence of ketosis in the former condition (11). However, Arieff and Carroll reported an average insulin concentration of 17.2 $\pm$ 7.2 μU/ml in their patients, significantly greater than the very low values which have usually been recorded in diabetic ketoacidosis (1). Vinik et al. reported plasma insulin values from 0 to 50 μU/ml in seven patients with hyperosmolar coma (31). Deficiency of factors which mobilize fatty acids might also contribute to the absence of ketosis. Gerich et al. observed that plasma growth hormone and cortisol values were significantly lower in patients with hyperosmolar coma as compared to those with diabetic ketoacidosis (11). None of the patients with hyperosmolar coma had growth hormone values exceeding 4 mμg/ml. Suppressed growth hormone values were also recorded by Vinik et al. (31) while plasma cortisol levels were significantly raised. However, Arieff and Carroll felt that growth hormone, which ranged up to 11.5 mμg/ml, was not deficient in their patients (6). Furthermore, Milloy reported development of hyperosmolar coma in a patient with acromegaly and a plasma growth hormone level of 122 mμg/ml (19). Catecholamines have not been reported in patients with hyperosmolar coma nor has their plasma FFA response to the acute administration of various lipolytic agents. It is thus possible that decreased mobilization of plasma FFA may be an important contributing factor to the absence of ketosis in this syndrome.

Dehydration and hyperosmolarity have also been implicated in the lack of ketosis. Passmore and Johnson demonstrated that dehydration diminished the ketosis induced by exercise (21). In addition, Gerich et al. observed that dehydration decreased levels of plasma FFA and ketone bodies in fasted rats (12). These changes were not associated with any significant modifications of plasma insulin compared to control animals fasted but not dehydrated. Dehydration impaired glucose tolerance, the insulin response to glucose, and the FFA response to epinephrine. Dehydration consequent to mannitol administration also lowered FFA and ketone body concentrations in the plasma of fasted rats. In vitro, hypertonic mannitol also significantly reduced release of FFA from rat adipose tissue and insulin from rat pancreas. Although defective hepatic production of ketone bodies from FFA could also contribute to the absence of ketogenesis, such patients have not usually had evidence of hepatic dysfunction. Furthermore, Vinik et al. interpreted their observation of elevated triglyceride concentrations in such patients as evidence for hepatic utilization of FFA (31). Gerich et al. reported that hyperosmolarity induced by mannitol did not inhibit ketone body formation in the isolated, perfused liver (12). Thus, these studies suggest that dehydration and hyperosmolarity could impair the insulin response to hyperglycemia allowing the development of even higher levels of blood glucose. Although the dehydration might tend to inhibit lipolysis, the diminished insulin secretion would have the opposite effect. Even in the face of reduced insulin, lipolysis might be diminished by the increased glucose metabolism in adipose tissue induced by hyperosmolarity (15) and hyperglycemia. However none of these factors appear to provide a satisfactory explanation for the absence of ketosis in patients with hyperosmolar coma since similar dehydration and hyperosmolarity is present in patients with diabetic ketoacidosis. The role of vasopressin, catecholamines, glucagon, and potassium depletion have not as yet been systematically evaluated in the syndrome of hyperosmolar coma (11).

Although hyperosmolar coma is not as common as ketoacidosis, the seriousness of this entity is underscored by its very high mortality. In most series, the mortality has been approximately 50 percent (2,18,26), but in some more recent series, it has been significantly lower than this (7,11). Frequently death is attributable to the underlying or associated disease which precipitated the hyperosmolar coma (2,11). Infection with gram negative organisms was especially associated with a grave prognosis since 11 of 12 such patients reported by Arieff and Carroll did not survive (2). Significant reduction in renal function also carried an unfavorable prognosis since seven of nine patients with creatinine clearances of less than 40 ml/min died. Of the 34 fatal cases of hyperosmolar coma analyzed by McCurdy (18), about half of the deaths were due to the preexisting or precipitating illness while the other half reflected the severe dehydration of the patients. Eight of these latter patients died of rapidly progressive shock. Another seven responded to therapy initially, but then subsequently died from thromboembolic phenomenon, undoubtedly initiated during the period of severe dehydration. Thrombosis of leg and pelvic veins has been a common finding at autopsy. An occasional patient has died of potassium depletion. McCurdy compared the laboratory values of the 34 patients who died with the 50 who survived (18). The fatal cases had higher blood sugar, sodium and plasma osmolarity values, but there was a considerable overlap and the differences were not significant.

The high mortality rate associated with this syndrome clearly emphasizes the inadequacies of the therapeutic approach to the problem. Deaths attributed to the underlying or precipitating disease may be difficult to prevent until there have been substantial improvements in the therapy

of such diseases. However, reports of mortality as low as 15-25 percent (11,17) indicate that appropriate therapy can be life-saving. General agreement exists that the hyperglycemia of hyperosmolar coma is relatively sensitive to insulin, and therefore insulin should usually be used in smaller amounts than is required for treatment of ketoacidosis. Gerich et al. compared the amounts of insulin required to lower the blood glucose to less than 300 mg percent during the initial 8 hours of treatment in patients with hyperosmolar coma and diabetic ketoacidosis (11). The former group received an average of 133 units in contrast to 359 units in the latter group. However, some cases of hyperosmolar coma have manifested significant insulin resistance (6,26). One such patient received 4675 units of insulin during the first 24 hours of therapy which reduced the blood sugar from 1824 mg percent to 172 mg percent. Occasionally patients have demonstrated extreme sensitivity to insulin or have been successfully treated without administration of any insulin (11). It should be emphasized that this is probably not very common, and all patients should receive a moderate dose of insulin (about 50 units) as soon as diagnosis is established. The best guide to the patient's sensitivity to insulin and an indication of the magnitude of subsequent doses of insulin can be obtained by assessing the hypoglycemic response to the initial insulin dosage 2 to 3 hours later. The initial dose of insulin should be administered intravenously since the dehydration and shock may interfere with absorption of insulin injected subcutaneously or even intramuscularly. The absence of a reduction in blood glucose in response to the initial dose of insulin should alert the physician to the possibility that the patient may be insulin resistant and require larger than usual amounts of insulin. This may be especially true of the patient who has received steroid therapy as a contributing cause to the development of hyperosmolar coma.

Of equal or even greater importance in the treatment of patients with hyperosmolar nonketotic coma is fluid replacement. Matz attributed the low mortality rate in his series to the early use of massive amounts of hypotonic, multi-electrolyte solutions at the rate of 1-2 liters/hour (17). He also stressed the vigorous administration of plasma volume expanders, especially in those patients in shock at the time of admission. McCurdy emphasized the initial use of isotonic saline rather than hypotonic saline for the patient in shock as a more effective way of expanding the vascular volume and reestablishing normal renal hemodynamics (18). In view of the significant number of deaths attributable to shock and dehydration (18), it is obvious that treatment of this condition should receive the highest priority. The rate of fluid administration must be individualized, based on a variety of factors including the patient's cardiovascular status. A urine flow of at least 50 ml/hour should be maintained. After shock has been successfully treated, hypotonic saline (0.45 percent) should be used as a more effective way of reducing the plasma hyperosmolarity. The possibility of hemolysis must be kept in mind, especially if such hypotonic fluid is administered very rapidly. Gerich et al. administered as much as 8 liters of 0.45 percent NaCl over 4 hours without evidence of any complications (11). Although 5 percent glucose in water presents the theoretical advantage of providing more available free water, its use cannot be recommended for several reasons. Administration of glucose will obscure evaluation of the effectiveness of administered insulin in reducing the hyperglycemia. Further elevation of the blood glucose as a consequence of glucose administration can also accentuate the osmotic diuresis which has already been responsible for the patient's dehydration. Since most of the patients have normal serum potassium on admission, addition of potassium to the intravenous fluid will probably be necessary early in the course of therapy. Treatment of the patient with insulin and fluids

will decrease the serum potassium both as a consequence of hemodilution and as potassium returns to the intracellular space with reestablishment of normal glucose metabolism. The amount of potassium administered should be monitored by frequent determinations of the serum potassium and evaluation of the EKG. It is important to maintain an adequate urinary output during potassium administration.

Initially there is marked intracellular dehydration, but the possibility that rapid reduction in plasma osmolarity might be associated with osmotic disequilibrium across the central nervous system and the development of cerebral edema has been raised by the recent studies of Clements et al. (5). Cerebral edema has also been reported in patients treated for diabetic ketoacidosis (33), but its frequency is not known. During the period of hyperglycemia, glucose is metabolized to sorbitol and fructose in the central nervous system. Since these sugars are not as freely permeable as glucose, they can accumulate intracellularly and exert an osmotic effect not balanced by glucose in the plasma and extracellular space if there has been a rapid reduction in the hyperglycemia (24). Arieff and Carroll followed eight patients with serial lumbar punctures and did not find any severe imbalance between plasma and cerebrospinal fluid osmotic concentration. In response to therapy, both the plasma and cerebrospinal fluid osmolarity declined at essentially similar rates (2). However, in studies in rabbits where it was possible to also measure the osmotic gradient between brain and plasma, Arieff and Kleeman found that rapid reduction of plasma glucose induced by insulin caused an osmotic gradient between brain and plasma (3). Cerebral edema developed when the plasma glucose was reduced below 14 mM. Measurements of sorbitol and other metabolites of glucose did not support the concept that sorbitol and fructose accumulation via the polyol pathway was responsible for the osmotic disequilibrium. The source of the idiogenic osmoles which contributed to the development of cerebral edema was not elucidated. Thus, although there is good animal data to consider the development of cerebral edema as an undesirable complication of rapid reduction of the hyperglycemia in patients with hyperosmolar coma, the importance of this clinically is not known. None of the patients who were autopsied in the series reported by Arieff and Carroll had cerebral edema (2). At autopsy, some of the patients reported by Rubin et al. (26) had cerebral edema but since they had been mechanically respirated, the significance of this finding is unclear. In consideration of this possibility, the plasma osmolarity should be reduced at a more moderate rate in order to avoid development of this complication.

The economic impact of hyperosmolar coma is sufficient that it would justify additional funding to prevent the development of this entity. Foster has estimated that the cost of hospitalization and medical care for the treatment of diabetic ketoacidosis is at least $5,500,000 per year, not including the cost of time lost from work (9). Since hyperosmolar coma is approximately one-sixth as common a cause of coma in the diabetic as ketoacidosis (11), the medical cost of this entity would be approximately $920,000, assuming equal periods of hospitalization for both entities. Since patients with hyperosmolar coma have generally been more severely ill and have had serious underlying diseases, the length and daily cost of hospitalization tends to be greater than that of ketoacidosis. The higher mortality rate of this entity further accentuates the economic impact of this entity, especially in terms of lost income, although such an unfavorable outcome might be associated with a shorter period of hospitalization.

It is obvious from the increasing frequency with which hyperosmolar coma is being reported and the very high mortality rate that new information is urgently needed to provide improved

acute care for such patients which should significantly reduce the mortality rate. Clearly, prevention of the syndrome offers the best solution to its unacceptably high mortality. Such prevention depends primarily on the patient's physician being able to recognize the entity at a much earlier stage of its genesis. Many physicians are completely unaware of the existence of this syndrome and even more have only a superficial understanding of it. An educational program is needed which will reach a large number of physicians who are providing primary patient care. In addition to making them aware of hyperosmolar coma, the educational program should emphasize those situations which predispose to it so that the physician is able to anticipate its development. Examination of the urine for glucose is a simple, inexpensive procedure which can be done in the physician's office and can provide important information alerting the physician to the possible development of this entity. Thus, all patients who present themselves to the physician with any symptoms or physical findings of dehydration should certainly have their urine tested for glucose. In addition, all patients with symptoms of serious disease should be considered as potential candidates for development of hyperosmolar coma and have frequent urine examinations for glucosuria. If the physician is aware of this entity and the circumstances under which it develops, he can be on the lookout for it and detect the glucosuria and hyperglycemia early enough to prevent the serious clinical problems of the full-blown syndrome. As soon as glycosuria has been detected, appropriate treatment for it should be initiated and the patient followed closely to assess its effectiveness. The success of such an educational program is related to the larger problem of the continuing education of the practicing physician. An even more difficult problem is patient education so that they will seek medical attention at the onset of their symptoms. No matter how aware the physician is of the entity of hyperosmolar coma, he cannot prevent its development unless the patient comes to him early in his illness.

New information concerning the pathogenesis of the syndrome could also be valuable in designing more rational therapy for it. A better delineating of the factors causing hyperglycemia such as the relative roles of increased gluconeogenesis, decreased peripheral utilization of glucose, and reduced renal excretion of glucose could be helpful in treatment of patients. The alteration in a varity of hormones need to be better defined both in terms of the development of hyperglycemia and the absence of ketosis. Modifications of such hormonal changes could also be very useful in treatment. The possible deleterious effect of rapidly reducing extracellular hyperosmolarity by treatment with insulin has been alluded to previously. The importance of this should be established in carefully controlled animal and perhaps even patient studies. The source of the idiogenic osmoles which are responsible for the osmotic disequilibrium across the cells in the central nervous system should be clarified. The controversy concerning the use of isotonic versus hypotonic saline solutions early in treatment, especially in patients in shock, should be resolved by carefully controlled experiments. Although the hyperosmolarity and dehydration can be treated by insulin and fluid therapy, it is much more likely that even more rational therapy could be devised if the pathogenesis of the syndrome were fully understood.

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12

LACTIC ACIDOSIS: INTERRELATIONSHIPS WITH DIABETES MELLITUS AND PHENFORMIN

Robert Alan Kreisberg

INTRODUCTION

Lactic acidosis is a well-known and challenging problem. It is generally associated with disorders in which decreased cardiac output, hypotension, and hypoxemia are common denominators (28, 60, 38). When lactic acidosis occurs in patients with diabetes mellitus, shock and other underlying predisposing factors known to be associated with this syndrome are usually apparent (38). However, there is a relatively small group of patients with lactic acidosis, referred to as "spontaneous" or "idiopathic" in whom lactic acid accumulation and acidosis supervenes before alterations in cardiovascular dynamics or tissue perfusion are observed (28). The presence of diabetes mellitus in 50 percent or more of the patients with idiopathic lactic acidosis (38) raises the question of whether anatomic and/or metabolic abnormalities associated with diabetes predispose to the development of this disorder.

Biochemistry of Lactic Acid

Lactic acid is a strong organic acid (pKa;3.86) that is completely ionized at body pH. For every mmole of lactic acid produced, one mmole each of hydrogen ion and lactate anion are added to body fluids. The dissociation and release of hydrogen ion from lactic acid results in the reduction of body buffer base and when of sufficient magnitude, acidosis. While the lactate anion has no detrimental affect on acid-base homeostasis, its concentration may be used to indicate the extent to which excessive production of lactic acid has occurred. In this regard, lactate represents the "slag" or anionic residue of previously buffered lactic acid.

Lactic acid is a normal metabolic product of glycolysis. Its formation from pyruvic acid is regulated by a pyridine nucleotide-linked extra-mitochondrial enzyme, lactic acid dehydrogenase (LDH):

$$\text{Pyruvic acid} + \text{NADH} = \text{H}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{Lactic Acid} + \text{NAD}^+ \quad (1)$$

The transformation of pyruvic to lactic acid permits extramitochondrial re-oxidation of NADH. At thermodynamic equilibrium, the reaction can be transformed to its mass action form:

$$[\text{Lactate}^-] = [\text{Pyruvate}^-] \times k \frac{[\text{NADH}]\ [\text{H+}]}{[\text{NAD}]} \quad (2)$$

At any given concentration of pyruvate, the concentration of lactate is dependent upon the prevailing redox state of the NAD-NADH couple and the hydrogen ion concentration. Under most circumstances the lactate concentration is determined by changes in the pyruvate concentration alone or in combination with changes in cellular redox (27). An attempt has been made to differentiate lactic acid production due to increased pyruvic acid production from that due to altered redox by relating the increase in lactate concentration to that occurring in pyruvate (27). When the increase in lactate exceeds that which could be attributed to pyruvate, then an alteration in cellular redox must have occurred and Huckabee refers to the lactate, not accounted for by pyruvate, as "excess lactate." An increase in pyruvate unaccompanied by any change in cellular redox would be associated with an increase in blood lactate concentrations, maintenance of a normal L/P ratio and no "excess lactate." In patients with lactic acidosis, both the supply of pyruvate

and cellular redox state are altered so that even when the L/P ratio is increased and "excess lactate" is present, pyruvate concentrations are also increased (28). The concept of "excess lactate" has been criticized because cellular redox can be altered independent of oxygenation (39) however, it has been clinically useful and has allowed a classification of lactic acidosis.

BLOOD LACTATE MEASUREMENTS

The measurement of lactate is subject to a number of problems that may result in spuriously elevated values. Peripheral venous blood lactate concentrations reflect local tissue metabolism, primarily skeletal muscle, and are generally 30-50 percent higher than the arterial concentrations (28). The difference between arterial and venous concentrations can be further accentuated when samples are obtained in the presence of stasis. Although arterial blood is preferable preferable for measurement of lactate since the concentration reflects the mean body lactate uninfluenced by regional metabolism, venous samples may be used since they are seldom artificially increased to the level observed in lactic acidosis (28). Blood samples should be obtained with a minimum of stasis and promptly deproteinized in chilled acid. Plasma lactate concentrations are 20 percent greater than whole blood concentrations (27), and plasma can be used without much of a sacrifice in accuracy if the sample is quickly centrifuged and the plasma removed.

LACTATE HOMEOSTASIS

Although lactic acid is a product of the metabolism of virtually all tissues, brain, erythrocytes and skeletal muscle account for most of the lactate produced with minor contributions by leukocytes, platelets and the renal medulla (11). In the basal state, arteriovenous lactate differences across brain and skeletal muscle are negative (11, 40, 64) indicating net lactate production while arteriovenous differences across the splanchnic bed, which primarily reflect hepatic metabolism, are positive indicating net lactate utilization (20, 45, 64). Splanchnic lactate extraction or utilization at rest accounts for approximately 40-60 percent of the total available lactate (20, 45), thus emphasizing the importance of the liver in maintaining lactate homeostasis. The utilization of lactate by liver and skeletal muscle is a concentration-dependent process (18, 21, 30, 54, 64). When systemic lactate concentrations are elevated as a result of a simple infusion of lactate or by selective limb exercise, net lactate balance across resting muscle becomes positive (21) indicating that the overproduction of lactic acid by a single tissue or part of a tissue will not produce hyperlactatemia because of increased utilization by other tissues.

Because blood lactate concentrations reflect the balance between lactate production and utilization, it has been stated that lactic acidosis and hyperlactatemia can be the result of either the overproduction of lactic acid or the underutilization of lactate or both (9, 38). It has also been claimed that the capacity of the liver to utilize lactate greatly exceeds the ability of other tissues to produce it (9), implying that decreased lactate utilization by the liver is important in the geneses of lactic acidosis. The experimental data which bear on this proposal are not clear. Decreased hepatic removal of lactate has been observed in patients with liver disease but basal concentrations of lactate are often normal (24). Lactic acidosis is seen in patients with liver disease only when there is associated hemodynamic instability and impaired perfusion of the microcirculation. In rats, exclusion of the liver from the circulation, by portal vein and hepatic artery ligation, is associated with hyperlactatemia and acidosis

(1) indicating that in this species lactic acidosis accompanies decreased hepatic lactate utilization. One of the most convincing pieces of evidence against the proposal that decreased hepatic lactate utilization produces significant lactic acidosis or hyperlactatemia is the observation that arterial lactate concentrations in dogs do not rise excessively when hepatic lactate extraction is acutely inhibited by hypoxemia and hypoperfusion (56).

Under certain circumstances the liver may be a site of lactic acid production and thereby contribute to the development of lactic acidosis just as any other tissue. In experimental shock in dogs, a 20-34 percent reduction in blood volume is associated with hepatic and lower extremity lactate production and with more severe hemorrhage renal lactate production (53). In patients with clinical lactic acidosis net hepatic lactic acid production may be observed although measurements of regional lactate concentrations suggest that the production of lactic acid is generalized (53). There is no doubt, based on studies of patients with glycogen storage disease, that the liver can be a significant source of lactic acid in humans under certain circumstances (51).

The production of lactic acid by tissues adds both the hydrogen ion and the lactate anion to body fluids. The ability of the kidneys to excrete metabolically derived hydrogen ion is limited (50-100 meq/day), therefore maintenance of normal pH depends upon both renal reabsorption of filtered bicarbonate and continued generation of bicarbonate through metabolic processes. Although it is not clear how organic anions cross cell membranes and enter metabolic pathways, one could argue that the metabolism of lactate is necessary for acid-base homeostasis and interference with its utilization could produce a metabolic acidosis. If lactate is extracted by tissues in its anionic form, then its subsequent oxidation and/or conversion to glucose would result in the net production of bicarbonate. Alternatively, if lactate gains access to the cell as lactic acid, then its uptake would, by removal of hydrogen ion, generate bicarbonate at the cell surface. In either situation bicarbonate is a by-product of lactate utilization by tissues and it balances the loss in bicarbonate that occurs when lactic acid is generated. Even in this formulation, the production of lactic acid is seen as the event leading to the loss of bicarbonate while the utilization of lactate is seen as repairing the deficit. In this situation the major cause of lactic acidosis is viewed as the overproduction of lactic acid.

DIAGNOSIS OF LACTIC ACIDOSIS

There are no established criteria for the diagnosis of lactate acidosis, consequently, it is difficult to obtain precise data concerning the incidence of this disorder or its relationship to diabetes mellitus, ketoacidosis, and phenformin. The minimum changes in lactate concentration and pH that should be used for the diagnosis of lactate acidosis are by necessity somewhat arbitrary and the levels at which such changes become diagnostic are not known. Huckabee (28) observed that while random venous lactate measurements on hospital patients seldom exceed 1.3mM, higher values were occasionally seen particularly with hyperventilation. Because improper handling of blood samples can result in moderate increases in the blood lactate concentrations, it seems that the values of 1.3mM used by Peretz (42) and 2.0mM used by Oliva (38) are too low and may result in the overdiagnosis of lactic acidosis. The use of a pH value of less than 7.37 as proposed by Oliva (38), though sensitive, may be relatively nonspecific; while the statement that there should be a "significant reduction in pH" (60) is inadequate without a more precise definition of "significant." The sensitivity of the bicarbonate measurement is such that blood lactate concentrations

must be at least 5 mM before acidosis can be detected (28). Peretz (42) observed an 85 percent mortality rate in patients with hyperlactatemia and shock when the lactate concentrations were in excess of 8.9 mM. In a series of 32 patients with lactic acidosis, 90 percent with blood lactate values above 7 mM died (62). Use of blood lactate concentrations in excess of 7 mM as recommended by Tranquada (60) and pH values of less than 7.3 as recommended by Peretz (42) seem appropriate and reasonable, although milder cases of lactic acidosis may be overlooked.

The diagnosis of lactic acidosis is not difficult in patients in whom there is a severe metabolic acidosis in the presence of known causes of this disorder and the absence of diabetes mellitus. The diagnosis of lactic acidosis in a patient with diabetes mellitus may be difficult. Unfortunately, blood lactate, pyruvate, β-hydroxybuty-rate and acetoacetate measurements, even when available, generally cannot be provided with the speed that is necessary for prompt diagnosis and management. The diagnosis of lactic acidosis must be made quickly and therapy undertaken before the results of such measurements are known. A presumptive diagnosis of lactic acidosis can usually be made when there is an abrupt onset of a severe metabolic acidosis characterized by an increased "anion gap" in the absence of other known causes for an increase in unmeasured anions (66). Resistance to alkali therapy and spontaneous variations in blood pH and bicarbonate concentrations in the absence of alkali therapy can be important retrospectively. An increased "anion gap" $[(Na + K) - (Cl + HCO_3) > 15]$ is not diagnostic of lactic acidosis but may also be seen with renal failure, salicylate intoxication, methanol poisoning, ethylene glycol ingestion, and diabetic ketoacidosis. If lactic acidosis is superimposed on or associated with an acidosis characterized by an increased "anion gap," the diagnosis may be difficult. This is particularly true when there is coexistent diabetes mellitus and ketoacidosis. At the present time, there is no way to make the diagnosis of lactic acidosis in the presence of ketoacidosis without a blood lactate measurement. On the other hand, the presence of only trace amounts of plasma ketones does not necessarily indicate lactic acidosis since significant ketoacidosis can exist in the presence of weakly positive plasma acetest reactions. The failure of a patient with ketoacidosis to improve despite adequate therapy or the persistence of acidosis which is resistant to alkali should suggest coexistent or superimposed lactic acidosis.

The onset of lactic acidosis is traditionally described as precipitous, particulary when it occurs in the hospital as a consequence of severe underlying disease and altered tissue perfusion. Whether the onset of idiopathic lactic acidosis or lactic acidosis in patients receiving phenformin is acute, is not known. Our experience, and that of others with diabetic patients who are found in the emergency room to have idiopathic lactic acidosis, is that a history of prodromal symptoms developing over several days can often be obtained (62). This suggests that diabetics, particularly those receiving phenformin, should be closely observed for the development of the nonspecific symptoms and if present and unexplained, the possibility that subtle lactic acidosis is present and/or developing must be considered.

DIABETES MELLITUS AND LACTIC ACIDOSIS

The occasional occurrence of nonketotic acidosis and coma in patients with diabetes mellitus has been known for many years (44). Appel and Cooper (5) reviewed the subject and attributed the acidosis to impaired renal excretion of ketones. Lexow (36) described three diabetic patients with coma and acidosis without ketosis and suggested that the acidosis was due to lactic acid. However, it was not until Daughaday (14) described an additional three diabetic patients with

nonketotic acidosis, that excessive accumulation of lactic acid could be established as the cause for the acidosis. Because of the frequent occurrence of diabetes in patients with idiopathic lactic acidosis, a causal relationship between diabetes mellitus and lactic acidosis has been suggested.

There are no obvious metabolic defects in experimental diabetes mellitus in animals (22) nor spontaneous diabetes mellitus in humans which predispose to the development of lactic acidosis. Blood lactate concentrations in 83 acutely ill patients with diabetes studied by Traquada (62) were normal or slightly elevated with a mean value of 1.22 mM. Although Shreeve (50) observed decreased lactate-^{14}C clearance in diabetes mellitus lactate disposal, as reflected by the rate of removal of an exogenous oral lactate load, it is not prolonged (4). The administration of glucose to normal subjects and insulin to patients with diabetes results in increased glycolysis but only a slight increase in blood lactate concentrations (27, 57).

The incidence of lactic acidosis in patients with ketoacidosis seems relatively small. In a series of 12 patients studied by Strangaard (55) blood lactate and pyruvate concentrations were within normal limits and lactate increased only slightly in some patients with treatment. Furthermore, these investigators observed an inverse linear relationship between total blood ketone and bicarbonate concentrations indicating the absence of lactate and other unmeasured anions. In another series of 23 patients with diabetic ketoacidosis studied by Watkins (67) the contribution of lactate to the anion gap and the acidosis was small and an excellent correlation was also observed between the concentration of β-hydroxybutyrate and pH, again emphasizing the unimportance of lactate in this study. However, in seven of these patients, the blood lactate concentrations were in excess of 3.0 mM and in three patients were 4.2, 7.3, and 19.4 mM, respectively. Although blood lactate concentrations were, with the three exceptions mentioned, either within normal limits or only minimally elevated blood lactate concentrations increased during therapy of the ketoacidosis in 13 of 17 patients in whom it was measured, two of whom died. One of the latter had a severe predisposing disorder that ordinarily would be associated with lactic acidosis and invocation of diabetes mellitus is unnecessary. In two patients in this series, the acidosis appeared to be due to lactic acid. Alberti and Hockaday (1) have reported lactate and pyruvate measurements in a series of 50 patients with diabetic ketoacidosis. The mean lactate and pyruvate concentrations were 2.49 and 0.14 mM, respectively, and the L/P ratio was 19. Based upon the changes that occurred in blood lactate with insulin treatment, these patients were subdivided into two groups. Approximately one-third of the patients demonstrated a fall in blood lactate, the magnitude of which inversely correlated with the initial lactate concentration (i.e., the higher the concentration, the greater the fall). This group was characterized by higher initial lactate concentrations and greater degrees of hyperglycemia, hyperketonemia, and acidosis. They also required more insulin than did the other group, suggesting that the diabetic ketoacidosis was more severe. In the remaining patients, the blood lactate concentrations transiently increased during therapy. In none, however, was severe or progressive lactic acidosis detected. Although there is a gradation of lactate concentrations in diabetic ketoacidosis, these authors have observed only four patients in a series of 55 in whom the lactate was greater than 7.0 mM (26). It would thus appear that significant lactic acidosis is not a common problem in patients with ketoacidosis nor does insulin therapy, despite its ability to increase blood lactate concentrations in normal and diabetic subjects result in lactic acidosis.

Most diabetics with lactic acidosis are in shock or have severe underlying diseases or disorders known to be associated with the syndrome (62). Nonetheless, there are patients with lactic acidosis, in the absence of known predisposing factors, in whom diabetes mellitus or some disturbance of carbohydrate metabolism is present (38, 62). It is not clear whether such abnormalities in carbohydrate metabolism are etiologically related to the acidosis or just coincidental. The prognosis of patients with idiopathic lactic acidosis, with and without diabetes mellitus, is poor. In Oliva's review article on lactic acidosis (38) only 5 of 15 patients described in the literature with both lactic acidosis and diabetes mellitus had survived while the mortality rate was 100 percent in patients with lactic acidosis in the absence of diabetes. In a series of 58 patients, both with and without diabetes mellitus, reported by Tranquada (60), the mortality rate was 90 percent. The studies of blood lactate concentrations in patients with ketoacidosis do not indicate the presence of metabolic lesions that predispose to the development of lactic acidosis. However, because these patients are younger and therefore less likely to have vascular disease, the possibility remains that there may be an anatomic lesion which, when associated with diabetes predisposes to the development of this syndrome. The available information concerning lactic acidosis and coexistent diabetes mellitus reveals that the patients are generally older and demonstrate only mild to modest hyperglycemia despite severe stress. These individuals could perhaps be best described as having maturity onset diabetes mellitus and may be more at risk because of age and accompanying vascular disease, which is more extensive than in nondiabetics, rather than because of any biochemical predisposition.

PHENFORMIN AND LACTIC ACIDOSIS

The role of phenformin in lactic acidosis is highly controversial and the high incidence of diabetes mellitus in patients with lactic acidosis who are not receiving phenformin confounds the issue and makes the definition of this relationship difficult. Shortly after its introduction, attention was drawn to the occurrence of severe and fatal metabolic acidosis in a series of diabetics who were receiving phenformin (65). Subsequent publications (8, 10, 12, 15, 59) have inferred a direct relationship between phenformin and lactic acidosis. In 1973 there were approximately 200 cases (documented in the scientific literature or reported directly to the manufacturers) of lactic acidosis occurring during phenformin treatment (63). Analysis of these reports reveal that 70 percent of the involved individuals were females, 55 percent were between the ages of 60 and 80 years, and 90 percent were older than 41 years. Virtually all patients were receiving between 100-150 mg per day. These patients were found to differ in a number of ways when compared with a randomly selected series of 181 patients with lactic acidosis who were not receiving phenformin. The mean age of the patients who were receiving phenformin was 61 years in contrast to 51 years for the nonphenformin group. In 58 percent of the phenformin series, the lactate concentration was greater than 6.5 mM and the pH was less than 7.30-7.33. Known causes of lactic acidosis were present in 56 percent of the cases and in an additional 17 percent, conditions were present which are known to contribute to the development of lactic acidosis. In only 30 percent of the group receiving phenformin was lactic acidosis present without known or contributing causes. Recovery occurred in 50 percent of the phenformin-treated group, but in only 22 percent of the nonphenformin group. In a series of 21 patients with lactic acidosis who were receiving phenformin, described by Bengtsson (8) 67 percent survived. However, the survival rate in the nine patients described by Cleaver and Carretta (12) was only 22 percent.

The incidence of lactic acidosis in phenformin-treated diabetes is unknown but considering that 400,000 patients were estimated as receiving phenformin in 1972, the occurrence must be relatively rare. In opposition Bengtsson and co-workers observed 21 patients in a large diabetic clinic over a period of 39 months in whom lactic acidosis developed during oral antidiabetic treatment in which phenformin was being used alone or in combination with sulfonylurea agents or insulin (8). Cleaver and Carretta (12) have observed nine cases of lactic acidosis in diabetic patients receiving phenformin and although they have claimed that there is a more common problem than previously appreciated, the time interval over which their cases were accumulated was not stated.

The intense concern over the potential relationship of phenformin to lactic acidosis is a direct result of observations in animals and humans that it can increase lactate production and/or elevate the blood lactate concentration. In initial in vitro studies, phenformin was shown to inhibit oxidative phosphorylation, increase anaerobic metabolism and the production of lactic acid in skeletal muscle (51). Subsequently, it has been demonstrated to inhibit gluconeogenesis from lactate in rat liver slices (41), renal cortex (41) and in isolated perfused rat and guinea pig livers (3, 58), indicating that the hyperlactatemia in intact animals may be theoretically due to both increased production and decreased utilization. Although the effects of phenformin on cellular metabolism *in vitro* have often been demonstrated at concentrations considerably in excess of those seen therapeutically, the observations cannot be dismissed because certain tissues (skeletal muscle, liver and the gastrointestinal tract) concentrate phenformin (25) and tissue levels of the drug may be greatly in excess of that expected from plasma concentrations. More recently, inhibition of gluconeogenesis by rat liver has been achieved with concentrations of phenformin that are within the therapeutic range (58). In normal human volunteers, therapeutic doses of phenformin have produced mild elevations of blood lactate and have accelerated glucose turnover and its conversion to lactate while also increasing the turnover and incorporation of lactate into glucose (31, 32). Similar changes have also been observed in patients with diabetes mellitus (47). Increased glucose conversion to lactate may simply reflect enhanced glycolysis and does not imply an increase in anaerobic metabolism. Increased concentrations of blood pyruvate in phenformin-treated subjects (13) and normal or slightly increased glucose oxidation (31, 47) supports such a proposal.

Dembo and associates (16) have suggested that the changes in pyruvate metabolism that accompany relative insulin deficiency may, in the presence of phenformin, lead to lactic acidosis. In this scheme insulin deficiency and fasting result in the overproduction and underutilization of pyruvate. The former from enhanced protein breakdown and provision of three carbon precursors and the latter from preferential utilization of fat and inhibition of pyruvate oxidation. The shift to a more reduced cellular redox state as a consequence of increased fatty acid oxidation may be further accentuated by the accumulation of excessive amounts of phenformin which occurs with renal impairment and its further effect on cellular redox. Recently, Searle (48) has studied the metabolism of lactate-^{14}C in a patient who developed lactic acidosis with the administration of phenformin and demonstrated that lactate oxidation did not increase sufficiently to keep pace with the increased rate of lactic acid formation that occurred. He suggests that the imbalance in lactate metabolism is responsible for the lactate acidosis that accompanies phenformin therapy.

Because of the high incidence of diabetes mellitus in patients with idiopathic lactic acidosis, it is particularly difficult to determine whether phenformin exerts an independent effect, synergizes in some way with the abnormality in carbohydrate metabolism present in diabetes, or is coincidental and unimportant as a cause of lactic acidosis. The possibility must be seriously considered; however, that under certain circumstances phenformin predisposes to the development of lactic acidosis. It may be speculated that in the presence of marginal tissue oxygenation, phenformin may increase or potentiate lactic acid production beyond that which would have occurred, thus converting a situation in which compensation could be maintained or spontaneously reversed into progressive acidosis. Studies in rats on this point are conflicting. Lacher (35) demonstrated impairment in the disposal of both endogenous and exogenous lactate loads in phenformin-treated rats, while Ruggles (46) was unable to show potentiation of hyperlactatemia resulting from hypoxemia. In humans, phenformin does not potentiate exercise-induced hyperlactatemia (23, 49). Other predisposing factors, particularly ethanol, should not be minimized since ethanol and phenformin act synergistically on blood lactate concentrations (34).

Phenformin is eliminated from the body by the kidneys (6) and in the presence of renal disease impaired excretion and elevation of blood levels of phenformin may be expected. This is important since there is a rough relationship between the dose of phenformin used in maturity onset diabetic patients and the increase observed in the blood lactate concentration. Hyperlactatemia does not occur with phenformin doses of less than 75 to 100 mg daily, while greater increments in lactate are observed with doses of 175 to 250 mg daily (13, 19). In view of the relationship between the dose of phenformin and the blood lactate concentration, it is reasonable that impaired excretion of phenformin results in drug concentrations that produce or predispose to lactic acidosis. The development of lactic acidosis with suicidal overdoses of phenformin would also suggest that blood concentations may be an important factor in the genesis of this syndrome (15, 43). The vast majority of patients who develop lactic acidosis while receiving phenformin have evidence of impaired renal function (62, 63). There may be certain individuals with mild or modest degrees of impairment in renal function in the absence of azotemia, in whom alterations in hydration alter renal perfusion and result in the retention of phenformin. Consequently, it seems justifiable to recommend that phenformin not be used in patients who have evidence of renal impairment and that creatinine clearance measurements be considered in all patients with obvious renal disease preliminary to the initiation of therapy with this drug.

TREATMENT

There is very little that can be said concerning the treatment of lactic acidosis. Recovery is usually determined by the response of the basic underlying disorder to specific therapy. Bicarbonate treatment is not successful, even when correction of pH can be obtained, unless the basic defects can be identified and corrected. Unfortunately, even when the underlying pathology can be defined, the disorder may not be reversible and the prognosis for survival is poor. Since the pathogenesis of idiopathic lactic acidosis is not known, no specifically directed therapy is possible. Recovery of patients with idiopathic lactic acidosis is uncommon, unpredictable and in most instances when it occurs, unexplained. The mortality of both nondiabetic and diabetic patients with idiopathic lactic acidosis is high (28, 30, 62). Patients who develop lactic acidosis while receiving phenformin may have a somewhat better prognosis (8, 63).

Unfortunately, there have been few large series of patients with idiopathic or phenformin-associated lactic acidosis which allow evaluation of therapy. The effectiveness of any therapeutic regimen is difficult to evaluate because of differences in diagnostic criteria and therefore the severity of the acidosis. In most instances, the requirement for bicarbonate is high and use of large quantities cannot reverse the acidosis. However, the early aggressive use of sodium bicarbonate with careful monitoring of pH may be more advantageous than the use of similar doses of bicarbonate over longer periods, recognizing that rapid alkalinization can decrease oxygen delivery to tissues and intensify lactic production (7). As a result of the resistance to bicarbonate, indicative of continuing overproduction and/or underutilization of lactic acid, problems relating to sodium and volume overload are superimposed upon and compounded by the deleterious effects of continuing acidosis on cardiovascular function. Although dialysis is not an effective means of correcting the acidosis, it offers a mechanism for control of volume and body sodium content and therefore may be an important therapeutic adjunct (17). While there is no evidence on this point, dialysis may also be useful in the treatment of lactic acidosis in patients in whom phenformin is thought to play an etiologic role since removal of phenformin by this route may be of benefit. In extreme cases, methylene blue, an oxidizing agent which accepts protons and alters cellular redox state, can be used (64), however, in most cases it has not been effective (38). When lactic acidosis occurs with hypoglycemia the infusion of glucose and correction of the hypoglycemia has been associated with correction of the acidosis (37). Rare patients with diabetes mellitus and lactic acidosis have also had a beneficial response when insulin was used to treat accompanying hyperglycemia (29). In a recent review Dembo (16) observed that the recovery of patients with phenformin associated lactic acidosis who were treated with insulin was higher (65 percent) than those who were not so treated.

RECAPITULATION

The pathogenesis of idiopathic lactic acidosis and its relationship to diabetes mellitus is incompletely understood. Additional epidemiologic and basic information must be obtained before this relationship can be clarified. High priority must be given to studies which establish the true frequency of idiopathic lactic acidosis in acutely and chronically ill, hospitalized and ambulatory, diabetic and nondiabetic populations to determine whether the apparent predisposition of the diabetic patient to lactic acidosis is more apparent than real. Basic studies must also be undertaken to more thoroughly understand the factors which regulate lactic acid production and lactate homeostasis in animals and man before the mechanisms responsible for the development of idiopathic lactic acidosis and the role of diabetes can be fully defined. The importance of phenformin in this syndrome, particulary if it is to be retained as a therapeutic agent, will require continued investigation into its mechanism of action. The role of other drugs, such as ethanol and of drug-drug interactions in the pathogenesis of idiopathic lactate acidosis must also be considered in future research.

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13

ACUTE COMPLICATIONS OF THE DIABETIC STATE

Joseph Silva and F. Robert Fekety, Jr.

INFECTION

The Frequency and Importance of Infection in Diabetes Mellitus

It is generally believed that diabetics have more infections than nondiabetic persons, and that infections in diabetics tend to be more severe and difficult to manage (62,67). However, this is still controversial and some believe the relationship has been overemphasized. While we believe that anyone who treats diabetics will soon be convinced of the inordinate frequency, devastating effects, and great importance of infections, the arguments on both sides should be presented and put into perspective. Unfortunately, little scientific evidence exists to prove that diabetics have a generally increased rate of infection and a clinically significant impairment of resistance to most infectious agents. There is even some evidence that the increased frequency of certain infections in diabetes is attributable not to the diabetic state per se but instead to harmful things that happen to diabetic patients. This line of reasoning is attractive because some of these things may be eliminated. For example, it is maintained that the high incidence of urinary tract infections in diabetics is attributable to the frequency of bladder cathetrization during acidosis or to relieve a distended neurogenic bladder. Similarly, frequent skin infections may be attributed not to difficulty in handling microorganisms, but instead to obesity and poor personal hygine, to insulin injections using unsterile equipment or to the inability to care for oneself because of diabetic retinopathy or cataracts. Infections of feet and legs may be attributed to arteriosclerosis and vascular insufficiency instead of to diabetes. Unquestionably, these factors are important, but they incompletely explain all these infections. More importantly, some infections are clearly more common in diabetics even in the absence of such factors. Most investigators conceded that Candida infections of the perineum and vagina, and Phycomycotic infections of the orbit and brain are more common in the presence of diabetes.

Another erroneous concept that needs to be dispelled is that infectious diseases have become infrequent and relatively unimportant both in normals and in diabetics. The reasons proposed for this include the ready availability of potent antibiotics and vaccines, and an increasingly good standard of living associated with a decline of contagious diseases thriving on poverty, bad housing, and lack of sanitation. For example, Younger (67) has pointed out that with insulin therapy, but prior to the discovery of antibiotics, the average duration of life in juvenile diabetics was about seven years, and that death was usually related to sepsis. With insulin plus antimicrobial chemotherapy, the prognosis is better than two-thirds of normal life expectancy. However, it should be emphasized that this represents a major shortening of life. In addition, she goes on to point out that although the prognosis for diabetics as a group is now favorable, for many individuals within the group it is devastatingly unsatisfactory. Younger maintains that microangiopathy is the major factor associated with a bad prognosis and that infection is an important contributory factor to angiopathy. The role of infection in aggravating microangiopathy is not well established or clearly understood and needs further study.

In another related study, Robbins and Tucker (53) noted that when the causes of death of 307 diabetic patients over the age of 12 were compared with those of 2800 *consecutive* nondiabetic patients in 1944, the relative incidence of pulmonary and other infections found at autopsy was approximately the same in both groups. One major defect in this study is that the patients were apparently not matched according to age, race, and sex, and the data really prove little more than that infections are frequent in people who die from any cause. Another defect is that the data do not touch upon the tremendous morbidity and suffering from nonfatal infections that diabetics must experience and endure. Nonetheless, two relatively common and serious infections, acute pyelonephritis and infections of the extremities were observed more frequently among diabetics in this study. The concept that infections are not important in diabetics is invalid.

Two recent studies merit attention because they vividly document how infections are still of great significance to patients with diabetes. The first concerns patients who were admitted in diabetic ketoacidosis to the Birmingham General Hospital in the United Kingdom between January 1968 and October 1972. The patients were treated in an intensive care unit by a team skilled in the management of diabetic acidosis (59). The mortality rate in 258 episodes of diabetic ketoacidosis was 6 percent. Infection was responsible for ketoacidosis in 78 (38 percent) of the 190 episodes in which a factor precipitating the episode could be identified. The next most common precipitating factor, error in insulin dosage, was only half as frequent as infection. Seven (10 percent) of the 73 patients with infection died, while only 5 percent of those with ketoacidosis, not complicated by infection, died. Infection accounted for 7 (43 percent) of the 16 deaths in this study. One of the fatal cases had meningitis, three had influenza, and three had pneumonia. The mean age of the patients in this report was only 41 years, emphasizing the importance of infection as a serious complication even in relatively young diabetics. Similarly, Muller and his colleagues (44) reported on 26 consecutively admitted patients with diabetic ketoacidosis in Dallas during 1969 and 1970, and indicated that 20 (77 percent) of the 26 had infection as the cause of diabetic ketoacidosis. A wide variety of common and mundane infections that are supposedly no longer important were implicated in their patients.

What should be emphasized in assessing the significance of these recent reports is that research in the infectious diseases that harrass and kill diabetics has not been of major interest to most physicians and funding agencies concerned with endocrinology and metabolism. Quite understandably, they have been more concerned with important biochemical and cardiovascular features of the diabetic state. Therefore, in the future, research funding agencies interested in alleviating suffering in diabetic patients ought to take into consideration the present scarcity of funds for research in infectious diseases. Recently this has become increasingly dependent upon pharmaceutical companies. Furthermore, it should be stressed that research in the broad field of infectious diseases is likely to yield significant benefits to diabetic patients, because they suffer most from the common infections afflicting everyone.

Looking to the future, it is likely that infections will be even more serious and costly. For example, Kjellstrand and his colleagues (33) noted that uremia is a common cause of death of patients with juvenile-onset insulin-dependent diabetes, and that renal transplants are being performed in them with increasing frequency. In reporting their experience with 50 patients, they stated that the overall results were encouraging and suggested many of the symptoms of advanced diabetes may be caused by uremia rather than the diabetic process. On the other hand, 15

of their 40 patients died, and the main cause of death was infection.

Thus, while it is clear that effective antibiotic treatment of infection is second only to insulin in increasing the life span of a diabetic patient, infections cannot be discounted now or in the foreseeable future as a major cause of morbidity and mortality. They should be a major subject of further research, which should not be left to the marketplace needs of the pharmaceutical industry. In order to emphasize areas of special importance for future research, we will discuss the more noteworthy specific infections.

Infectious Processes Associated with the Onset of Diabetes

More and more attention is being given to the probability that the diabetic state may be caused, precipitated, or accelerated by viral or other infectious diseases causing inflammation of the pancreas. We believe this is an important area for further research, which is mentioned only in passing because it is discussed in detail in another section.

Skin and Soft Tissue Infections

It is probable that staphylococcal skin infections are more common in diabetic patients. This predilection may be related to their frequent contact with antibiotics and hospital reservoirs of resistant staphylococci, and to the apparent increased rate of nasal carriage of staphylococci in diabetics. It may also relate to the use of unsterile needles and poor personal hygiene. Generalized hypersusceptibility to staphylococci in diabetes is not proven, and diabetes does not appear important in increasing the rate of staphylococcal post-operative wound infections (12). Diabetes is frequently implicated in staphylococci bacteremia requiring hospitalization (11). Further definition of the responsible mechanism(s) is needed if prevention is to be made possible. Staphylococcal infections are especially worthy of further study because of the great frequency and severity of these infections in diabetes.

Peripheral vascular disease is a major factor contributing to staphylococcal and streptococcal infection of the legs in the diabetic. These organisms are particularly important causes of ulcers, gangrene, osteomyelitis, and other infections of the feet and legs that ultimately may result in amputations. Antimicrobial as well as surgical therapy of these infections must be improved if extremities are to be saved and mobility and independence preserved. Minor infections of the extremities often become serious and life-threatening to diabetics if treated improperly in early stages. Prevention of infection by means of optimal foot care and avoidance of burns and injuries in patients with diabetic neuropathy is most important.

Candidiasis (Moniliasis) is a yeast (fungal) infection commonly afflicting diabetics. It results in marked redness and swelling of the genitalia, perianal region, medial aspects of the thighs and other moist, intertriginous areas. Small superficial pustules are usually present at the edges of the involved areas. Itching and soreness are usually severe. The process may involve the oral mucous membranes (thrush) and occasionally becomes generalized. Candidiasis is common in patients with poorly controlled diabetes, and is often the initial symptom of the disease. It is well recognized that all patients suffering from Candidiasis should be tested for diabetes. When localized to the skin, Candidiasis is relatively minor and easily controlled, especially if the diabetes is well-managed, but occasionally these infections become generalized and systemic in seriously ill, hospitalized diabetic patients. These disseminated infections are extremely refractory to treatment and are associated with a high mortality rate.

Pneumonia

There is little published data on pneumonia in diabetics, even though most clinicians believe these are serious illnesses for them. In a study of 62 patients admitted to the Johns Hopkins Hospital in 1965-1966 because of pneumococcal pneumonia, diabetes was evident in only four patients, and was no more frequent than it was in a carefully matched control population (23). Nonetheless, another recent publication reporting on 112 diabetics with pneumonia called attention to the impact of this infection (31). The mortality from pneumonia in this study was 39 percent and the frequency of prolonged infection with such dangerous pathogens as Staphylococci and Klebsiella was high. This calls attention to the problem of hospital-derived pulmonary infections in diabetics. These probably are increasing in frequency, and better ways of preventing and managing these problems are needed.

Tuberculosis

Tuberculosis has long been a serious problem in association with diabetes mellitus, but the availability of effective antituberculous chemotherapy has significantly improved the prognosis for this disease. The physician's emphasis now should be upon adequate detection of early tuberculous infections by means of annual tuberculin skin testing and periodic chest x-rays. This will result in early treatment, with a high probability of success. Guidelines for managing diabetics who develop tuberculosis are well established and diabetic patients usually respond quite satisfactorily to appropriate chemotherapy. Tuberculosis should no longer be a major threat to the life of patients with diabetes. This is an outstanding example of how progress in a distant area can have profound effects upon diabetes.

Urinary Tract Infections

Catheterization, neuropathy with bladder paralysis, nephrosclerosis, and Kimmelsteil-Wilson disease contribute to the problem of urinary tract infections in diabetics. This infection commonly results in pyelonephritis and may eventually progress to renal failure. Urinary hyperosmolarity attributable in part to the excretion of glucose may result in impaired leukocyte function in the medulla of the kidney (10) and may be of importance in the pathogenesis of pyelonephritis and necrotizing renal papillitis.

Necrotizing papillitis ascribed to local ischemia and accompanied by septicemia was demonstrated in 10 of 266 post-mortem examinations of diabetic patients who died during a 5-year period (66). Obstructive uropathy is important in its pathogenesis, as is the use of analgesics such as phenacetin and possibly aspirin. The clinical picture is not always fulminating, and papillitis should be entertained in every diabetic patient with infection and worsening renal function.

Gellman and his associates (25) found chronic pyelonephritis in approximately 10 percent of 63 renal biopsies in diabetic patients. The incidence of pyelonephritis was even higher in autopsied cases, and in some series has ranged as high as 40 to 55 percent (67). The disease seems more frequent in females than males, a fact which is probably related to the short urethra of women, to pregnancy, and to the high frequency with which women are catheterized.

The frequency of asymptomatic bacteriuria in diabetics is also apparently higher than in matched nondiabetic patients, but the statistics vary widely from place to place. Asymptomatic bacteruria is thought to be an important but not inevitable precursor of chronic pyelonephritis

and uremia. Indwelling urethral catheters with their inherent risk of asymptomatic or overt urinary tract infection and sepsis are important causes of serious morbidity and mortality from infections in diabetics. Many already well established measures and concepts are influential in reducing the frequency of this complication, but they need further refinement and development to insure popularity, practicality, and wide application. For example, the value of using closed sterile drainage systems or continuous bladder irrigation with antibacterial agents in patients with indwelling catheters needs to be emphasized. There is a need for further research and development of new preventive measures in this important area.

Gram-Negative Bacteremia and Sepsis

It is difficult to substantiate the belief that Gram-negative bacteremia is more common in diabetics, and that shock and mortality rates are increased. Diabetes was found in 18 (20 percent) of 88 patients with Gram-negative sepsis at the Johns Hopkins Hospital (35), and the case fatality rate for diabetics (39 percent) was higher than for the entire group of septic patients (24 percent). The special hazard of these infections probably is related at least in part to exposure to hospitals and their attendant hazards such as intravenous and urinary catheters, to unconsciousness with aspiration, and to inhalation therapy with the increased risk of development of pneumonia (62,67). While a poorly explained phenomenon, it is well documented that persons over 55 with serious underlying diseases such as diabetes have a higher than average mortality rate from Gram-negative sepsis, and this condition is thus an important problem for further research in pathogenesis and treatment.

Phycomycosis (Mucormycosis)

Phycomycosis is a rare fungus infection that is caused by saprophytic organisms of the genus Phycomyces (including Rhizopus, Mucor, and closely related species frequently found on fruits and vegetables). It occurs most often in patients who are acidotic or receiving adrenal steroid therapy and is associated with diabetes mellitus in the majority of instances. After colonizing the nasal passages, these organisms sometimes invade the adjacent tissues, thrombosing blood vessels and producing necrosis and gangrene of the tissues of the nose and eye. Cerebral extension of the process occurs in about two-thirds of the cases, producing paralysis of eye muscles and signs of diffuse cerebrovascular disease and is usually fatal. The condition seems to be related to ketosis more than to acidosis, and has been correlated experimentally with a brief delay in leukocyte mobilization at the site of primary invasion by the fungus (55). This delay permits a brief period of unchecked proliferation of the organism and is probably illustrative of an important defect which is operative in many other serious infections in diabetes. Mucormycosis is treatable with amphotericin B, but it has a high fatality rate, and extensive and disfiguring surgery to remove necrotic and gangrenous tissues is often required. Blindness in the involved eye is usual, and prolonged and expensive hospitalization is the rule.

Host Factors Related to Hypersusceptibility to Infection in the Diabetic

While it is still unproven that diabetics have a general increase in susceptibility to infection, there can be no doubt that infections are of tremendous importance to them. Because a wide diversity of organisms are involved at different body sites, the conclusion is inescapable that deficiencies in many of the cellular and humoral immune systems are probably implicated. Furthermore, these immune mechanisms may be deranged in different ways for different infectious

diseases. Many studies designed to elucidate these abnormalities have yielded conflicting and inconclusive results. In most cases, the mechanisms have not been established, and although many interesting leads have been developed, much further work is needed. As a probable first step in elucidating these mechanisms, prospective studies of the rates of various types of infection in diabetic populations and matched controls should be performed. Those infections shown to be clearly more frequent can then be studied in more depth to determine the responsible factors.

Factors that already are considered potentially important in the pathogenesis of infection in diabetes will be presented according to the following outline: (a) factors resulting in increased exposure to microorganisms, (b) factors facilitating entry of microorganisms into susceptibel diabetic tissues, (c) specific defects in cellular and humoral defense mechanisms in diabetes, and (d) metabolic abnormalities which may nonspecifically contribute to hypersusceptibility.

1. Factors increasing exposure to organisms. Healthy human beings harbor large numbers of bacteria and fungi on the skin, and within the mouth, nose, pharynx, and gastrointestinal tract. This is the so-called normal or resident flora, which may be found at these sites in numbers which vary from 10^7 to 10^{11} organisms per gram or square centimeter of tissue sampled. These organisms are in a continual competitive struggle with one another. Antibiotics and certain underlying diseases can alter the balance between species, and despite their weak pathogenicity, disease may result if the numbers of organisms markedly increases.

Alternations in the composition of resident flora are seen in some diabetics, but the reasons for them are unknown. Samplings of diabetic skin, stool, and mouth flora have demonstrated an increased rate of carriage of Candida (29) and of staphylococci in the nose (57). There is little information concerning whether the adequacy of diabetic control influences the quantitative load of bacteria or fungi at various sites.

Another factor influencing the acquisition of pathogenic bacteria is that diabetics have frequent contact with hospitals, clinics, and health personnel. These locations and individuals may serve as reservoirs of microorganisms that are more pathogenic and resistant to antibiotics. For instance, the acquisition of *Staphylococcus aureus* infections which are resistant to penicillin is much more frequent in hospitalized patients than in outpatients. Infection with such organisms may be far more serious than with an organism acquired at home.

2. Factors facilitating the entry of microorganisms into susceptible tissues. The diabetic patient may also be compromised in defending against infection because vascular and neurological complications of the diabetic state permit organisms to gain a foothold in normally inaccessible tissues. For instance, ketoacidosis leads to coma and dehydration, which decreases the production of oropharyngeal and tracheal secretions. These secretions are important in providing a mucous blanket which defends against penetration of bacteria and also washes them away. Not surprisingly, pneumonia frequently occurs during coma because of aspiration of organisms and failure to clear them.

The neurological complications of diabetes include a variety of neuropathies. A motor or autonomic neuropathy may lead to incomplete drainage of the bladder, which causes stasis and urinary retention requiring catheterization with occasional introduction of organisms and subsequent urinary infection. Autonomic neuropathies of the gastrointestinal tract may produce a malabsorption syndrome. Vitamin deficiencies and malnutrition may then occur, which further predispose to recurrent infections in poorly understood ways. Sensory neuropathies cause sensory

deprivation leading to neglect of wounds, contusions, abrasions, and lacerations, which are susceptible to bacterial invasion.

The vascular diseases which are so frequent in diabetes may produce varying degrees of ischemia or gangrene in peripheral tissues. The frequency of gangrene in diabetes is well-known and much feared by laymen, for gangrene predisposes to extensive infection of extremities and bacteremia and may result in amputation or death. Organs or skin deprived of a vascular supply are much more prone to infection. Local defenses are compromised because of poor delivery of exudative substances such as antibodies and inflammatory cells, and of antibiotics.

Thus, these vascular or nervous system complications subject the diabetic patient to the hazards of urinary and intravenous catheterizations for drainage or for the delivery of fluids, electrolytes, and insulin. Indwelling intravenous catheters are being associated with infections at an increasing frequency. These may vary from focal phlebitis to bacteremia and septic shock. The responsible organisms are usually derived from the patient's skin, and may gain entry to the tissues via the penetrating catheter which may be left in place for long periods. Urinary catheters become colonized within a week of placement and can serve subsequently as a focus resulting in pyelonephritis or bacteremia. Thus, the poorly managed ketoacidotic diabetic patient requiring various catheters is subjected to an increased risk of infections that he is poorly able to withstand. Furthermore, when faulty techniques are used, abscesses or cellulitis leading to bacteremia can occur at sites of insulin injection.

3. Specific defects in cellular or humoral defense mechanisms. The inflammatory response has three major components: (a) antibodies and other chemical substances such as histamine, complement, and prostaglandins, (b) phagocytes (granulocytes, macrophages), and (c) lymphocytes. The first two components dominate the acute inflammatory reaction, whereas the latter component is especially involved in the more complicated cell-mediated response of a delayed type. Defense against pyogenic infections is usually dependent upon the first two components, whereas defense against some fungi and bacteria such as *Mycobacterium tuberculosis* is usually attributed to the delayed cellular immune response. These systems seem to overlap and may reinforce one another, especially if one component is defective.

Some of the metabolic abnormalities that occur in the diabetic patient and may influence these immune systems include hyperglycemia, alterations in serum and tissue osmolarity, and increased serum levels of ketones, lactic acid, and lipids. Investigators have examined the influence of these variables on some aspects of inflammation, mainly in vitro. There are only a few in vivo studies of immune responses in diabetic patients. The resultant information concerning inflammatory responses related to hypersusceptibility in the diabetic is sparse and often contradictory.

Alterations in Antibody Mechanisms in Diabetes. Antibodies are gamma globulins which may be "natural" or nonspecific, while others are produced against specific invading organisms. These antibodies kill organisms in a variety of ways, such as by direct lysis (bactericidal antibodies), by enhancing bacterial ingestion by phagocytes (opsonizing antibodies), or by agglutinating or precipitating clumps of organisms, thus allowing more effective clearance by circulating and fixed phagocytes. Many of these reactions are enhanced by low molecular weight proteins which comprise the complement systems (including properdin). Data concerning antibody deficiencies in diabetic animals and patients are conflicting and inconclusive.

Studies on the ability of diabetic patients to form antibodies to a variety of antigens have

indicated either an impairment (17,52) or no abnormalities (38,63). Studies of gamma globulin levels (36) and the properdin system (27) showed no abnormalities in the well-controlled diabetic patient. Powell and Field (51) and Balch et al. (6) found diabetic patients had increased levels of serum complement, indicating that these circulating proteins are probably not defective in diabetics.

Because of antibodies and other substances, blood from normal persons is bactericidal for many organisms. The addition of glucose (up to 10 times normal serum concentration) to blood from normals, does not decrease its bactericidal properties, nor increase the rate of growth of most microorganisms in culture media (50). In contrast, Richardson (52) found that the growth of several types of bacteria in artificial media was increased in the presence of whole blood from diabetic patients but not in the presence of normal blood. The mechanism of this effect is unknown and deserves further study.

Other factors besides deficiencies in antibodies may alter the bactericidal activity of blood. Dubos (19) showed that keto-acids protected bacteria from the natural bactericidal action of lactic acids. This may relate to the clinical observation that diabetics are especially prone to infection when ketoacidosis prevails. Studies utilizing experimental models of diabetes suggest that the serum bactericidal capacity may be deficient in some circumstances. Cruickshank and Payne (15) showed that blood of alloxan diabetic rabbits had a modest decrease in bactericidal capability for pneumococci of low virulence, but further experiments using more virulent pneumococci, staphylococci, and *M. tuberculosis* showed no abnormalities (14).

Balch et al. (6) in a well controlled study of diabetic patients (with an without infection) found no alterations in serum bactericidal activity which correlated with glucose or ketone levels. Furthermore, these patients showed no consistent depression of serum bactericidal activity when their insulin was withheld for 48 to 96 hours.

These data suggest that blood from most diabetic patients has a normal bactericidal capacity. More sensitive methods of studying antibody formation and function than were used in these studies are now available and should be applied to the question of whether antibody responses are defective in diabetics.

Deficiencies in Phagocytic Metabolism. Investigations of leukocyte morphology in diabetes have indicated few defects in clinical importance. The total numbers of blood leukocytes are not abnormally low in diabetes. In fact, leukocytosis and basophilia are common.

Phagocytes have a variety of functions that can be studied in vitro: (a) random migration, (b) specific and nonspecific chemotaxis (purposeful migration) which can be measured in chambers or in the skin, and (c) ingestion and digestion of bacteria and fungi. In addition, metabolic events such as the rates of hydrogen peroxide generation and oxygen and glucose consumption can be studied in leukocytes. Certain enzymes such as nitroblue tetrazolium (NBT) reductase have been studied because of their potential importance in the intracellular killing of bacteria.

Normal phagocytes (including polymorphonuclear leukocytes and monocytes) metabolize glucose via the Krebs-Meyerhof pathway (anaerobic glycolysis) in order to migrate and ingest orgnisms. Glucose metabolism shifts to aerobic pathways following ingestion of organisms or particles. This shift in glucose metabolism seems important, as bactericidal complexes are generated intracellularly in the process, and phagocytes lose their ability to kill bacteria when placed in anaerobic environments. During this process, oxygen is consumed and hydrogen peroxide is produced. The hydrogen peroxide (H_2O_2) then combines with a halide and an enzyme (myeloperoxidase)

to form a powerful bactericidal complex.

Some metabolic observations relevant to this system have been made utilizing diabetic phagocytes. Martin et al. (40) found decreased glucose utilization and lactate production in leukocytes obtained from diabetic patients in good control. Dumm (20) also found that leukocytes obtained from nonacidotic diabetic patients exhibited a depression of glycolysis which could be corrected by insulin. This is interesting because Esmann (22) showed that the phagocytic membrane does not require insulin for glucose transport. Consequently, functional defects in diabetic phagocytes, if attributable to an insulin deficiency, probably involves insulin's intracellular actions rather than a membrane effect. Insulin is known to regulate several rate-limiting glycolytic enzymes such as phosphofructokinase, pyruvokinase, and glucokinase (65).

The oxidative metabolism of leukocytes from diabetic patients has been analyzed by direct oxygen measurements and NBT reduction (64). Diabetic leukocytes were found to have a lower degree of NBT dye reduction and a higher oxygen consumption than control leukocytes. These abnormally low NBT tests are interesting because these diabetic patients also had a reduction in phagocytic activity. The NBT reduction test is similarly abnormal in children with the syndrome of chronic granulomatous disease. In this condition, phagocytes are unable to kill certain microorganisms and the patients usually die of infection at an early age. These studies in diabetics are important and should be confirmed.

Fixed tissue macrophages in diabetes have been described as having increased glycogen and cholesterol (46), which may relate to a potentially significant abnormality in these phagocytic cells. The effects of the intracellular accumulation of lipid, although described in diabetics in 1925, has to our knowledge not been fully analyzed and may be an important aspect of a compromised reticulo-endothelial system.

The proof of the importance of any leukocyte defect lies in the answers to two questions: (a) do leukocytes migrate into an area of microbial invasion normally, and (b) do phagocytes ingest and kill microorganisms normally at these sites?

Deficiencies in Chemotaxis (Migration) by Phagocytes. Several studies have shown that chemotaxis is not altered when leukocytes from non-diabetic individuals are incubated in glucose concentrations varying from 200 to 1000 mg percent. However, Perillie, Nolan, and Finch (49) noted a delayed and diminished migratory response of granulocytes in diabetic patients, as measured by the skin window technique. This effect was noted when the diabetic patients were acidotic but not when well-controlled. Furthermore, the deficient mobilization of these important defenses returned to normal following correction of the acidosis.

In vitro chemotactic defects have been noted in phagocytes from adult (7,26,43) and juvenile diabetic patients (41). There was no correlation between the chemotactic defect and levels of plasma insulin, or serum glucose, carbon dioxide, or urea nitrogen. The addition of insulin to the cell suspension corrected these chemotactic defects in one study (43), but not in others (26,41).

The reasons for these observed chemotactic defects are not known. Similar defects in chemotaxis have been found in patients with multiple myeloma and macroglobulinemia and have been attributed to abnormal amounts of surface immunoglobulins. However, Mowat and Baum (43) could not demonstrate any abnormal coating of diabetic leukocytes with immunoglobulin.

Some of the chemotactic defects demonstrated in human diabetes have been noted in experimental models of diabetes. Cruickshank (14) noted that the inflammatory response in the skin following challenge with staphylococci was diminished in alloxan-diabetic, ketotic rabbits. Similarly, Briscoe and Allison (8) showed that the formation of peritoneal exudates was reduced in experimental peritonitis in nonketotic diabetic rats. Rabbits developed a significant reduction in the intensity of their inflammatory reactions following relatively short periods of glucose infusion (2). This abnormality could be dissociated from hyperosmolar or acidifying effects of glucose infusions. Thus, the mechanism of these defects is unclear and should be further evaluated.

Other exudative factors such as pH that may relate to chemotactic defects are also worthy of consideration. The pH of inflammatory exudates in diabetic rabbits is lower than in non-diabetic rabbits. Hutchins and Sheldon (28) have shown that the reduced concentration of hydrogen ion at sites of injury in diabetic rabbits is associated with profound alterations in the immune defense. Furthermore, there is a direct correlation between the development of mucormycosis in the rabbit and the delay and paucity of the inflammatory response during ketosis (55). Organisms were noted to grow and spread through tissues before an effective inflammatory response could be mounted. These defects are especially significant because many investigators believe that the initial defensive reactions determine whether an infection will be established following the lodgement of organisms in tissues, and that the issue is usually settled within the first 30 minutes of tissue invasion.

Deficiencies in Phagocytic Ingestion and Digestion. The data are conflicting about whether defects occur in ingestion and digestion by phagocytes in diabetes mellitus. Bybee and Rogers (9) reported that leukocytes of ketoacidotic, diabetic patients demonstrated a reduction in phagocytosis in vitro. The defect disappeared when the acidosis was corrected. Ingestive capacity of the diabetic leukocyte was restored by suspending the cells in normal instead of diabetic serum. However, leukocytes from normal individuals failed to develop the abnormality after being suspended in sera from ketoacidotic diabetic patients. Similarly, Bagdade et al. (5) showed that phagocytes from poorly controlled diabetics demonstrated a combination of inadequate ingestion and bactericidal activity. Insulin treatment reversed these abnormalities, and phagocytic efficiency was inversely correlated with the fasting glucose level. Bagdade et al. (4) subsequently compared a group of nondiabetic patients with eleven poorly controlled diabetic subjects studied before and after treatment. Leukocytes from these patients demonstrated a defect in killing type 25 pneumococci which was related to an impairment in ingestion of bacteria rather than to their bactericidal capacity. The degree of impairment also correlated closely with the fasting glucose level and was corrected by appropriate treatment of the hyperglycemia and ketoacidosis. These studies have important implications, as they suggest that insulin corrected a potentially serious defect in leukocyte function. However, the test organism used was relatively avirulent. Therefore, these studies should be repeated with more virulent organisms and in particular with those bacteria and fungi which commonly infect diabetics.

Recently, Tan et al. (60) found that the neutrophils of a diabetic patient who was suffering from an infection with *Staphilococcus aureus* were defective in killing this organism. However, Crosby and Allison (13,41) found that the bactericidal and phagocytic activities of leukocytes from diabetics who did not have ketoacidosis were not different from those of control subjects.

Better controlled study of several different kinds of microorganisms using leukocytes from diabetics in varying degrees of control are obviously needed to answer the question of whether clinically significant intrinsic defects of phagocytic function occur.

Studies of leukocytes obtained from animals with induced diabetes have shown as variable results as those from humans. Drachman et al. (18) showed a defect in the ability of circulating and lung phagocytes obtained from diabetic, nonketotic rats to kill type 25 pneumococci. While suggesting this deficiency was due to an intrinsic leukocyte abnormality, they believed the alveolar macrophage defect was related to serum hyperosmolarity attributable to hyperglycemia. However, Briscoe and Allison (8) could not find a defect in phagocytosis in nonketotic diabetic rats. Using avirulent pneumococci, Cruickshank and Payne (15) showed a decrease in bactericidal capacity of phagocytes obtained from alloxan diabetic rabbits which was related to ketoacidosis. Thus, these data from experimental models of diabetes mellitus suggest that intraleukocytic phagocytic defects in killing bacteria may occur and be related to the severity of diabetic control.

4. Nonspecific defects and metabolic abnormalities relating to infection. Many physicians have been attracted to the notion that hypersusceptibility in diabetes has been related to hyperglycemia and a saturation of tissues with glucose, leading to a more favorable environment for growth of microorganisms. The factors which compromise immunity in diabetes seem more complex than just hyperglycemia. Supplementation of serum or bacteriological media with glucose does not enhance bacterial growth rates appreciably (50). Several parameters of the immune system are more dramatically affected by ketoacidosis than by hyperglycemia. The ill effects of the hyperosmolarity which occurs in the ketoacidotic state may be the important factor.

Sbarra et al. (54) and Allison and Lancaster (3) have shown the dramatic adverse effects of hyperosmolarity on phagocyte function. However, the ranges of hyperosmolarity studied exceed those usually noted in the blood stream and tissues, except for the renal medulla (33). This may be important because diabetics have an increased rate of chronic pyelonephritis, which may be related to altered leukocyte function in the renal medulla (10). Alterations in complement fractions may also occur in this region and may interfere with effective opsonization and ingestion of bacteria. Another potentially important factor is that the diabetic patient may develop renal failure and uremia because of vascular disease or pyelonephritis. Uremic patients seem more prone to infection, but the responsible mechanisms are obscure (42).

Malnutrition secondary to a chronic catabolic state may be another important factor in relation to susceptibility in diabetes. Recent studies of phagocytes from humans (56) and lymphocytic-mediated cellular immunity (58) have shown defects related to severe malnutrition. Possibly lesser degrees of malnutrition as occurs frequently in diabetics may also affect leukocyte function.

The relationship between the pathogenesis of infections and alterations in serum lipids is as yet unexplored. Abnormalities in lipid and fatty acid composition and production seem important in the pathogenesis of cutaneous infections, such as boils and acne. A variety of infections, such as cholera and influenza, and administration of bacterial endotoxin are associated with characteristic lipid alterations (24). These have not been related to changes in lipids in diabetes as yet, but recent work suggests lipids influence the immune response to Coxsackie virus in

mice (37). This is an area worthy of further study.

Summary of Abnormalities in Host Defense Mechanisms

We believe that there are definite pathologic mechanisms operating in the diabetic patient, which predispose to recurrent infections. There are studies to support the presence of defects in chemotaxis, mobilization, and possibly ingestive and digestive capacities of phagocytes in diabetes.

The ready availability of potent antibiotics has dampened enthusiasm for investigative efforts on the immune defects of diabetics. There are almost no data concerning host defenses against viral infections in diabetics. Whether cellular immunity, delayed hypersensitivity, and other lymphocyte-mediated immune events are intact in diabetics is largely unexplored. The importance of the potential defects is underscored by the recent data showing that diabetic patients with ketoacidosis frequently die of infections with organisms in which these systems play an important defensive role (59).

Further investigations should be conducted on the pathogenesis of these infections in diabetes. Thomas (61) has indicated that research directed at corrective problems ("half way technology") is enormously expensive and often fails to affect disease, but that research directed at an understanding of disease processes ("high technology") is often rewarded with successful disease management and gratifying long-term benefits.

The Adverse Effects of Infections Upon Metabolic Processes in Diabetes Mellitus

While it is recognized that worsening of the diabetic state can precipitate infections such as mucormycosis, the reverse is probably more frequent. That is, infection increases insulin requirements and is important in worsening the diabetic state and precipitating ketoacidosis. As mentioned earlier, in two recent studies infection was the most frequent and important factor precipitating episodes of ketoacidosis (44,59). The exact mechanism by which infection is diabetogenic and aggravates the disease is unclear, but there are several interesting clues.

In the first place, infection is stressful and associated with an increased activity of adrenal steroid and epinephrine-like substances, both of which have profound effects upon carbohydrate and fat metabolism and commonly cause hyperglycemia. Second, fever in infection increases the rate of metabolic processes and increases caloric and water requirements.

Third, relatively high levels of circulating glucagon have been found in humans with diabetes (1,48) or infection. Glucagon is an insulin-opposing hormone. Glucocorticoid treatment in man has been shown to result in hyperglucagonism (39), and the increased endogenous steroid activity associated with infection has already been noted.

Muller, Faloona, and Unger (44) have presented evidence suggesting that infection is associated with hyperglucagonemia which may thus worsen the diabetic state. They studied 26 patients with diabetic ketoacidosis. Infection was the precipitating event in more than half of these. Plasma glucagon levels average 390 pg/ml, which was significantly greater than the fasting level of 118 pg/ml in diabetic subjects without ketoacidosis. Absolute hyperglucagonemia was present in 16 of the 26 patients. Insulin was present in the plasma in six of seven ketoacidotic patients; the level was "normal" in four.

These studies of Muller and his associates (44) may explain, at least in part, the remarkable degree of insulin resistance which almost universally characterizes the early hours of severe diabetic ketoacidosis. While severe insulin deficiency can cause hyperglucagonemia (45), they

believed absolute insulin lack was probably not responsible for the metabolic deterioration noted in most of the patients in their study, since four had normal levels of insulin during their acute illness. If their data and reasoning are correct, an infection-related stimulus caused an inappropriate release of glucagon. The mechanism of this is as yet unknown and seems worthy of further study. Unger and his associates reported that dialysates of infected tissues stimulated glucagon secretion in rats. Other mechanisms might be proposed, such as that hyperglucagonemia could reflect a reduced effectiveness of insulin upon alpha cells as well as upon other sensitive tissues. They also pointed out that hyperaminoacidemia and hyperkalemia might also account for the increased plasma glucagon levels, since these conditions are associated with increased glucagon levels in serum and are also found in diabetic ketoacidosis.

On the other hand, Cryer et al. (16) have reported that hypoinsulinemia and hyperglycemia develop promptly during *E. coli* septicemia in the nondiabetic baboon. They believed that insulin resistance was not a major determinant of the hyperglycemia associated with septicemia. They suggested that the occurrence of hyperglycemia is not an obligatory response to hormones other than insulin. The plasma half-time (a measure of utilization and excretion) of exogenous insulin was significantly prolonged during septicemia in their animals. Thus, they believe the observed fall of endogenous plasma insulin concentrations during *E. coli* septicemia must represent a decrease in pancreatic insulin secretion.

It is obvious that studies of the metabolic alterations in infected diabetics are incomplete, of great interest, and far from conclusive at present. Response patterns probably vary according to the nature and severity of the infection. This is an important area, and one in which significant advances immediately applicable to the care of diabetics with infection might result. It deserves high priority in future work.

Miscellaneous Aspects of Infection in Diabetics

Lerner and Weinstein (34) have shown that penicillin is adsorbed abnormally slowly following intramuscular injection in diabetic patients over 50 years of age. Maximum serum levels are lower than in nondiabetic controls, and occur one hour after injection in contrast to one-half hour in control subjects. Total excretion of penicillin in the urine was the same in diabetic and nondiabetic subjects after intramuscular drug administration, but there was a slght delay in excretion in the diabetic. Diabetic and normal individuals responded in similar fashion to an intravenous injection of penicillin, as evidenced by identical serum curves and similar urine excretion patterns. The data suggest that delayed adsorption of penicillin from intramuscular depots may be related to diabetic microangiopathy, although other factors have not been excluded. Not all diabetics demonstrated this defect. Similar results were observed with sulfisoxazole, but they were not statistically significant.

Under most circumstances this phenomenon probably has no clinical significance. However, it might be important in particularly stubborn or refractory infections. It is therefore noteworthy that the original observations which led to this study concerned two patients with diabetes mellitus and alpha streptococcus endocarditis who failed to respond to ordinary intramuscular doses of penicillin but did respond to greatly increased doses. These pharmacological studies need to be extended to other antibiotics (and indeed to many other important classes of medications). More attention needs to be given to the potential clinical significance of delayed adsorption of drugs in patients with diabetes.

The treatment of an infected wound in a diabetic by local application of insulin has been recommended by Paul (47) and others. Many patients with wound infections treated in this way with favorable results have been reported anecdotally, but we are unaware of controlled studies either proving or disproving the worth of this practice. Such a study needs to be done because many physicians, particularly surgeons, utilize this practice empirically. The favorable effect has been assumed to be related to an effect of insulin upon glucose metabolism in the wound, but may be related instead to the presence of zinc in the insulin preparation. Zinc is believed to have profound effects upon wound healing. While a seemingly minor adjunct to treatment of wound infections, the value of this measure could be determined very quickly, and if a definite effect was proven, further interesting studies are suggested.

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LONG-TERM COMPLICATIONS

14

DEMONSTRABLE METABOLIC ABNORMALITIES IN DIABETES MELLITUS THAT MAY CONTRIBUTE TO THE PATHOGENESIS OF SPECIFIC LATE COMPLICATIONS

Albert I. Winegrad and Rex S. Clements, Jr.

INTRODUCTION

The development of effective methods for the prevention of the long-term complications of diabetes mellitus will probably require the identification of the specific biochemical mechanisms responsible for each of the diverse clinical syndromes to which this term is applied, since there is little evidence to support the concept that a single pathological process is responsible for their development. It is recognized that diabetes mellitus may be a group of diseases with heterogeneous etiologies that have as their common characteristics the development of an impaired insulin secretory mechanism and an abnormality in the regulation of plasma glucose concentration that permits diagnosis. Since with rare exception a specific etiology cannot be assigned in any given patient with diabetes mellius, the possibility that specific complications are primarily associated with specific etiologic forms of diabetes remains to be excluded. However, none of the long-term complications of diabetes appears to be restricted to what is presently termed genetically determined diabetes. (Some workers still dispute the association of diabetic retinopathy and nephropathy with diabetes associated with chronic pancreatitis, hemachromatosis, and pancreatectomy; this is considered in subsequent contributions to this section.) It is therefore reasonable to consider the metabolic abnormalities that are commonly associated with diabetes mellitus to determine whether they may contribute to the development of specific long-term complication. The development of clinical manifestations is clearly preceded by a prolonged period of asymptomatic pathologic change in many of the long-term complications of diabetes mellitus, and the search for detectable metabolic abnormalities that influence their development is an essential part of any program for the development of effective preventive therapy.

Until quite recently efforts to determine whether the commonly demonstrable metabolic development of specific late complications have been restricted to clinical studies whose interpretation remains a matter of dispute. This subject is considered in detail elsewhere in this volume; however, it is appropriate to comment that the data derived from the clinical trials reported to date do not exclude the existence of relationships between the demonstrable metabolic abnormalities in diabetes mellitus and the pathogenesis of any of the late complications.

Hyperglycemia

Abnormalities in the daily fluctuations in plasma glucose concentration are universally present in patients with clinically detectable diabetes mellitus, and may be present intermittently in some patients over a prolonged period (29). The degree of abnormality in the regulation of plasma glucose in many diabetics has probably been underestimated since the random blood sugars obtained from these patients are almost invariably evaluated in terms of the blood or plasma glucose concentrations observed in normal subjects after the ingestion of a large load of pure glucose (usually 50 to 100 grams). Molnar et al. (74) have recently monitored the plasma glucose fluctuations that occur over a 24-hour period in normal subjects eating a mixed diet; these fluctuations

are restricted to a much narrower range than those observed in normal subjects during an oral glucose tolerance test. Abnormalities in the range and pattern of plasma glucose fluctuations are present in diabetics eating a mixed diet (74); however, these cannot be adequately assessed by isolated plasma glucose determinations or by reference to the values observed in normal subjects during a glucose tolerance test.

It should be noted that the value of "normalizing" plasma glucose fluctuations in patients with diabetes mellitus has never been assessed in large scale clinical trials and may not be feasible in many patients with the therapeutic methods presently available. Thus the possibility that hyperglycemia may contribute to the pathogenesis of specific late complications cannot be excluded on the basis of the clinical data presently available.

Recent studies suggest that increased activity of the polyol pathway may serve as a model for biochemical mechanisms by which hyperglycemia might produce significant derangements in the metabolism of tissues that are the sites of pathological changes in long-term diabetics. The polyol pathway of glucose metabolism consists of two enzymatic reactions by which free (i.e., non-phosphorylated) glucose is converted to free fructose:

$$\text{(1) D-Glucose + NADPH + } H^+ \xrightarrow[\text{(aldose reductase)}]{\text{Alditol:NADP Oxidoreductase}} \text{Sorbitol + } NADP^+$$

$$\text{(2) Sorbitol + } NAD^+ \xrightarrow[\text{(sorbitol dehydrogenase)}]{\text{L-Iditol:NADP Oxidoreductase}} \text{D-Fructose + NADP + } H^+$$

In this sequence glucose is initially reduced to its polyol (polyhydric alcohol) derivative, sorbitol, through the action of aldose reductase which requires NADPH as a co-factor. Sorbitol is subsequently oxidized to fructose by enzymes resembling hepatic sorbitol dehydrogenase for which NAD^+ is the preferred co-factor (49, 50). These reactions appear to occur in the cytoplasm, but in most tissues the subcellular localization of the pathway has not been rigorously examined.

The polyol pathway is unusual in that glucose utilization by this sequence does not involve the initial formation of glucose-6-phosphate as is the case in the quantitatively major pathways for glucose metabolism in mammalian tissues. Although the individual reactions are potentially reversible, the polyol pathway has been found to operate in an essentially irreversible fashion in those tissues in which it has been most thoroughly studied. This behavior is probably the result of the large decrease in free energy that is associated with cytoplasmic sequences in which NADPH is utilized for the reduction of NAD^+, which is one of the net effects of glucose utilization via the polyol pathway (2).

The polyol pathway provides the mechanism for the synthesis of seminal fluid fructose in the accessory glands of the male genital tract (49, 50). (Fructose appears to be the preferred substrate for glycolysis in most mammalian spermatozoa including man.) The polyol pathway also provides the mechanism for the synthesis of fructose in the placenta which is the source of the significant concentrations of free fructose found in fetal plasma (20,50,90). Until quite recently it was believed that aldose reductase, and hence polyol pathway activity, was restricted to these tissues. However, recent studies indicate that aldose reductase activity is widely distributed in mammalian tissues (19) and at present there is evidence that the polyl pathway is normally operative in human seminal vesicles, placenta, lens, brain, peripheral nerve, aortic

intima and media, and erythrocytes (19,20,27,75,84,90,121). (Other tissues have not been examined as yet.) Although the function of the polyol pathway in tissues other than the seminal vesicles and placenta is obscure, the presence of this pathway in tissues in which the intracellular transport of glucose is not subject to insulin regulation and is not rate limiting for glucose concentration may result in increased polyol pathway activity with the development of associated derangements in metabolism and composition. This was first recognized as a consequence of studies of experimental cataract formation by van Heyningen (114).

Lens. A number of observations suggest a relationship between elevated plasma levels of glucose and other aldoses (e.g., galactose) and the development of cataracts. "Snowflake" cataracts occur primarily in adolescents and infants with symptomatic hyperglycemia (15). Although senile cataracts occur in both nondiabetics and diabetics, cataract extraction is more frequently performed in adult diabetics (i.e., over age 40) (15). Cataracts are also a well recognized complication of human galactosemia either of the classic variety or resulting from galactokinase deficiency (93).

In rats with experimental diabetes, cataracts develop with great regularity and there is an inverse relationship between the degree of hyperglycemia and the time required for cataract development. Lowering the blood sugar by treatment with phloridzin delays or prevents cataract formation without correcting the insulin deficiency (82). Van Heyningen (112, 113, 114) demonstrated the enzymes of the polyol pathway in rat lens, and called attention to the accumulation of the polyol derivatives of glucose, galactose, and D-xylose in the lenses of rats in whom cataract formation resulted from alloxan diabetes, or from feeding diets with high galactose or D-xylose contents. These observations suggested that increased polyol formation is a common feature of experimental "sugar cataracts."

The intracellular transport of glucose is not rate limiting for glucose phosphorylation in the lens, and direct effects of insulin on glucose utilization have not been consistently demonstrated in this tissue (60,113,114). Elevated plasma glucose levels result in increased concentrations of free glucose in the lens in human and experimental diabetes (84, 113). Lens aldose reductase exhibits a high Km for free glucose and the intracellular glucose concentration is a major determinant of the rate at which it is reduced to sorbitol (47,54,113,114). Although sorbitol is a normal constituent of the lens, its concentration is significantly elevated in both human and experimental diabetes (84,113).

A relationship between increased polyol pathway activity in lens and the development of cataracts is well established. Mice of the CFW strain have lenses which are deficient in both aldose reductase and sorbitol dehydrogenase activities; cataracts do not develop when these mice are made diabetic with alloxan despite the presence of high concentrations of free glucose in the lens (59). Similarly the cataracts that develop when rabbit lenses are maintained in tissue culture in the presence of high medium glucose concentrations are prevented by the addition of an inhibitor of lens aldose reductase, 1,1-cyclopentanediacetic acid (18).

The association between increased polyol formation and the development of cataracts has been attributed to the osmotic effects of increased intracellular concentrations of sorbitol or other polyol products of aldose reductase activity (54). Tissues other than liver appear to lack facilitated transport systems for sorbitol and other acyclic hexitols, and even in the face of large concentration differences their rate of transport across the cell membranes is very slow

(62, 116). The turnover of sorbitol in the lens has been assumed to be slow, and the magnitude of the increases in lens sorbitol concentration observed in experimental diabetes would exert a significant osmotic effect and account in part for the associated increases in lens water. Increased medium osmolality minimizes the defects in the active transport of K^+, amino acids, and myoinositol that have been demonstrated in lenses exposed to high concentrations of galactose under conditions that result in cataract formation; these observations have also been used to support the osmotic hypothesis for the development of sugar cataracts (54, 55, 56).

There are, however, significant limitations to the osmotic hypothesis. Recent electron microscopic studies indicate that even in its earliest phases the formation of a sugar cataract involves much more than the simply "hydropic swelling" of the lens fibers which Friedenwald and Rytel (34) observed by light microscopy. The earliest changes occur in the anterior lens epithelium which exhibits a marked increase in free and membrane-bound ribosomes and subsequently proliferates to form a multicellular layer (61). Similarly, the data of Stewart et al. (100) suggest that the increased lens water content in rats fed a high galactose diet may not result solely from the osmotic effects of the accumulation of galactitol in the lens.

Erythrocyte. In considering the possible consequences of increased polyol pathway activity in other tissues there has been a tendency to approach the question in terms of the osmotic hypothesis. This is scarcely justified on the basis of the data available, since there is evidence that metabolic derangements resulting from increased polyol pathway activity need not be restricted to those predicted by the osmotic hypothesis. Thus in human erythrocytes polyol pathway activity accounts for approximately 1.8 percent of the glucose uptake at a physiological medium glucose concentration but may increase to 11 percent during incubation with 50 mM glucose (75, 108). Under these conditions, the increased utilization of NADPH for glucose reduction to sorbitol results in increased pentose phosphate activity as a consequence of changes in the redox state of the $NADP^+$/NADPH couple. In addition, the markedly increased rate of sorbitol oxidation to fuctose results in an increased free NADH/NAD^+ ratio with resultant changes in the steady state levels of the glycolytic intermediates proximal to the glyceraldehyde-3-phosphate dehydrogenase reaction and a significant fall in erythrocyte 2,3-diphosphoglycerate (108). Thus in the human erythrocyte increased polyol pathway activity may significantly alter the redox state of both free pyridine nucleotide couples. It is of interest that van Heyningen originally suggested that such changes might contribute to the development of experimental "sugar" cataracts (113).

Aortic Wall. The enzymes of the polyol pathway have been demonstrated in human thoracic aorta and in the intima and media of rabbit thoracic aorta (19, 76). The latter tissue provides a convenient in vitro system for the study of the effects of elevated glucose concentrations on the metabolism of the arterial wall (118). Intracellular transport does not appear to be rate limiting for glucose utilization in the arterial wall since free intracellular glucose can be demonstrated in tissue from both normal and alloxan diabetic rabbits that have been incubated with a physiological glucose concentration (5mM) (118). In addition, the free intracellular glucose concentration has been shown to rise following incubation with elevated glucose concentrations (76). Direct or immediate effects of insulin on glucose metabolism have not been demonstrated in aortic intima and media with the exception of minimal increases in the recovery of ^{14}C from ^{14}C-glucose in glycogen (118). Increasing the medium glucose concentration from 5 mM to 20 mM, which is within the range observed in the plasma of human diabetics, results in a

threefold increase in polyol pathway activity in the aorta (76). Polyol pathway activity is best assessed by the determination of aortic fructose production, since in this tissue as well as in the human erythrocyte there is a very significant turnover of endogenously synthesized sorbitol (75, 76).

Increased polyol pathway activity results in striking alterations in the composition and metabolism of aortic intima and media. Incubation with 20 mM or higher glucose concentrations results in an increase in the water content of the aorta within two hours that is similar in magnitude to that observed in the aortae of chronically hypertensive rats (76, 107). This occurs in the face of a decrease in the extracellular volume of the aorta as measured by the inulin space. The increase in aortic water content is too large to be explained as a consequence of the observed increases in aortic sorbitol content (76). However, there is no doubt that it is a consequence of increased polyol pathway activity since it is prevented by the inhibition of aortic aldose reductase and polyol pathway activity with 1,1-cyclopentanediacetic acid (77). Moreover, incubation with elevated concentrations of galactose, which is also a substrate for aortic aldose reductase, also results in increases in aortic water content (76).

Although the aorta characteristically exhibits a high rate of aerobic glycolysis (i.e., glucose conversion to lactate), it has a significant oxygen uptake and a modest Pasteur effect can be demonstrated (115). Acute exposure to elevated glucose concentrations does not alter the oxygen uptake of aortic intima and media, but within 30 minutes, a period sufficient to result in a detectable increase in water content, the oxygen uptake begins to decline (76). After a two-hour pre-incubation with 20 mM or higher glucose concentrations, the oxygen uptake of the aorta is significantly less than that of paired samples incubated with 5 mM glucose, its rate of lactate production is increased, and there is an increase in aortic lactate/pyruvate ratio reflecting a change in the free $NADH/NAD^+$ ratio. These changes, which suggest inpaired oxygenation of the aortic wall, occur in medium saturated with a physiological oxygen tension. Increasing the oxygen tension (by saturating the medium with 95 percent oxygen) restores oxygen uptake and lactate production to levels similar to that observed in tissues incubated with 5 mM glucose (76). Thus exposure to elevated glucose concentrations appears to result in hypoxia of aortic intima and media which appears to result from impaired oxygen diffusion. The addition of 1,1-cyclopentandiacetic acid in concentrations sufficient to produce a 40 percent inhibition of polyol pathway activity in aortic tissue incubated with 20 mM glucose prevents the development of both the increase in aortic water content and the associated impairment in oxygen diffusion at a physiological oxygen tension (77). These in vitro effects of elevated glucose concentrations on the composition and metabolism of the aortic wall may have in vivo counterparts since both an increase in water content and impaired respiration have been demonstrated in the aortic intima and media of alloxan diabetic rabbits (76).

The nature of the alterations in aortic metabolism that result from exposure to elevated glucose concentrations and increased polyol pathway activity is of interest in light of current speculation concerning factors that may adversely affect the response of this tissue to exogenous factors operative in the pathogenesis of arterial lesions. It has been noted that the arterial intima and media of larger vessels are devoid of capillaries and are dependent upon diffusion from the lumen for the provision of their oxygen requirements (115). Further, it has been noted that the ability to provide the oxygen requirements of aortic intima and media by diffusion

is limited by the distance through which this process must occur, and that minimal increases in diffusion distance would be expected to result in hypoxia of the aortic wall in the face of a physiological oxygen tension in arterial blood (115). Haust (45), Robertson (88), and Whereat (115) have all speculated on the possible significance of hypoxia of the arterial wall in potentiating the effects of other factors operative in inducing arterial lesions. Whether hypoxia is a significant factor in the proliferation of arterial smooth muscle, which Ross and Glomset (91) have postulated to be the key event in the pathogenesis of atherosclerotic lesions remains to be determined. It is, however, apparent that hyperglycemia itself can induce significant alterations in the metabolism and composition of the arterial wall that are similar to those which are thought to favor the development of arterial disease.

Peripheral Nerve. Current evidence suggests that the development of the symmetrical distal polyneuropathy associated with diabetes mellitus results from a metabolic derangement rather than from occlusive disease of nutrient blood vessels. By sufficiently sensitive techniques widespread abnormalities in peripheral motor and sensory nervous function can be demonstrated in newly diagnosed diabetics without evidence of clinical neuropathy (17). These abnormalities include decreased motor and sensory nerve conduction velocity. The latter are usually interpreted as indicating a derangement in myelin function, i.e., segmental demyelination or changes in the resistance or capacitance of internodal and paranodal myelin. Insulin deficiency or hyperglycemia may contribute to the changes that occur in peripheral nerve for experimental diabetes produced by pancreatectomy, alloxan, or streptozotocin results in reduced conduction velocity in rat sciatic nerve and in changes in the electrical properties of its myelin sheath (25,35). The polyol pathway is operative in peripheral nerve and elevated levels of glucose, sorbitol and fructose are present in the peripheral nerves of rats with experimental diabetes (37,101,103, 104. Polyol pathway activity in peripheral nerve appears to be regulated in part by ambient glucose concentration, and lowering the blood sugar of alloxan diabetics by the administration of insulin results in a rapid fall in the glucose, sorbitol and fructose contents of peripheral nerve (104). The localization of polyol pathway activity within the peripheral nerve has not been clearly established; however, the sharp rise in the fructose content of peripheral nerves undergoing Wallerian degeneration at the time which coincides with rapid multiplication of Schwann cells suggests that a significant fraction of polyol pathway activity is localized within those cells (102).

The intrinsic relationship between the Schwann cell and the myelin sheath of myelinated nerves has led to speculation that derangements in the metabolism of the Schwann cell resulting from increased polyol pathway activity may contribute to the pathogenesis of diabetic neuropathy (36,37). As yet there has been no clear demonstration that hyperglycemia as distinct from insulin deficiency can induce abnormalities in peripheral nervous function.

Plasma Lipids and Lipoproteins

It is commonly assumed that atherosclerotic disease in diabetics is accelerated as a consequence of associated hyperlipidemia (see Section on Atherosclerosis). However, the evaluation of plasma lipids in diabetics is difficult because of the existence of independently determined factors that may contribute to any observed abnormality. This point has been clearly established by the recent studies of Goldstein et al. (41, 42, 43) on the genetics of the hyperlipidemia observed in survivors of myocardial infarction. Hyperlipidemia was present in 33 percent of 500 survivors

of a myocardial infarction. By detailed family studies three distinct inherited lipid disorders (familial hypercholesterolemia, familial hypertriglyceridemia, and a newly recognized entity, familial combined hyperlipidemia) were shown to be present in 20 percent of the survivors less than 60 years of age, and in 7 percent of all older survivors. These studies clearly indicate that a high fraction of the hyperlipidemia associated with coronary artery disease is genetically determined. The frequency of diabetes mellitus in the 500 survivors was examined using a fasting blood sugar greater than 120 mg percent or current therapy with insulin or an oral antihyperglycemic agent as the diagnostic criteria. By these rigorous criteria 12 percent of the survivors were found to be diabetic. The frequency of diabetes in these survivors in whom an inherited lipid disorder had been established by family study was 22 percent in those with familial hypertriglyceridemia, 15 percent in those with combined hyperlipidemia, and 6 percent in those with familial hypercholesterolemia. Although the criteria employed would probably have underestimated the true frequency of diabetes in these survivors, it would appear that diabetes mellitus frequently co-exists with familial hypertriglyceridemia in survivors of myocardial infarction and that the frequency with which it co-exists with familial combined hyperlipidemia in such populations deserves further study. Family studies are currently required to document the presence of a genetically determined disorder of lipid metabolism since the lipoprotein phenotypes (32) do not appear to be qualitative markers in a genetic sense but rather quantitative parameters which may vary among different individuals with the same genetic lipid disorder (48). The lack of a specific genetic marker for diabetes mellitus and the failure to define the distribution of genetically determined lipid disorders in the patients studied seriously restricts the interpretation of most of the data presently available concerning plasma lipids and lipoproteins in diabetic populations.

Most diabetics have normal serum triglyceride and cholesterol concentrations (78,99,120). The highest reported incidence of hyperlipidemia (either hypercholesterolemia or hypertriglyceridemia) in diabetics approximates 30-35 percent when compared with arbitrary limits derived from age and sex matched normals (9). The fact that diabetes mellitus and hyperlipidemia co-exist more frequently than would be expected by chance is generally accepted. However, although some have assumed that a causal relationship must exist, this has thus far been demonstrated only in restricted instances.

In ketoacidosis the serum triglycerides and cholesterol levels may be markedly elevated and in these instances there is a marked increase in the plasma very low denisty lipoprotein (VLDL) concentration which may be accompanied by increases in chylomicra (24,46). These abnormalities in plasma lipids are corrected by treatment with insulin. Hyperlipidemia resembling that observed in diabetic ketoacidosis can be induced in insulin-dependent juvenile diabetics by the cessation of insulin administration (5). The hypertriglyceridemia associated with ketoacidosis has been attributed, in part, to an acquired deficiency of lipoprotein lipase activity (LPL). VLDL and chylomicra are triglyceride-rich lipid particles which appear to share a common saturable removal mechanism (13). The hydrolysis of the triglycerides of chylomicra and VLDL is mediated by LPL, which is found in particularly high concentrations in the capillary endothelium of adipose tissue (31). In rats experimental diabetes results in a marked reduction in adipose tissue LPL activity, which is corrected by treatment with insulin (51,89). Tissue LPL activity is difficult to assess in intact man. The intravenous administration of heparin appears to displace LPL from

tissue sites to which it is bound, and post-heparin plasma catalyzes the hydrolysis of triglycerides in chylomicra and VLDL. This post-heparin lipolytic activity (PHLA) acts on artificial triglyceride emulsions and assays of this activity have been used as an indirect measure of LPL (33). Insulin deficiency has also been implicated in the marked increases in plasma triglyceride-rich particles, primarily chylomicra, that are infrequently observed in chronic symptomatic diabetes with minimal ketosis (3, 4). These patients exhibit subnormal PHLA which can be restored to normal by treatment with adequate quantities of insulin; this is accompanied by improved triglyceride removal and a reduction in plasma triglyceride levels.

In less severe diabetics with mild fasting hyperglycemia PHLA is usually normal; however, in some of these individuals with associated hypertriglyceridemia the LPL-related maximal triglyceride removal rate (as measured in vivo during prolonged heparin infusion) is low (9,11,85). Brunzell et al. (13, 14) found that diabetics with normal PHLA but with impaired triglyceride removal exhibit a fall-off in PHLA activity after the second hour of a 5-hour infusion of heparin, which differs from the normal sustained response to heparin infusion. They have related this "PHLA depletion" to varying degrees of insulin deficiency, but as yet the reversibility of this abnormal response to heparin infusion following insulin treatment has not been fully documented. Thus in ketoacidosis and in the two instances described above, it is clear that insulin deficiency can contribute directly to the development of hypertriglyceridemia.

Hypertriglyceridemia is also observed in diabetics without fasting hyperglycemia and often with only mild impairment of glucose tolerance. Although the increase in plasma triglyceride levels which results from the substitution of a high carbohydrate diet for a normal diet appears to be exaggerated in this group, the response is now considered neither unique nor abnormal (9). In some of these subjects an abnormally high triglyceride influx rate into the circulation can be demonstrated (11,79,86). No abnormalities of PHLA or triglyceride removal kinetics have been uncovered, but fractional removal rates may be reduced (9). Although both free fatty acids and glucose are major precursors for endogenous triglyceride production, there is no evidence that substrate flux is accelerated in the type of individuals under consideration. The lipoprotein pattern most frequently associated is one in which pre-β-lipoproteins are the only triglyceride-rich lipoproteins present in increased amounts, however, in patients with the highest pre-β-lipoprotein concentrations chylomicronemia may also be present. This is believed to result from a saturation of the LPL-related plasma triglyceride removal system (9,13,14,86).

The causes of the hypertriglyceridemia associated with mild glucose intolerance are unknown. Since the necessity for family studies to establish the existence of the common inherited disorders associated with coronary artery disease has only recently been recognized most of the available data is derived from studies of populations that are not sufficiently well characterized to permit firm conclusions. It has been suggested that the association between hypertriglyceridemia and mild glucose intolerance may be explained by the common coexistence of obesity (10, 12). Correlations between plasma triglyceride concentrations and obesity have been observed frequently (1,28,30,44,52,87). Obesity is known to be associated with true hypersecretion of insulin in both normal and diabetic subjects, i.e., the insulin secretory response to comparable stimuli is greater than that seen in normal weight nondiabetic and diabetic subjects (57). In a variety of populations serum insulin, both basal and after stimulation, correlates with plasma triglyceride concentrations (12,86,92). (Although lipoprotein phenotypes are nonspecific,

Glueck et al. (40) failed to observe a positive correlation between insulinogenic indices and serum triglycerides in 80 patients with Type III, IV, or V patterns; obesity was not a significant factor in these patients but a high percentage had abnormal glucose tolerance.) This has led to speculation that insulin may be one of several factors promoting hepatic triglyceride sythesis (68) and thus aggravating the accumulation of triglyceride-rich lipoproteins in the plasma of subjects with endogenous lipemia (9,12,79). Weight reduction has been shown to result in correction of glucose intolerance, "hyperinsulinism," and hypertriglyceridemia in some individuals (12). However, defective early insulin responses to glucose are similar in hypertriglyceridemic and normolipidemic subjects with comparable degrees of mild glucose intolerance (6); this has led to the suggestion that early diabetes with its associated abnormalities in early insulin release is not etiologically related to hypertriglyceridemia (9).

It is apparent that the classification and delineation of the abnormalities in lipid metabolism present in the diabetic population is still in a developmental stage. However, the frequency with which inherited abnormalities in lipid metabolism are encountered in survivors of myocardial infarction, and the frequency with which some of these disorders coexist with diabetes mellitus (as defined by fairly rigorous criteria) suggests that failure to define the distribution of such patients seriously limits the value of all the reported clinical trials in so far as cardiovascular mortality is concerned.

It should be noted that Havel (46) has expressed doubt that the increased morbidity and mortality from ischemic coronary and peripheral vascular disease in diabetic subjects can be accounted for solely by an augmented rate of atherogenesis (see Section on Atherosclerosis).

Glycoproteins and Mucopolysaccharides

Winzler (119) has reviewed the extensive literature which indicates that the level of total serum protein-bound carbohydrate tends to be elevated in human diabetics. Most of the studies which attempted to relate alterations in serum glycoproteins and the presence of vascular complications in diabetics are difficult to interpret; this results from the large number of glycoproteins in plasma and the failure to isolate and quantify specific glycoprotein components (119). Thus, although elevation of the serum α_2-globulin fraction has been observed in diabetics, the components of this fraction may vary independently, and a correlation between an elevated α_2-globulin fraction and diabetic retinopathy or nephropathy has not been clearly established (69,119). Elevated concentrations of serum α-globulin glycoprotein components have been noted by Cleve et al. (21). McMillan (70) has recently reported a qualitative alteration in the composition of α_1-acid glycoprotein in diabetic serum; this purified component was reported to have an increased content of fucose. The data on plasma mucopolysaccharides in diabetics are fragmentary.

Earlier speculation that the lesions in the retina or renal glomeruli of diabetics might result from the deposition of plasma glycoproteins has been discounted (119). The present evidence on the composition of the glomerular basement membrane in long-standing diabetics indicates that it clearly differs from that of circulating glycoproteins (7, 8, 95).

The liver is known to be the source of most of the plasma glycoproteins with the exception of the immunoglobulins and the glycoprotein hormones (95). Insulin deficiency does not significantly alter the utilization of glucose for the synthesis of the glucosamine components of glycoproteins in rat liver even though glucose utilization for glycogen synthesis is markedly impaired (94). This and related observations led Spiro (94) to suggest that glucose utilization

for the synthesis of the carbohydrate components of glycoproteins (most of which are derived from glucose) might be increased in human and experimental diabetes as a consequence of decreased glucose utilization by insulin-dependent pathways and the increased availability of glucose. Recent evidence suggests that the glomerular basement membrane of long-standing human diabetics contains increased quantities of a disaccaride containing galactose and glucose linked to the hydroxyl group of hydroxylysine residues (78). The biochemical mechanism(s) responsible for these changes have not been clarified; however, Spiro and Spiro (96, 97, 98) have presented evidence that the activity of the "glucosyltransferase" of rat kidney cortex which catalyzes the transfer of glucose from uridine diphosphoglucose to galactose linked to hydroxylysine is increased in alloxan diabetic rats when compared with age-matched controls, and can be reduced by treating the diabetic rats with insulin. (The structure and synthesis of glomerular basement membrane are considered in detail in a subsequent section of this volume.)

Earlier speculation that insulin deficiency and/or hyperglycemia might favor the addition of carbohydrate units to specific amino acids in peptides or proteins has found unexpected support in recent studies of hemoglobin A_{1c}. The mean values for hemaglobin A_{1c} are nearly twofold greater in diabetics than in normal subjects (109). Structurally hemoglobin A_{1c} is a condensation product between one molecule of hemoglobin A (the major component of hemoglobin in normal adult erythrocytes) and one or more hexoses. The presence of increased concentrations of hemoglobin A_{1c} does not appear to be related to the patient's age, duration of disease, or the presence of specific clinical complications (109). However, in contrast to hemoglobin A, the oxygen affinity of hemoglobin A_{1c} does not appear to be significantly influenced by 2,3-diphosphoglycerate which is an important regulator of the oxygen affinity of normal erythrocytes. Present evidence indicates that the increased concentrations of hemoglobin A_{1c} found in some diabetics represents a modification of normal hemoglobin as a consequence of the diabetic state. Dixon (23) has recently concluded that the glucosylvalylhistidine (Glc-Val-His) formed by the reaction of glucose and Val-His (in pyridine-acetic acid at pH 6.2) corresponds to the substance found to be released from the amino terminus of the α-chains of hemoglobin A_{1c} by Schroeder. Dixon (23) has suggested that the dissociation constant of this reaction is such that under the conditions existing in human erythrocytes no more powerful glycosylating agent than an increased blood glucose level need be postulated to explain the increased concentrations of hemoglobin A_{1c} in diabetics. These studies also suggested that a low degree of glycosylation might possibly be expected for other α-amino groups that are exposed to an elevated glucose concentration (23). Since the glycosylation of hemoglobin A to A_{1c} has been shown to be associated with a loss of normal regulatory function, the possibility that similar alterations in the structure and function of other biologically important peptides in the diabetic obviously requires consideration. However, no systematic study of this problem has, as yet, been undertaken. There are a number of obvious candidates for study including the apoproteins of the circulating lipoproteins.

Hormones

There has been recurrent speculation that abnormalities in growth hormone secretion may contribute to the pathogenesis of diabetes mellitus or its complications; however, the early studies of plasma human growth hormone concentrations (HGH) by radioimmunoassay (122) failed to demonstrate any significant abnormality in fasting HGH or in the response to glucose ingesion in diabetics. (Clinical trials of the value of hypophysectomy in the management of diabetic

retinopathy are considered in another contribution to this volume; these studies provide no direct evidence that an abnormality in growth hormone secretion is a significant factor in the pathogenesis of this condition.) Lundbaek (63, 64, 65) has proposed that an abnormality in growth hormone secretion is a causal factor in the development of "diabetic angiopathy." These studies have been primarily concerned with documenting the existence of an abnormality in HGH secretion in human diabetics. Plasma HGH in normal adult subjects may exhibit wide acute fluctuations (16,39). Known stimuli to growth hormone secretion include fasting hypoglycemia, a rapidly falling blood glucose concentration in the absence of hypoglycemia, physical exercise, surgical and emotional stress, the ingestion of a protein meal, and the intravenous infusion of specific amino acids (arginine is particularly effective). In addition, a significant peak of plasma HGH often occurs at the onset of deep sleep (16,22,39). Lundbaek and his co-workers (63, 64) compared the HGH levels in young newly diagnosed male diabetics and in normal male medical students in serum samples obtained at 30-minute intervals through a 24-hour period in which a standardized pattern of eating, physical exercise, smoking, and bed rest was imposed. The average serum HGH in the diabetics was 3 to 4 times higher than that of the controls, and the pattern of serum HGH fluctuations in the diabetics was characterized by the presence of many more peaks throughout the day, with little apparent relationship to fluctuations in blood glucose. The same workers reported that a controlled exercise stress which failed to induce a significant rise in serum HGH in normal male controls caused a rise in male diabetics with disease of recent onset to 30 years' duration. Following treatment, which lowered the fasting blood glucose levels to a normal range (60 to 100 mg percent), newly diagnosed male juvenile diabetics exhibited a decreased HGH response to standardized physical exercise (63, 64).

Knopf et al. (58) have reported that there was a modest positive correlation between fasting blood glucose and plasma HGH concentrations in 315 patients who attended a diabetic outpatient clinic over a six-month period. The fasting plasma HGH in diabetic males was significantly higher than that of young male controls (58). Female diabetics with retinopathy had a mean fasting HGH that was significantly higher than that of normal young females, and higher, although not significantly so, than that of female diabetics who did not have retinopathy. In male diabetics the presence of retinopathy was associated with a significantly higher fasting level of both plasma HGH and blood glucose (58).

Although the normal response to an oral glucose load is an initial fall in plasma HGH, a rise has been observed in association with a variety of diseases as well as in the normal newborn infant (32). A number of investigators had suggested that a similar response to glucose ingestion is observed in mildly diabetic patients (123) and in juvenile diabetics (64). Knopf et al. (58) found that this "paradoxical" response of plasma HGH to glucose ingestion is observed in 10.5 percent of 57 healthy controls, in 8.3 percent of 84 patients with latent diabetes, and in 14.8 percent of 27 subclinical diabetics (positive family history of the disease, normal glucose tolerance but abnormal cortisone glucose tolerance). The "paradoxical" increase was not a reproducible response in any given patient and was not observed following the intravenous administration of glucose to normal control or latent diabetic subjects. Knopf (58) concluded that the pattern of suppression of HGH secretion during hyperglycemia induced by the administration of glucose is similar in patients with latent diabetes, in close relatives of diabetics, and in healthy controls.

The existence of a consistent abnormality in plasma HGH fluctuations in diabetics requires further documentation. The data available, however, do suggest that an elevated plasma HGH is more frequently present in male diabetics and in female diabetics with retinopathy than in young healthy subjects of the same sex. Further, it would appear unlikely that a fixed abnormality in the regulation of HGH secretion is present in human diabetics. There is some evidence that the degree of abnormality may be related to the range of blood glucose fluctuations present in that patient and can be altered by a closer approximation of the range of blood glucose fluctuations present in normal subjects. The causes for the apparent association between increased fasting plasma HGH levels and the presence of retinopathy remain to be determined, but a causal relationship has not been suggested (58).

Merimee et al. (72, 73) have noted the frequent occurrence of abnormal glucose tolerance in sexual ateliotic dwarfs who have a monotrophic deficiency of human growth hormone. These workers distinguished two groups: (Type I) exhibited a decreased plasma insulin (IRI) response to the ingestion of glucose, a mixed glucose-beef meal, or to the intravenous infusion of L-arginine; (Type II) had a normal or greater than normal increase in plasma insulin after these stimuli. (The question of what would constitute appropriate controls for these responses has been frequently raised.) Since the insulin responses to provocative stimuli and glucose tolerance improved in Type I dwarfs given HGH replacement therapy, Merimee (71) concludes that these dwarfs do not have genetic diabetes. Retinopathy has not been observed in sexual ateliotic dwarfs (71). From collaborative studies with Siperstein (73) it was concluded that sexual ateliotic dwarfs do not exhibit abnormally thickened muscle capillary basement membranes. Merimee (71) has concluded that carbohydrate intolerance and the other metabolic abnormalities associated with diabetes are relatively unimportant in the development of microangiopathic lesions in the absence of genetic diabetes and the chronic absence of HGH. The unique populations studied by Merimee are of great interest and deserve further exploration. His conclusions, however, have not gone undisputed. Williamson (117) has noted the potential fallacy of equating microaneurysms in the retinal circulation and capillary basement membrane thickening, since the processes may be distinct and subject to independent degrees of modification by growth hormone deficiency. Further, he has suggested that the values for muscle capillary basement membrane in the ateliotic dwarfs are suggestive of a bimodal distribution, and that with one exception the dwarfs with the thickest CBM had the most abnormal glucose tolerance tests. He concludes that it is not justified to conclude that an association between hyperglycemia and increased capillary basement membrane thickness may not exist in these dwarfs (117). Although abnormal glucose tolerance is observed in a significant number of patients with acromegaly, it has not been possible to draw meaningful conclusions concerning the possible contribution of excess HGH secretion to the pathogenesis of complications in diabetes from the data available.

Hyperinsulinism. The existence of true hyperinsulinism in association with diabetes mellitus is disputed (57). As noted in the discussion of lipid abnormalities in diabetics, the relationship between diabetes mellitus, obesity, hyperinsulinism and hypertriglyceridemia requires clarification. The situation is further complicated by the suggestion (80, 81) that one can identify within the nonobese survivors of myocardial infarction a group of individuals with "true hyperinsulinism" without impaired glucose tolerance. Similar observations have been reported by Tzagournis et al. (110, 111), Peters and Hales (83), and Gertler et al. (38). As noted by

by Nikkili et al. (81) all evidence for the possible role of hyperinsulinemia in the pathogenesis of arterial disease is thus far derived from cross-sectional studies and is more suggestive than conclusive. However, Stout (105, 106) has presented evidence which he interprets as indicating that insulin stimulates the synthesis of lipids in the arterial wall; the significance of these observations is disputed (66). A lipase has been identified in arterial tissue which appears to be distinct from lipoprotein lipase and whose activity with tributyrin or tripalmityn as substrate is increased by 3',5'-cyclic AMP, adrenaline, glucagon, cortisol, and growth hormone (66, 67). In rat aorta this activity is markedly increased following the induction of alloxan diabetes and is reduced by pretreatment of the animals with insulin (66). In human arterial tissue insulin decreases the lipolytic activity observed in homogenates of aorta, femoral, and coronary intima and media exposed to 5×10^{-5} M norepinephrine (67). It has been suggested that hyperinsulinemia during the late postprandial phase may impair the disposition of lipids derived from the plasma in the arterial wall (66, 67).

Other Hormones. Abnormalities in the secretion of other hormones in diabetes mellitus are considered in other sections of this volume. Data relating such abnormalities to the pathogenesis of the late complications of diabetes are at present fragmentary.

Information Required through Research Now and in the Future

A. An essential deficiency which must be corrected is the lack of information concerning the normal metabolism of the tissues that are the sites of pathological lesions underlying the late complications of diabetes and the manner in which they are affected by human and experimental diabetes.

The studies required include:

(1) Delineation of the normal metabolism of human and mammalian capillaries including those found in the retinal, renal glomerular, and peripheral circulations.

(2) Delineation of the metabolism and normal function of the components of the capillary including the pericyte (termed mural cells in the retina).

(3) Delineation of the processes of capillary basement membrane synthesis, modification, and degradation in the renal glomerular, retinal, and peripheral circulations.

(4) Delineation of the normal metabolism and function of the components of the arterial wall: the factors regulating myoepithelial cell migration and proliferation; the synthesis and degradation of elastin, glycosaminoglycans, and collagen within the arterial wall; the means by which lipoproteins cross the endothelial barrier and their subsequent disposition in the arterial wall.

(5) Delineation of the normal metabolism of the Schwann cell and axon and their metabolic interrelationships.

(6) Direct examination of the manner in which the above are affected by insulin and hyperglycemia, and in tissues obtained from human diabetics and animals with various forms of genetically determined and pharmacologically induced diabetes.

B. Information is required concerning the distribution within the whole population of patients with diabetes mellitus of independent, genetically determined factors that may modify the course with regard to the development of specific complications.

(1) Delineation of the frequency with which the recognized inherited derangements in lipid metabolism associated with coronary heart disease coexist with diabetes mellitus. Delineation of the frequency of clinical events related to atherosclerotic vascular disease in diabetics with a

coexistent inherited abnormality in lipid metabolism as compared with nondiabetics with the same inherited defect, and diabetics in whom the presence of an inherited lipid disorder has been excluded as best as possible.

(2) Efforts to delineate other subgroups of diabetics with presently unrecognized coexistent independent factors influencing the course of diabetes mellitus.

a. Studies to determine whether the development of rapidly progressive diabetic retinopathy, nephropathy, or neuropathy is more frequently associated with diabetes of presumed infectious etiology or with other diseases; efforts to determine whether these events are more frequently encountered within specific family groups.

b. Continued pursuit of leads derived from the studies outlined in A to determine whether the development of specific late complications may be determined by coexistent independent factors.

To cite specific examples:

When the processes of basement membrane synthesis and degradation are better understood, it should be possible to examine the possibility that inherited variations in the enzymes concerned with basement membrane synthesis or degradation exist in humans; the extent to which these might account for the development of specific microvascular complications in the diabetic population could then be examined. Similarly in the future it should be possible to determine whether inherited variation in polyol pathway activity within specific tissues may decrease or increase the likelihood of the development of specific complications.

c. Information is required concerning the normal range of plasma glucose fluctuations throughout the day in nondiabetic males and females of varying ages eating normal mixed diets, as well as the alterations that are associated with obesity, subclinical, and latent diabetes.

d. The relationships between diabetes mellitus, abnormalities in plasma lipids, obesity, diet, and altered patterns of insulin secretion must be clarified.

e. A continued search is required for evidence of possible relationships between hormonal abnormalities in the diabetic and the development of specific complications.

Personnel and Financial Requirements

(1) It is apparent that much of the basic information required has immediate application to major health problems other than diabetes mellitus and may to some extent overlap the requirements for cardiovascular disease, blindness, renal disease, and peripheral nervous disorders. However, traditionally the study of the metabolism of individual tissues has developed in large part as a consequence of studies concerned with diabetes, and this combined interest is frequently encountered in well qualified personnel.

(2) Much of the information required is in essence applied biochemistry, and few major biochemistry departments would presently consider the metabolism of isolated tissues and their alterations in human and experimental diabetes appropriate research activities for a significant fraction of their senior faculty. It is therefore necessary to continue to develop individuals with suitable training who will enter this field and to provide means to maintain their continued work in this area; provisions are required both for individuals who will restrict their studies to laboratory investigations and need not have clinical training, and for physician investigators.

Value of the Proposed Research

The diabetic population appears to be heterogeneous not only with regard to etiology but also with regard to the existence of independent factors that may alter the probability that a specific late complication of diabetes will develop in time. It would therefore seem unlikely that any

single therapeutic approach will prove effective for all diabetics. The proposed research should permit an understanding of the processes responsible for the development of specific late complications and the identification of possible contributing factors. This should permit a segregation of the diabetic population into well defined groups. This is essential for meaningful clinical trials. Moreover, the proposed research should provide an indication of the specific biochemical processes which must be influenced if the development of complications is to be prevented, and will provide systems in which a search for effective pharmacological agents can be carried out.

The extent to which the metabolic consequences of an impaired insulin secretory mechanism, including hyperglycemia, may contribute to the development of the late complications can only be assessed in studies of well defined populations. Current efforts to develop an artificial pancreas and to explore the feasibility of islet transplantation must be justified, in part, on the assumption that a correction of the metabolic abnormalities associated with diabetes mellitus will influence the development of specific complications. The development and testing of these modalities of therapy should be accompanied by efforts to define the appropriate populations in which such therapy may be warranted, and in which the value of these therapies can be appropriately tested. Information on the normal range and pattern of plasma glucose fluctuations in normal life is an obvious prerequisite for such studies.

The significance and origin of lipid abnormalities in diabetics in whom there is not a coexisting genetic disorder in lipid metabolism must be defined before appropriate studies to determine the need for specific forms of therapy and to test the value of any therapy.

The large number of patients with diabetes mellitus, the chronic nature of the disease, and the time required for the development of related late complications suggest that any economically feasible program to prevent complications will require the development of reliable methods of predicting the likelihood that specific complications will develop. In this manner emphasis can be placed on those subgroups of diabetics in whom the risk is greatest. An essential prerequisite to the development of these methods is the data to be derived from the proposed studies.

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15

DIABETES, HYPERGLYCEMIA AND ATHEROSCLEROSIS: NEW RESEARCH DIRECTIONS

Leon D. Ostrander, Jr. and Frederick H. Epstein

A. THE ASSOCIATION BETWEEN DIABETES, GLUCOSE INTOLERANCE AND CARDIOVASCULAR DISEASE

Atherosclerosis has been recognized as a complication of diabetes mellitus for more than a century (152), but the acute complications of ketoacidosis and infection commanded most attention until well into the insulin era. After the introduction of insulin, advances were rapid in both treatment and diagnosis. Tests of glucose tolerance (35,81,110) revealed that diabetes is a common chronic disease which often remains undiagnosed for may years (54).

With expansion of the recognized diabetic population because of more sensitive diagnostic tests and virtual elimination of death from ketoacidosis or infection, chronic vascular disease has become the principal cause of death and disability among diabetics, with atherosclerotic cardiovascular disease accounting for most of the excess morbidity and mortality (45,90,103).

The diabetic's tendency to develop gangrene of the feet has long been attributed to extensive atherosclerosis of the peripheral arteries, but neuropathy, microangiopathy, and susceptibility to infection may be as important (15). In other respects peripheral arterial disease does not differ appreciably from that of nondiabetics except for earlier onset, more extensive involvement of the vessels below the knees and a greater tendency for medial calcification (18,59,132,163).

While peripheral arterial disease accounts for much morbidity among diabetics, mortality is due largely to atherosclerotic heart disease. Clinical studies (14,20,29,100,124), mortality statistics (45,90,103), and necropsy findings (18,32,69,144,145) attest to the diabetic's greater frequency and higher fatality rate from severe, premature coronary atherosclerosis.

Women with diabetes appear to have nearly as high a prevalence of ischemic heart disease as diabetic men in necropsy and clinical studies. Nondiabetic women have a substantially lower incidence and prevalence of myocardial infarction than nondiabetic men. In a large autopsy series, Clawson and Bell (32) found that 19.5 percent of diabetic men and 17.4 percent of diabetic women past 40 years of age died of atherosclerotic heart disease. The proportions for nondiabetic men and women were 10.0 percent and 5.8 percent, respectively. Goldenberg and associates (69) found myocardial infarction at autopsy in 56 percent of men and 43.9 percent of women with diabetes, in contrast to 23.3 percent of men and 13.8 percent of women without known diabetes. Partamian and Bradley (124) reported 258 serial myocardial infarctions among patients from a diabetic clinic. Fifty-six percent occurred among women and 44 percent among men. The percentages correspond closely to the proportions of men and women in the base population.

Evidence for a greater prevalence of cerebral vascular disease among diabetics is less convincing although suggestive of a relationship (18,34,60,101,114). Diabetes appears to be related to cerebral infarction but not to hemorrhage (6). Pathologic studies demonstrated earlier and more extensive cerebral artery atherosclerosis in diabetics than in nondiabetics (6,60,73). While cerebral infarction tends to occur at an older age than peripheral or coronary arterial manifestations, it is an important cause of prolonged disability and ultimately death.

An extensive literature indicates, therefore, that atherosclerosis is an important complication of overt diabetes. In the past 25 years interest has gradually increased in the converse relationship, the frequency of abnormal carbohydrate tolerance among the very large number of persons with manifestations of atherosclerosis. Goldberger and associates (68) reported abnormal glucose tolerance in 10 to 14 patients with recent myocardial infarction. Waddell and Field (167) directed further attention to this area of research when they reported that 78 percent of 47 persons with severe manifestations of atherosclerosis were diabetic according to the cirteria of Fajans and Conn (54), while another 7 percent were probable diabetics. Other reports indicate that diminished glucose tolerance occurs in 33 percent to 46 percent of patients with healed myocardial infarction (5,156,168) and from 32 percent to 75 percent of persons with occlusive arterial disease in the legs (17,79,171). Although these observations suggest an excessive prevalence of reduced glucose tolerance and, by inference, mild diabetes among persons with atherosclerotic cardiovascular disease, appropriate control groups were not studied, the effect of age on glucose tolerance was not appreciated (8) and little consideration was given to the possibility that the vascular event itself, particularly myocardial infarction, could influence glucose tolerance (41,98). Reaven and associates (137) reported a carefully controlled study of patients with healed myocardial infarction in which 41 percent had abnormal glucose tolerance tests according to the criteria of Fajans and Conn (54), and an additional 34 percent had abnormal glucose tolerance tests after cortisone administration (53). Ten percent of controls had diabetes according to standard glucose tolerance tests, and another 23 percent had abnormal tests after cortisone.

In partial answer to criticisms that glucose intolerance may develop only after myocardial infarction, a few studies of other manifestations of ischemic heart disease revealed similar, significant associations with diabetes or specified levels of hyperglycemia (20,51,169).

These clinical observations are supplemented and reinforced by epidemiological investigations based on defined populations. The Tecumseh epidemiological study was instituted to investigate the prevalence and incidence of chronic diseases and their precursors in a total, natural community. Participation of the residents approached 90 percent for each series of examinations. Cardiovascular disease and diabetes were studied with particular emphasis. Men and women with coronary heart disease, peripheral or cerebral vascular disease, or asymptomatic T wave inversion in the electrocardiogram had a significantly higher prevalence of hyperglycemia than the population as a whole (120). Hyperglycemia was defined as the upper quintile of age- and sex-specific blood glucose values. The relationship of hyperglycemia to cardiovascular disease was largely independent of blood pressure level or cholesterol concentration (49). Keen and associates (89) then reported similar observations from the epidemiological study in Bedford, England. There is substantial evidence that heart disease of any etiology, particularly when associated with congestive heart failure, may suppress insulin secretion and cause hyperglycemia (50,75,153). Therefore, reports of a higher than expected incidence of ischemic heart disease among hyperglycemic but otherwise healthy participants in the Tecumseh and Bedford studies have strengthened the hypothesis that hyperglycemia is a true precursor of atherosclerotic heart disease (87,118). Stamler and associates (158) reported similar findings from their large epidemiological study of employed men in Chicago. A recent analysis of data from several epidemiological studies of cartiovascular disease, including that in Framingham, strongly suggests that hyperglycemia is an important precursor of atherosclerotic coronary artery disease (48).

The prevalence of hyperglycemia and its association with atherosclerosis differ greatly among certain ethnic groups. Diabetes and hyperglycemia are almost unknown among Eskimos (111), but diabetes according to clinical criteria is found in 50 percent of adult Pima Indians (22). Other ethnic groups have prevalence and incidence rates between these two extremes (62,142,143, 147,159). However, the frequency of atherosclerotic complications does not parallel the prevalence of diabetes (21,61,14,147,172). The relative immunity of certain ethnic groups with a high prevalence of hyperglycemia and diabetes may be related to factors discussed in the next section.

In summarizing this background information, the following conclusions appear justified: (1) Diabetics develop earlier and more extensive atherosclerosis of the coronary, peripheral, and cerebral arteries than nondiabetics. (2) Persons with atherosclerotic cardiovascular disease have a significantly higher prevalence of hyperglycemia than the general population. (3) Apparently healthy persons who develop ischemic heart disease have a significantly higher frequency of prior hyperglycemia than individuals who remain free of coronary events.

B. FEATURES THAT PREDISPOSE HYPERGLYCEMIC AND DIABETIC PERSONS TO ATHEROSCLEROSIS

Since overt diabetes and hyperglycemia predispose the individual to premature atherosclerosis, investigators have tried to identify characteristics of hyperglycemic persons which account for accelerated atherogenesis. Arterial hypertension, high serum lipid concentrations, hyperglycemia, increased coagulability of blood, and aberrations of insulin secretion are the major suspect conditions.

I. Arterial Hypertension

Much evidence suggests that diabetics have a higher prevalence of hypertension than nondiabetics (19,102,112,120,146). Pell and D'Alonzo (125) initially attributed most of the excess prevalence of atherosclerotic heart disease observed in the diabetic employees of a large industrial concern to their higher frequency of hypertension. In a subsequent prospective mortality study, hypertensive diabetics were particularly prone to death but among normotensive employees, diabetics also had a substantially higher incidence of cardiovascular death than nondiabetics (126). Most investigators agree that hypertension increases the risk of atherosclerosis among diabetics, but some assign it a major role (69,97,166), while others find the relationship less convincing (86,101,132,144,170). There are many hypotheses but no definitive studies to account for the high prevalence of hypertension among persons with diabetes or hyperglycemia.

II. Hyperlipidemia

Hyperlipidemia in the form of gross hyperchylomicronemia is frequently observed in markedly insulin-deficient diabetics. It is due to inadequate postheparin lipolytic activity (PHLA), which is partially insulin dependent (12). Diabetics in ketoacidosis may have profound elevations of all lipid fractions which clear rapidly with insulin therapy (67). The defect is primarily deficient clearance of triglycerides because of inadequate insulin (13). Even among milder diabetics, delayed removal of triglycerides may be due to subtle deficiencies in PHLA (9,27,130). Among a much larger proportion of diabetics and hyperglycemic persons, high concentrations of triglyceride-rich, very low density lipoproteins (VLDL) are found without evidence of marked insulin insufficiency or any tendency to ketoacidosis (2,99,115,149,174). Albrink and associates (2) reviewed clinical records of all diabetics in a single medical center who had serum lipid determinations between 1931 and 1961. They observed that serum triglyceride concentrations in-

creased among diabetics during that period and were associated with a greater prevalence of ischemic heart disease in the decade 1951-1961. Higher triglyceride levels were attributed to liberalization of dietary carbohydrate allowances, but the authors conceded that more frequent obesity of patients in later decades may have influenced the results. Unfortunately, data on adiposity were incomplete. Regardless of etiology, numerous reports implicate hyperlipidemia, principally hypertriglyceridemia, as an important factor in the development of atherosclerosis among diabetics (33,55,76,78,99,122,149,164). However, Carlson and Wahlberg (30), using an intravenous glucose tolerance test, could not demonstrate a significant relationship between glucose and lipid levels among patients with ischemic heart disease.

Carbohydate induction of hypertriglyceridemia is a distinct entity (1,94), but Bierman and Porte (24) and Ford and associates (64) presented evidence that obesity is commonly related to high triglyceride and glucose concentrations. Bierman and Porte suggested that hyperglycemia and hypertriglyceridemia usually coexist because of a common etiologic factor, adiposity, without an independent metabolic relationship. Studies among apparently healthy men in a random sample of the Tecumseh population lend some support to this hypothesis (121), although the relationship may be different in the diabetic or hyperglycemic person (119). Reaven and associates (138) and Kuo and Feng (95) presented evidence that high serum insulin concentrations are common among maturity-onset diabetics and persons with hypertriglyceridemia. They postulated that insulin probably stimulates production of triglyceride-rich VLDL. Nikkila and Taskinen (116) pointed out the extreme complexity of interrelationships between serum insulin concentration, glucose tolerance, adiposity and carbohydrate consumption and their association with hypertriglyceridemia. Most evidence relates adiposity to serum insulin concentration (11,85,127) so that identification of obesity as the most frequent cause of both hypertriglyceridemia and abnormal glucose tolerance is a useful generalization in spite of many exceptions (23).

The relationship of hyperlipidemia to diabetes was further complicated by the introduction of the typing of plasma lipoprotein patterns to characterize lipid disorders. Lipoprotein types III, IV, and V of Fredrickson and associates (65) include high concentrations of VLDL and are usually associated with hyperglycemia. Differentiation between primary hyperlipoproteinemia with associated carbohydrate intolerance and diabetes with secondary hyperlipidemia is largely arbitrary when this system of classification is employed and the differentiation is usually based on the relative severity of the lipid and carbohydrate manifestations. Adiposity, delayed insulin responses after carbohydrate challenge and later hyperinsulinemia are features common to types III, IV, and V hyperlipoproteinemia and to maturity-onset diabetes (93,165).

The approach to the study of inherited abnormalities of lipid metabolism associated with coronary heart disease was fundamentally altered by the recent studies by Goldstein et al. (70, 71) and the related publication by Hazzard et al. (77). These workers studied a large number of survivors of myocardial infarction and found by comparison with a large series of controls that 31 percent had hyperlipidemia; they then carried out studies of plasma triglyceride and cholesterol concentrations in the relatives and spouses of hyperlipidemic and normolipidemic survivors. The distribution of the fasting cholesterol and triglyceride values in the relative, together with segregation analysis, suggested the presence of five distinct lipid disorders. Three of these--familial hypercholesterolemia, familial hypertriglyceridemia, and a newly defined disorder, familial combined hyperlipidemia--appeared to represent dominant expression of three different autosomal genes, occurring in about 20 percent of the survivors below 60 years of age and 7 percent of older survivors. Their data also suggested that heterozygosity for one of these three

disorders may have a frequency in the general population of about 1 percent, thus being among the most common inherited metabolic abnormalities. In a related study Hazzard et al. (77) demonstrated that on an individual basis no lipoprotein pattern proved to be specific for any particular genetic lipid disorder, and that conversely in the population studied no genetic disorder was specific by a single lipoprotein pattern. Their studies indicated that lipoprotein phenotypes are not qualitative markers in the genetic sense, but are quantitative parameters which may vary among individuals with the same genetic disorder. The implication is that, for the moment, the genetic classification of the individual hyperlipidemic patient may be more meaningfully approached from a quantitative analysis of lipid levels in his relatives. In a follow-up, Brunzell et al. (28) examined the prevalence of diabetes mellitus in 397 adult first degree relatives of 91 index subjects with an autosomal dominant form of hypertriglyceridemia (familial hypertriglyceridemia or familial combined hyperlipidemia) which had been established by family studies. This study demonstrated that diabetes mellitus and these common familial forms of hypertriglyceridemia segregate independently, for they could find no evidence that the prevalence of diabetes mellitus was related to the presence of a specific inherited form of hypertriglyceridemia. They suggested that the frequently reported association between diabetes mellitus and hypertriglyceridemia may reflect preferential selection of patients with both diabetes mellitus and a familial form of hypertriglyceridemia, since the combination is more likely to be symptomatic than hypertriglyceridemia alone.

III. Abnormalities Related to Coagulation and Thrombosis

There are no convincing reports of an excessive incidence of thrombotic or thromboembolic events related to uncomplicated diabetes (25,66,105), although several aspects of coagulation are frequently abnormal in diabetics. Increased platelet adhesiveness is the most commonly reported abnormality (10,26,96,106,136,154). High concentrations of free fatty acids, a common finding in diabetes (135), causes platelet aggregation (36,37,80), but several studies suggest that increased platelet adhesiveness among diabetics is independent of fatty acid level (96,117). One report attributes platelet "stickiness" directly to the blood glucose concentration (26), but this observation has not been confirmed. Diabetics have higher concentrations of factor VIII, factor V, and fibrinogen than nondiabetics (43,106,136). Diabetics also appear to have lower than normal fibrinolytic activity (10,56).

Hypercoagulability, particularly increased platelet adhesiveness, has long been implicated as a factor in atherosclerosis among both diabetics and nondiabetics (113,128). Connor and associates (37) demonstrated that high concentrations of long chain saturated fatty acids cause marked platelet aggregation and massive thrombosis in experimental animals; they postulated that lower concentrations of the same fatty acids probably initiated enough platelet aggregates to form small mural thrombi, which in turn became sites of atheroma formation. Although still somewhat conjectural, platelet thrombi are probably a factor in atherogenesis. Diabetics are then at higher risk of atherosclerosis than nondiabetics in part because of abnormalities related to coagulation and thrombosis.

IV. Insulin and Blood Glucose Concentrations

Inadequate or excessive insulin release, inappropriate response to insulin, and abnormal insulins are suspected factors in the initiation of premature atherosclerosis in diabetes.

Winegrad and associates, whose studies are described elsewhere in this monograph, relate the

formation of polyols in the inner arterial wall directly to hyperglycemia and hypoinsulinemia (175). Sorbitol, the principal polyol formed in aorta, alters metabolism of the inner arterial wall and increases its susceptibility to atheroma formation.

Martin and Stocks (104) reported a significant relationship between atherosclerosis and a reduced hypoglycemic effect from intravenous insulin injections among first insulin dependent and later less severe diabetics (161). Sloan and associates (155) reported higher serum insulin responses and blood glucose levels after glucose challenge among apparently nondiabetic men with atherosclerotic peripheral vascular disease than among age-matched overtly healthy control subjects. On the other hand, Elkeles and associates (44) observed more frequent vascular complications among noninsulin dependent diabetics with poor endogenous insulin responses than in subjects with more appropriate insulin levels after oral glucose stimulation.

Diabetes was long attributed to hypoinsulinemia due to inadequate islet cell mass or function. This concept logically explains the pathophysiology of most insulin-dependent and many less severe diabetics (52,107,176). The maximum serum insulin response after stimulation occurs appreciably later in most diabetics than in nondiabetics, although the peak insulin concentration may be quite high (63,91,107,151). Some persons who are diabetic according to glucose tolerance criteria react promptly to stimulation with either normal or supernormal insulin responses (40, 83,140,139). Hyperglycemia in the presence of apparently adequate serum insulin concentrations suggests either a high proportion of biologically inactive insulin or resistance to the effect of insulin. Obesity is the most frequent cause of hyperinsulinemia because of the high concentrations required to overcome resistance to glucose transport into large fat cells (148). Glucocorticoids, glucagon, and growth hormone are insulin antagonists, but high concentrations of these hormones are probably not common factors in the etiology of diabetes or atherosclerosis. Catecholamines cause gluconeogenesis, a factor in hyperglycemia, and have been implicated in the development of heart disease (74,133,134). Epinephrine and norepinephrine also induce hyperglycemia by inhibiting insulin secretion (31,129), so that adrenergic excess results in hyperglycemia but, at the same time, hypoinsulinemia. Therefore, while hyperinsulinemia in nonobese diabetics may be due to insulin resistance, the mechanism is unknown.

The variety of glucose-insulin relationships observed among diabetics is inconsistent with a single pathophysiologic explanation. Furthermore, individuals may exhibit markedly different glucose or insulin responses when studied under similar conditions over periods of days, months, or years (52). Although a single hypothesis cannot reconcile all experimental observations, one can generalize that all diabetics have inappropriate insulin-glucose relationships, which are frequently unstable over time. Not only hyperglycemia but hyperinsulinemia may enhance atherosclerosis. Stout (162) reported that high concentrations of serum insulin increase lipid synthesis in the arterial intima and inner media. Epstein (47) earlier hypothesized that either insulin deficiency or excess could enhance atherosclerosis.

C. TREATMENT

1. Medical Treatment of Hyperglycemia

Blood glucose levels as close to normal as possible have been the logical but usually unattainable goal of diabetic management since before the insulin era. The frequent occurrence of both microangiopathy and premature atherosclerosis among persons with only mild abnormalities of glucose tolerance or well controlled overt diabetes suggests that factors other than meticulous

control of the glucose concentration are important (51,87,169). However, because of the chronicity of diabetes and the variability of glucose levels over long periods, a major effect of hyperglycemia cannot be discounted in spite of apparent stability of glucose concentrations during a particular interval of time.

The University Group Diabetes Program (92), a long-term, randomized, prospective intervention study, treated noninsulin dependent diabetics according to five regimens: fixed dose insulin, variable dose insulin, tolbutamide, phenformin, and placebo. The results continue to evoke controversy (39,58,108,131,150), but much of it concerns the significantly higher cardiovascular mortality in the tolbutamide treated group. The most important observation was the failure of any treatment regimen to reduce mortality from atherosclerotic events below the rate of the placebo group. It is not surprising that no regimen of treatment reduced the incidence of atherosclerotic cardiovascular events. Control, as determined by periodic serum glucose concentrations, was appreciably better only in the variable dose insulin group (108). Adherence to the program was lowest, only 45.3 percent, in this group. Perhaps members of the variable dose insulin segment achieved their mean 13 percent reduction in fasting glucose concentration by more regular insulin administration prior to clinic visits. Mean serum glucose levels probably varied little among any of the study groups.

The study design did not include standardized treatment of other precursors of atherosclerotic events, but evidence of vascular disease, renal insufficiency and rather marked hypercholesterolemia was taken into account in the characterization of the groups (92).

The University Group Diabetes Program participants were a heterogeneous collection of diabetics whose antecedents of macroangiopathy were incompletely defined and essentially untreated even when recognized. Consequently, the study revealed neither the relative importance of separate precursors of atherosclerosis nor the benefits, if any, of intervention.

Keen and associates (88) found no evidence of a harmful effect from tolbutamide in a primary intervention study among "borderline" diabetics. Lower morbidity from ischemic heart disease was observed among the treated participants, but the difference from the control group was not statistically significant. Paasikivi (123) reported that long-term tolbutamide treatment after first myocardial infarction postponed fatal and nonfatal recurrences among persons with abnormal intravenous glucose tolerance tests but without overt diabetes. The subjects of the three intervention studies were not comparable. The inconsistent results of tolbutamide treatment on atherosclerotic complications were probably due to subject selection. The effect of tolbutamide, whether favorable or deleterious, appears minor and inconclusive. Available evidence suggests that no current regimen to control blood glucose concentration has an appreciable effect on the atherosclerotic complications of diabetes.

II. Surgical Treatment of Atherosclerotic Complications

Surgeons vary in their optimism regarding the results of operations to alleviate peripheral arterial insufficiency in diabetics. Barker (15) makes a clear distinction between occlusive disease in diabetics that is similar to that found in nondiabetics and the more complicated vascular lesions associated with rest pain, gangrene, diffuse small vessel disease and neuropathy. He considers patients with the latter findings poor operative risks with minimal prospects for improvement with any form of treatment. Most surgeons agree that diabetics tend to have more extensive atherosclerosis of the tibial and peroneal arteries than nondiabetics and more frequent

medial calcification (15,16,38,173). Diabetics are operated upon more often for relief of rest pain or treatment of gangrene than nondiabetics. The preferred revascularization procedure is the saphenous vein bypass, which is said to be as effective in diabetics as in nondiabetics for relief of discrete occlusions (16,160,173). Even in patients with gangrene, revascularization often permits a more conservative amputation. In selected cases, sympathectomy and endarterectomy are still useful, but prosthetic tubes are no longer used extensively because of thrombosis, which is particularly common below the knee (15). At best, surgery is only palliative treatment for peripheral atherosclerosis in diabetes.

Aortocoronary saphenous vein bypass for treatment of atherosclerotic occlusive disease of the coronary arteries has gained popularity in recent years. Only limited information is available on the short-term results of operation and no assessment is available of large numbers of patients observed for 2 years of more. Immediate results from several centers are encouraging. Sixty to ninety percent of survivors obtain complete to moderate relief from intractable angina pectoris (4,42,109). The surgical mortality is about 5 percent and another 10 percent survive myocardial infarction as a complication of the operation. No distinction has been made between diabetics and nondiabetics in published reports. Poor results have generally been attributed to graft occlusion or inadequate myocardial function preoperatively (4,7,141). Lack of adequate follow-up and failure to perform controlled studies of the efficacy of the procedure make objective evaluation impossible at this time (157). It is probably a worthwhile palliative procedure for some patients but is not a proven treatment for occlusive disease of the coronary arteries.

D. RESEARCH NEEDS

I. Biological Definition of Hyperglycemic States

Since no biological marker is specific for diabetes, diagnosis depends on arbitrary criteria for classification of the glucose tolerance test, the insulin response to specific stimuli, and the presence of other clinical features that are characteristic of diabetes in varying degrees. Diabetes may represent the upper end of a continuous distribution of glucose tolerance and other variables; specific biological identification is then impossible. Further productive research on prevention or delay of atherosclerotic complications does not depend on a diagnostic marker of diabetes.

Diabetes in the broadest sense should be classified in a stratified fashion according to well-defined criteria that include: (a) blood glucose concentrations during the glucose tolerance test, (b) serum insulin concentrations at appropriate intervals after stimulation, and (c) levels of associated factors of suspected importance in the development of atherosclerotic complications (age, sex, adiposity, blood pressure, serum lipids, smoking, and coagulation factors). Classification would depend on a comprehensive examination and at least two and preferably three successive measurements of physiological variables and biochemical factors. It is inconceivable that maturity-onset diabetics, who differ substantially in the features enumerated, would exhibit similar biochemical interrelationships or experience the same risk of atherosclerotic complications. Therefore, multifactorial classification and data analysis are essential for further investigation of atherosclerosis in diabetes.

II. Clinical and Laboratory Research

Much additional information is needed to develop a comprehensive concept of the multiple

complex metabolic interrelationships between blood glucose, serum insulin, lipids, blood pressure, and coagulation factors in persons with different degrees of carbohydrate intolerance and various expressions of the other enumerated factors. Complex problems in intermediary metabolism would hopefully be clarified if not entirely settled by more precise classification of subjects.

Human and animal studies should be designed to determine environmental and genetic factors that predispose subjects with various glucose-insulin relationships to hypertension, hyperlipidemia, and coagulation abnormalities. Meticulous study of humoral and biochemical dynamics under strictly controlled experimental conditions should yield essential information about metabolic interrelationships and their correlation with atherosclerosis. Angioradiography will probably be an important adjunct to such research in humans, because it permits accurate assessment of subclinical arterial atherosclerosis. With appropriate precautions and skillful technique, risk to the subject is minimal, and arteriograms allow immediate correlation between metabolic features and degree of atherosclerosis in critical arteries.

III. Epidemiological Studies

Sufficiently large numbers of otherwise healthy hyperglycemic subjects with various combinations of glucose and insulin concentrations and other suspect characteristics and an appropriate control group must be followed for an adequate period of time to ensure that enough atherosclerotic events occur among the cohort for valid statistical determination of relative risk. Participants should range from 35 through 59 years of age, include approximately equal numbers of men and women, and all be free of evident cardiovascular disease. They should be geographically stable and willing to participate for 10 years. Approximately 500 myocardial infarctions or coronary deaths would be necessary to assess adequately the suspected precursors. It is estimated that population of 5,000 to 7,000 diabetics would be required in order to collect such a large number of definite atherosclerotic events within 10 years. The larger number is probably more realistic if one allows for attrition from causes other than coronary events. Such a study requires collaboration among investigators in many centers.

Although the logistics and cost are formidable, such a study should yield information that more than justifies the expense. Particular features or combinations of traits probably account for a disproportionately large segment of atherosclerotic events among hyperglycemic persons. Only a well-designed epidemiological study can test this hypothesis.

IV. Intervention Studies

The design and initiation of precise intervention studies require prior identification of specific precursors of atherosclerosis among different categories of hyperglycemics. Problems of design, population cooperation, and cost would be great, but the causative role of traits implicated as precursors of atherosclerosis in clinical, laboratory and epidemiological studies can only be established if successful intervention reduces the incidence of the disease.

V. Early Identification of Potential Diabetics

Individuals may have features of diabetes other than hyperglycemia for long periods before glucose intolerance is readily detectable (54). Sufficient numbers of such subclinical diabetics could be included in a prospective epidemiological study to ascertain their relative risk of atherosclerotic events. Further detailed study of the intermediary metabolism of such persons should elucidate more of the hormonal and biochemical interrelationships of early diabetes.

VI. Investigation of Ethnic Groups with a High Prevalence of Diabetes but Little Atherosclerosis

Intensive laboratory and clinical investigation of the physiological features and intermediary metabolism of appropriate samples of such populations should afford important clues to the origins of atherosclerotic complications in the general diabetic population.

VII. Summary

Classification of hyperglycemic persons according to the presence and severity of major factors of suspected importance in the development of atherosclerosis is essential to the proposed investigations. This research is based on the hypothesis that atherosclerosis is a multifactorial disease, in which the etiologic importance of abnormal carbohydrate and insulin metabolism has not been fully appreciated. The suggested epidemiological studies require standardized procedures and research sophistication far beyond any previously attempted among large numbers of participants. However, less ambitious studies are unlikely to yield enough new information to justify subsequent intervention trials or prevention programs.

E. APPLICATION OF PROPOSED RESEARCH TO PREVENTIVE MEDICINE

Complications of atherosclerosis are the leading cause of death and disability in the United States (57). Atherosclerotic events are not confined to the elderly but are the major cause of mortality and morbidity among middle-aged employed men (72). Current evidence suggests that atherosclerosis is caused by combinations of predisposing conditions (84). Epidemiological studies have revealed such a strong and consistent association between serum cholesterol level, arterial blood pressure, and cigarette smoking, singly and in combination, and the incidence of atherosclerotic heart disease, that they are generally accepted as important precursors (46,82). While the evidence is less extensive, diabetes and hyperglycemia are also significantly related to the risk of ischemic heart disease (48,87,118,158). The interrelationships of these precursors have been discussed at length in this article.

A clear categorization of diabetes, hyperglycemia, and associated conditions, investigation of basic hormonal and biochemical relationships, and determination of the relative risk of specific combinations of features in each category would provide the essential base for the rational application of prophylaxis against atherosclerosis. Much can be surmised from current knowledge, but the problem requires more precise definition before extensive preventive programs are feasible. Even a modest reduction in the incidence of atherosclerotic events by the application of information derived from the research suggested would more than justify the considerable effort and expense.

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16

OCULAR COMPLICATIONS IN DIABETES

Morton E. Smith and Bernard Becker

I. BACKGROUND

Visual disability in diabetes can be caused by cataracts, refractive errors, and glaucoma, but the most important ocular complication which gives rise to blindness is diabetic retinopathy; a term which encompasses all the pathologic phenomena in the retina directly related to the presence of diabetes. Diabetic retinopathy is further divided into two major categories--the nonproliferative type and the proliferative type; the hallmark of the latter being the presence of newly formed blood vessels (neovascularization).

A. Scope of the problem.

Diabetic retinopathy is the fourth leading cause of all legal blindness (visual acuity of 20/200 or worse) in the United States today (70). Furthermore, it is on the rise as a cause of blindness because senile cataracts and glaucoma are reduced through better delivery of health care and as more diabetics live longer. The latter point is appreciated by the historical fact that before the discovery of insulin by Banting and Best in 1922, reports of proliferative diabetic retinopathy were infrequent. In that era, diabetics did not live long enough to develop severe retinopathy or become blind from their disease. In 1930 less than 1 percent of newly reported cases of blindness in the United States were due to diabetes. Today, 15 percent of all new cases of blindness are due to diabetes; and in the 40 to 60 age group, diabetes is the most common cause of newly reported cases of blindness. Almost 40 percent of all diabetics have some degree of diabetic retinopathy, and 2 percent of all diabetics are blind (30).

There are many factors which determine the occurrence of clinically detectable diabetic retinopathy, but the most important are the duration of the diabetes and the age of the patient at the time of diagnosis. If a patient is diagnosed as a diabetic at age 30, then there is a 10 percent chance he will have some degree of diabetic retinopathy by age 37, a 50 percent chance by age 45, and a 90 percent chance by age 55. This concept is slightly modified by age, i.e., if the diabetes is diagnosed before the patient is 30 and the disease lasts 5 to 9 years, the risk of diabetic retinopathy is 2 percent per year, ... but if diabetes is established after the age of 30 and the disease lasts 5 to 9 years, the risk of diabetic retinopathy is 7 percent per year.

The mere existence of diabetic retinopathy does not necessarily portend a visual handicap or a poor visual prognosis. Many of these individuals have and retain excellent to adequate vision. In fact, one of the frustrations of the ophthalmologist is the inability to predict which diabetic patients will remain stable and which ones will deteriorate. Visual prognosis is

Note: It is estimated from an index of prevalence of economically disadvantaged persons from diabetic retinopathy in 1968, that the total costs, which included health services, lost productivity, and benefits, amounted to an absolute minimum figure of $260,000,000 per year. With rising inflation since 1968, the calculation would probably come to a 1974 minimal estimate of about $338,000,000 per year.

estimated not only on the duration of the disease but also on what the visual acuity is at the time of diagnosis and whether or not the retinopathy is nonproliferative or proliferative (55).

From the statistics available, a general picture can be drawn. If, at the time of diagnosis of diabetes, the patient has minimal retinopathy of the nonproliferative type and the vision is good (20/40 or better), then the risk of blindness in 5 years is less than 10 percent. The risk of blindness in 5 years is greater if the vision is already bad at the time of diagnosis (20/80 - 20/200), and especially if the retinopathy is of the proliferative type; this risk being about 50 percent. It has also been pointed out that if one eye is already blind from diabetic retinopathy, there is a 50 percent chance the other eye will have a significant loss of vision within 1 year (56).

The magnitude of the problem is further emphasized when the prevalence of blindness in the diabetic is compared to the prevalence of blindness in the general population. The prevalence of blindness from all causes at age 50 is 0.15 percent; but in any diabetic at age 50 who has had the disease for over 15 years, the prevalence of blindness is 3.5 percent, i.e., the diabetic is 23 times more likely to be blind than his nondiabetic counterpart (30).

The presence of severe diabetic retinopathy also bears on the prognosis for life. Berkow et al (5) made an interesting observation from reviewing records of patients who sought a guide dog at Seeing Eye, Incorporated, where it had been noted that blind diabetics rarely returned for a second dog. They found that there were 85 blind diabetics who had died since getting their first guide dog, and that the average life span after the onset of blindness was only 6 years.

In summary, diabetes is the most important systemic disease giving rise to blindness. The visual prognosis in a diabetic is influenced by the age of the patient and the vision at the time of diagnosis, and more importantly by the duration of the disease and the type of retinopathy, i.e., proliferative versus nonproliferative. Blindness due to diabetic retinopathy is an ominous finding in regard to the prognosis for life. One out of every 40 diabetics is blind, and two out of every 1,000 become blind each year. This represents over 10 times the risk of blindness from all causes in the general population.

B. Characteristic appearance and course of diabetic retinopathy.

1. Nonproliferative retinopathy (Figs. 1 and 2).

a. Pathology of the capillary bed.

Nonproliferative diabetic retinopathy (also commonly referred to as background retinopathy) is first manifested by the appearance of the microaneurysm. These globular or fusiform outpouchings occur from one side of the capillary wall, range from 10-200 microns, and vary in color from deep purple-red to yellow-white. Although microaneurysms can be seen by routine ophthalmoscopy, fluorescein angiography[1] has greatly facilitated their recognition and has shown that they are particularly prominent around the edge of areas of nonperfused, obliterated capillaries.

It is here in the capillary bed that the initial changes are believed to occur. The "shunt theory" (13) suggests that first there is loss of capillary pericytes,[2] resulting in loss of

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[1]A technique whereby fluorescein dye is injected into an arm vein and photgraphs taken when the dye reaches the retinal vessels.

[2]Special cells (also referred to as mural cells) in the walls of capillaries.

capillary tone, microaneurysm formation, and dilation of some capillaries which shunt or "steal" blood away from other capillaries, leading to areas of obliteration of adjacent vascular channels. Other investigators (18,3) feel that the sequence is reversed, i.e., capillary obliteration followed then by secondary dilation of vascular channels. Ashton postulated that the capillaries may actually be squeezed shut by the surrounding edematous retinal tissue, or that arteriolar perfusion pressure is altered as the result of degenerative changes in the arteriolar wall.

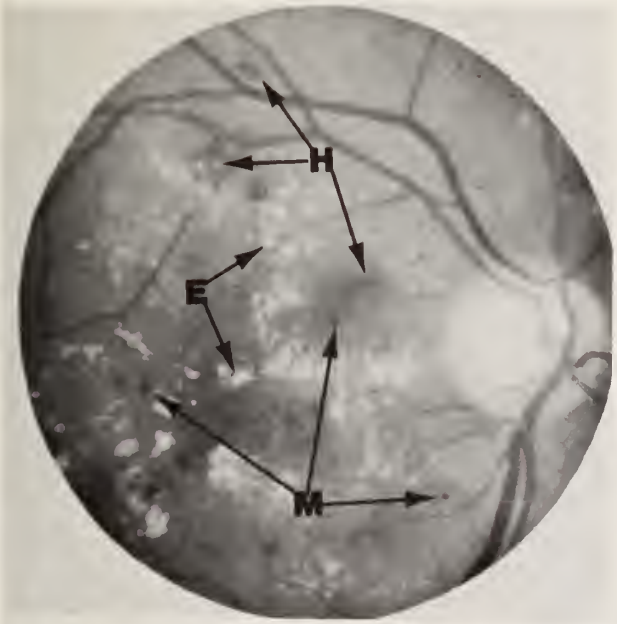

FIGURE 1. Ophthalmoscopic view showing hemorrhages (H), exudates (E), and microaneurysms (M).

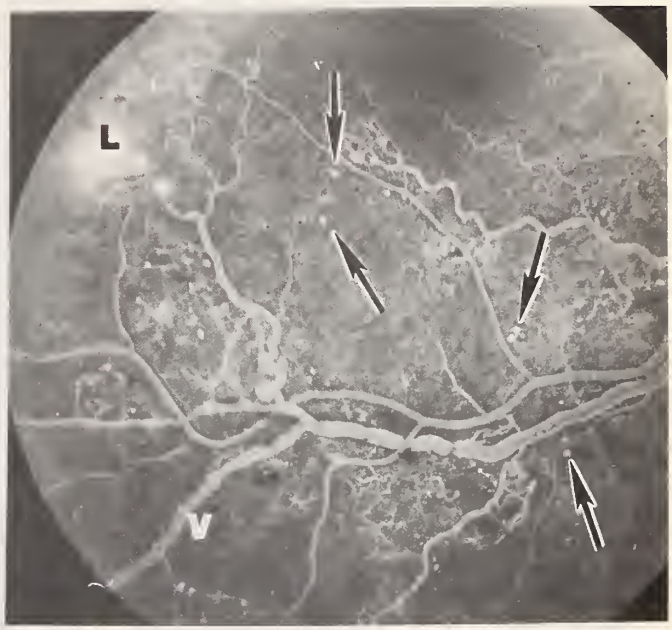

FIGURE 2. Fluorescein angiogram shows scattered microaneurysms which "fluoresce" (arrows). An area of fluorescein leakage is in the upper left of photo (L). The vein (V) shows "beading."

Regardless of the precise initial sequence of events, retinal capillary closure certainly occurs early in the natural history of diabetic retinopathy, thereby inducing tissue hypoxia which can be demonstrated by clinical and pathologic techniques. Aside from the microaneurysm, some of the other clinical manifestations include the following changes.

b. Edema, exudates, hemorrhages, arteriolar and venous changes.

Retinal edema is common in both nonproliferative and proliferative retinopathy. It often involves the macula[3] and is the most common cause of reduced vision in nonproliferative retinopathy. Clinically, the macula appears swollen and angiographs show diffuse intraretinal leakage of fluorescein; presumably due to altered permeability of surrounding capillaries.

"Hard, waxy exudates" is the term used to describe the pockets of extravasated protein and lipid which form in the deep layers of the retina, probably also as a result of altered capillary permeability. These discrete yellow-white flecks usually occur near areas of abnormal capillaries and microaneurysms, often becoming confluent to form a partial or complete circle around an edematous macula ("circinate retinopathy").

"Cotton wool spots," a lesion considered to be characteristic of hypertensive retinopathy, has recently been recognized as an early component of diabetic retinopathy without vascular hypertension (42). These grey-white lesions with indistinct borders result from microinfarctions in the superficial retinal layers; further support that closure of capillaries and/or terminal arterioles is a fundamental feature of this disorder.

[3]The region of the retina responsible for central vision.

Retinal hemorrhages in diabetic retinopathy can be flame-shaped in the superficial layers or the more common round or "blot" hemorrhage in the deeper retinal layers. The arterioles may show changes which are usually the result of generalized cardiovascular disease which may accompany the diabetes. Changes in the retinal veins are also considered an early sign of diabetic retinopathy and consist mainly of increased tortuosity and focal variation in caliber, referred to as "beading." These venous changes are often found in juvenile diabetics.

2. Proliferative diabetic retinopathy (6 percent of all cases of diabetic retinopathy or 2 percent of all diabetics) Figs. 3-6.

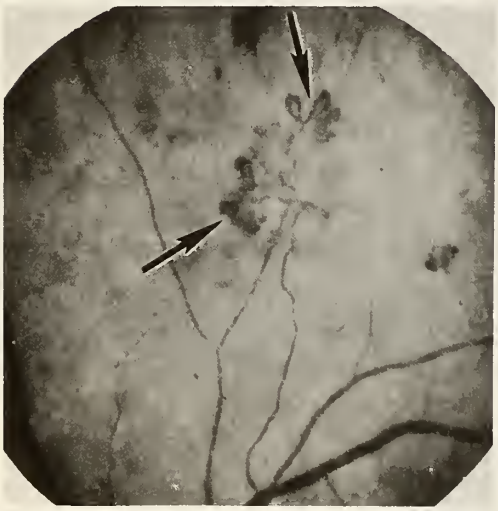

FIGURE 3. A tuft of new vessels (arrows) appears on the inner surface of the retina. (From Okun, E, et al. 1971) Reprinted with permission of C. V. Mosby, St. Louis.

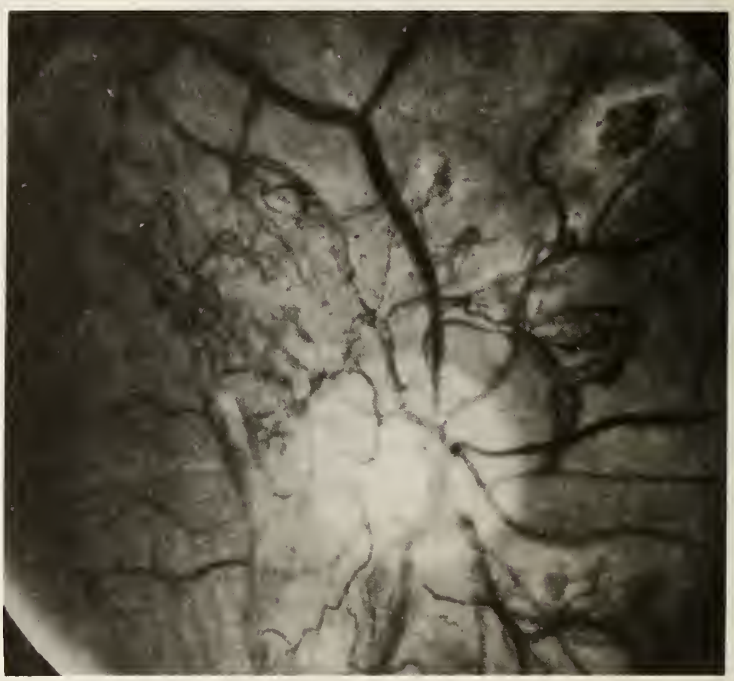

FIGURE 4. A network of neovascularization arises from the optic disk.

The hallmark of proliferative diabetic retinopathy is the appearance of neovascularization, which drastically changes the clinical picture as well as the prognosis. It is first appreciated ophthalmoscopically as a "brush" of fine capillaries in an area where the normal capillary bed has been damaged or destroyed. The site of predilection for neovasculatization are the optic disk, along the course of the main vessels, and in the equatorial region.

These new vessels form networks along the inner surface of the retina and follow a cycle of proliferation and regression. They evolve in three stages: 1) naked stage, in which these fine-walled vessels appear to have no connective tissue. After an interval of 1 to 4 years, they progress to 2) fibrous stage, in which there is an increase in the size of the vessels with formation of connective tissue around them. This progresses in 1 to 2 years to 3) regression and scarring when the connective tissue forms an avascular mass over the retina and disk, and the normal retinal arterioles become attenuated.

Adhesions form between this network of fibrovascular proliferation and the vitreous body. When the vitreous contracts, these fragile vessels are pulled forward often resulting in vitreous hemorrhage and/or retinal detachment, the two major causes of blindness.

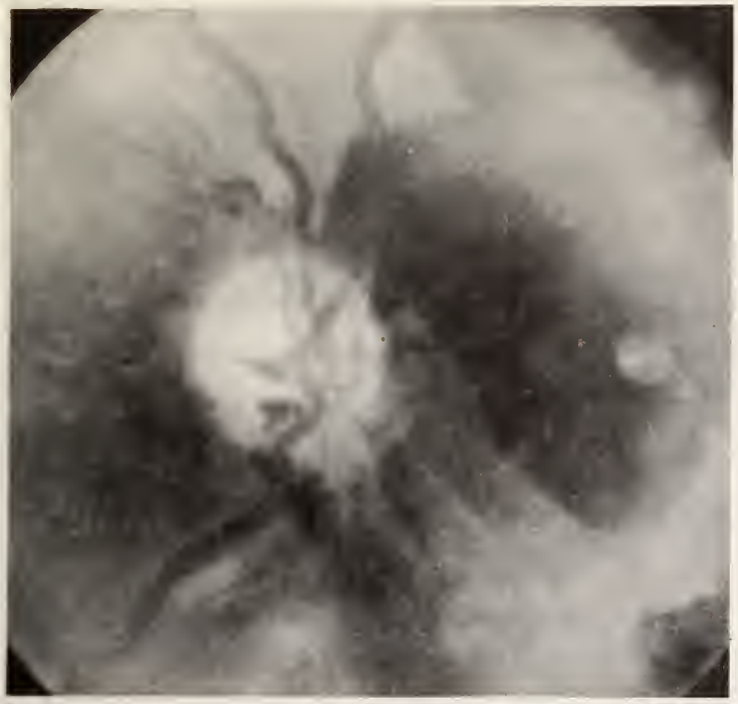

FIGURE 5. A large fresh hemorrhage into the vitreous is seen at the upper right corner of the photo.

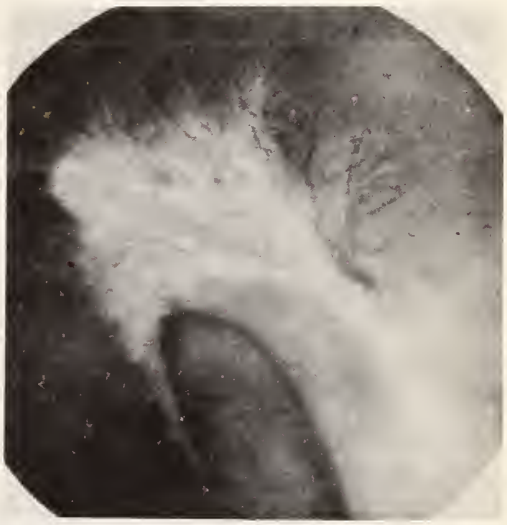

FIGURE 6. A dense connective tissue mass with neovascularization obscures retinal details. (From Okun, E, et al. 1971) Reprinted with permission of C. V. Mosby, St. Louis.

The rate of progression of proliferative retinopathy is variable. Occasionally it will go into a remission or "burned out" phase in which the vitreous contraction and the recurrent hemorrhages stop, and there is an overall ischemia of the retina.

In summary, capillary closure and tissue hypoxia occur early in diabetic retinopathy, followed by or coincident with edema, hemorrhages, exudates and eventually, in some cases, neovascularization. This latter ominous event may eventually go on to vitreous hemorrhage and retinal detachment with resultant blindness.

C. Therapy of diabetic retinopathy (53).

In order to evaluate the efficacy of various forms of therapy, it was necessary to establish a universally accepted classification of diabetic retinopathy. The most recent one, known as the Airlie House classification, is an attempt to present a framework for prospective studies with the hope that objective comparisons can be made. This classification is based on the photographic and ophthalmoscopic findings just described above (17).

1. Diabetic control.

Probably the most perplexing and frustrating question concerns the relationship between medical control of the diabetes and the occurrence and progression of diabetic retinopathy. Meaningful interpretation of data is hindered by the lack of criteria for what constitutes "good control." There is some statistical evidence which implies that better control of diabetes in patients under age 60 reduces the frequency or delays the appearance of retinopathy by a few years. Once retinopathy is established, however, control appears to have little or no effect (41).

2. Pituitary ablation (29).

In 1953, Poulsen reported a dramatic improvement in the retinopathy of a young diabetic woman after she developed Sheehan's syndrome[4] following the birth of a stillborn. Deliberate ablation of the pituitary by various means followed, and varying degrees of success have been reported (44). Assessment of these results, however, is difficult in light of the occasional spontaneous improvement of diabetic retinopathy which, of course, clouds the issue in evaluating any mode of therapy.

Selection of patients for this treatment is quite strict. These diabetics should be highly motivated individuals capable of following a demanding regimen of replacement therapy. Besides having a rapidly advancing proliferative retinopathy, but with macular function preserved in at least one eye, there must be absence of advanced renal, cardiovascular, and neurological disease.

The possibility of improvement must be balanced against the risks and disadvantages of the procedure, and with the data available, pituitary ablation cannot be considered the procedure of choice at this time except perhaps for a small number of carefully selected patients.

3. Photocoagulation (29).

Photocoagulation is the use of high intensity light from either a xenon arc or a laser to produce a "burn" with subsequent scarring of the tissues in the posterior portion of the eye. The method is thought to be beneficial in diabetic retinopathy is several ways: 1) areas of critical ischemia are destroyed, thus decreasing the stimulus to neovascularization; 2) new vessels are destroyed, thus removing the threat of hemorrhage; 3) leakage from permeable capillaries is lessened; 4) chorioretinal adhesions limit the ease of retinal detachment; 5) metabolic requirements of the retina may be diminished.

Photocoagulation is used primarily in proliferative retinopathy, but recently has been shown to be beneficial in nonproliferative retinopathy when the vision is impaired due to macular edema (60). In the latter instance, photocoagulation can aid by sealing leaks demonstrated by fluorescein angiography.

Results have been encouraging and most workers agree with the report of Okum (52) where in a small series of patients in which both eyes had symetrical involvement by proliferative retinopathy but only one eye was treated, almost half of the treated eyes improved while less than 10 percent of the untreated eyes improved. Long-term, randomized controlled studies are still needed, and an important step in this direction has been initiated by the Diabetic Retiopathy Study under the aegis of 16 medical centers in cooperation with the National Eye Institute (45).

4. Other modes of therapy.

Many other modes of medical therapy have been used against diabetic retinopathy, but because long-term controlled studies have been conspicuously lacking, these methods cannot be evaluated in any meaningful way and only brief mention is made here. These include rutin, testosterone, heparin, phenindione, vitamin B_{12}, para-aminosalicylic acid, corn oil, low fat diet, and clofibrate. It appears that the last three mentioned approaches do reduce the amount of exudation, but this is not accompanied by any visual improvement.

Calcium dobesilate, a drug being used in Europe, appears to show promise in retarding the progression of nonproliferative retinopathy by restoring normal capillary function (61).

[4]A syndrome in which the pituitary gland is destroyed.

In summary, the therapy of diabetic retinopathy is less than satisfactory at this time. No proven medical therapy exists, and the role of "good control" remains an enigma. Pituitary ablation does not appear to have proven its worth in light of the risks involved. Photocoagulation appears to be the most promising approach to palliation of proliferative retinopathy, but its true value is yet to be determined.

D. Cataracts, glaucoma.

1. Cataracts.

The association between cataracts and diabetes is not clearcut since it is not always possible to attrubute the presence of a cataract in an adult to his diabetes. It is customary and fair, however, to assume that cataracts occurring in diabetics under the age of 40 are presumably related to the systemic disease. Statistical evidence also exists to support the notion that cataracts in older individuals occur more frequently in diabetics than in nondiabetics, and that cataracts in diabetics mature more rapidly than cataracts in nondiabetics (8,51).

Although the cataracts can take any nonspecific form, the so-called "snowflake" pattern of opacity is considered characteristic in diabetes and may be the first manifestation of the disease. Many factors are probably involved but sorbitol[5] accumulation in the lens appears to play an important role in these diabetic cataracts (40,24,68). The osmotic effects of sorbitol accumulation in the lens may also be responsible for the transient myopia which so often occurs in diabetics. Therapy for the cataract consists of surgical removal and does not differ from conventional cataract removal.

2. Glaucoma.

a. Secondary glaucoma associated with neovascularization of the iris.

Rubeosis iridis is the clinical term used to describe new vessel formation on the anterior surface of the iris. This enigmatic phenomenon occurs in a variety of ocular diseases including diabetes where it is almost invariably associated with the presence of severe proliferative retinopathy. Estimates of the frequency of rubeosis iridis in diabetics vary widely, but a figure of 1 percent in all diabetics appears reasonable (49).

This fine network of new blood vessels can be seen clinically around the pupillary margin, over the surface of the iris, and in the anterior chamber angle (the anatomical angle formed by the peripheral iris and peripheral cornea). Eventually, this network of blood vessels and its accompanying fibrous tissue contracts and pulls the peripheral iris up over the outflow channels of the peripheral cornea to form dense adhesions, subsequent obstruction to the outflow of aqueous humor, and an intractable glaucoma (37). The treatment of this type of glaucoma is unrewarding and most of these already blind eyes need to be surgically removed because of unrelenting pain.

b. Open angle glaucoma and diabetes.

Primary open angle glaucoma ("chronic simple glaucoma") is the most common form of glaucoma that exists in the general population and is believed to be due to as yet unexplained degenerative processes occurring in the channels of the eye which allow for egress of aqueous humor.

Recent clinical studies (4) have shown the following statistical relationships to exist between primary open angle glaucoma and diabetes: 1) primary open angle glaucoma is more prevalent in diabetics than in nondiabetics; 2) proliferative retinopathy occurs less often in diabetic patients with primary open angle glaucoma than in nonglaucomatous diabetics; 3) diabetes

[5]Sugar alcohol analogue of glucose. See Chapter 14.

and positive glucose tolerance tests are more prevalent in the glaucoma population; 4) the glaucoma population with a positive glucose tolerance test appears to be more susceptible to glaucomatous visual field loss than those with a negative glucose tolerance test; 5) positive glucose tolerance tests are more prevalent in glaucomatous patients with lower intraocular pressures.

II. EXPERIMENTALLY INDUCED RETINOPATHIES.

Although diabetes can be induced in animals in a variety of ways, complete pathologic changes characteristic of human diabetic retinopathy seldom develop and most of the changes are minimal. This is also true of spontaneous diabetes in animals. Table 1 summarizes these findings.

Engerman (23) has shown that diabetic dogs kept in "good control" developed less retinopathy over a period of years compared to dogs purposely kept in "poor control."

III. CURRENT STUDIES, GAPS IN OUR KNOWLEDGE, AND THOUGHTS FOR THE FUTURE.

The exact initial sequence of events leading to diabetic retinopathy still remains a mystery. If, as Cogan and Kuwabara (13) have suggested, the initial event is the loss of the capillary pericyte, then what causes this selective "drop out" of these cells in diabetes? The exact function of this particular cell still needs to be determined. Although a vasomotor function has been attributed to this cell, evidence is lacking to support this contention. Recent studies have shown that this cell may have a phagocytic function (19).

If capillary obliteration is the initial event, and if surrounding edematous retinal tissue is responsible for this capillary shutdown (3), then it becomes interesting to speculate on possible causes for this retinal edema. We have seen how the sorbitol pathway is responsible for fluid accumulation in the lens. Could the same process be occurring in the retinal tissues? The need for further biochemical studies on the diabetic retina becomes obvious.

The nature of the microaneurysm needs further elucidation. Although most diabetic microaneurysms are thin walled, some have a thick wall giving an intense staining reaction for glycoproteins. Endothelial cell degeneration and proliferation, plus basement membrane thickening with vacuolization, are also part of the microscopic picture. The finding that fluorescent insulin binds specifically to these microaneurysms suggests the possibility of an autoimmune reaction, but this may be secondary rather than causal (15,46). Ashton has suggested that the microaneurysm may represent an abortive attempt at neovascularization.

The stimulus for neovascularization is still unknown, but it has been suggested that a "vasoformative factor" may be liberated from hypoxic foci in the retina (2). Such a factor may also be responsible for the development of rubeosis iridis.

What role does pressure play; both the pressure within the vascular system as well as intraocular pressure? It has already been pointed out that proliferative retinopathy occurs less often in diabetics with primary open angle glaucoma than in nonglaucomatous diabetics. Also, diabetics with no retinopathy tend to have a lower systemic blood pressure than diabetics with retinopathy. Furthermore, when the retinopathy is asymetrical in both eyes of a patient, the less affected eye is usually associated with a decreased retinal arterial diastolic pressure (26). Duane (21) correlated the progression of diabetic retinopathy with the ratio of the intraretinal arteriolar pressure to the intraocular pressure. Conditions which increased the numerator (intra-arteriolar pressure) such as systemic hypertension, or decreased the denominator

TABLE 1. Experimental Diabetic Retinopathy*

Animal	Mode of Production	Lesions	Authors
Monkey	Alloxan	Microaneurysms	Gibbs et al (1966)
Dog	Spontaneous	Microaneurysms, exudates, loss of pericytes	Patz and Maumenee (1962)
Dog	Spontaneous	Microaneurysms	Gepts and Toussaint (1967)
Dog	Spontaneous	Microaneurysms	Sibay and Hausler (1967)
Dog	Alloxan	Microaneurysms, pericyte loss, acellular capillaries	Engerman and Bloodworth (1965)
Dog	Growth hormone	Microaneurysms, pericyte loss, acellular capillaries	Engerman and Bloodworth (1965)
Chinese hamster	Growth hormone	Microaneurysms	Hausler et al (1963)
Rat	Pancreatectomy	Microaneurysms	Musacchio et al (1964)
Rat	Alloxan	Intravitreal new vessels	Toussaint (1966)
Rat	Alloxan and iminidiproprionitrile	Acellular capillaries	Heath and Rutter (1966)
Rat	Cortisone and growth hormone	Microaneurysms and acellular capillaries	Agrawal et al (1966)
Rat	Streptozotocin	Microaneurysms, basement membrane changes	Leuenberger et al (1971)
Carp	Spontaneous (Sekoke disease)	Dilation of vessels	Yokote (1970)
Carp	Alloxan	Dilation of vessels	Yokote (1970)
Carp	Hydrocortisone	Dilation of vessels	Yokote (1970)

*Partially adapted from Caird, F. I., et al, 1969, Diabetes and the Eye, Table 4.1, p. 54.

(intraocular pressure) as in postoperative hypotony, tended to aggravate the retinopathy. Although it is unlikely that changes in intraocular pressure or systemic perfusion pressure are causative, it is probable that these alterations influence the point at which vascular insufficiency and tissue damage occur. Direct measurement of blood flow in retinal arterioles holds promise in answering some of these questions (36).

Much recent attention has been directed toward the possible role of clotting factors, particularly intravascular platelet aggregation (34). Recent evidence implies that there is an "enhancing factor" in the plasma of diabetics with retinopathy which is responsible for increased intravascular aggregation of platelets which in turn may produce the retinal capillary obstruction. This "enhancing factor" is not present in diabetics without retinopathy or in nondiabetics. On the basis of these findings, preliminary clinical trials using aspirin (a known inhibitor of platelet aggregation) are being conducted on diabetics (11,20).

Other metabolic parameters which have been implicated as being altered in diabetics with retinopathy but not in diabetics without retinopathy or in nondiabetics, and which need confirmation and/or refinement include: erythrocyte aggregation, fibrinogen concentration, serum proteins (67), serum lipids (58), growth hormone (62), and serum prostaglandins (69).

A better animal model of diabetic retinopathy is urgently needed and progress will be made if conditions can be found which, when superimposed upon experimental diabetes, accelerate the rate of pathological change in the retina (33).

From the clinical point of view, it has been pointed out that photocoagulation of proliferative retinopathy is only palliative and has no effect on the basic disease process. Although improved instrumentation may improve the efficacy of photocoagulation, it is important to continue the search for better medical therapy which could retard the progression of diabetic retinopathy before the onset of the proliferative state. Reference has already been made to the use of calcium dobesilate for restoration of normal capillary function and inhibitors of platelet aggregation such as aspirin.

If better medical therapy can be found, or if the role of "good control" becomes better understood, it becomes important to recognize the very earliest clinical signs or symptoms of diabetic retinopathy, perhaps even prior to ophthalmoscopically apparent disease. Encouraging studies from this point of view include such findings as impairment of color vision (39), electro-oculography changes (35), electroretinography changes (64), decreased flicker fusion response (12), and the identification of small blind spots (scotometry) (59).

In summary, attention centers around: 1) the initial events leading to capillary obliteration and the exact nature of the microaneurysm; 2) what role do other parameters play, e.g., intraocular pressure, vascular perfusion pressure, intravascular clotting factors, serum constituents; 3) production of a better experimental mode; and 4) detection of the earliest functional changes prior to morphologic changes.

CONCLUSION

From the foregoing discussion it becomes obvious that any successful approach to the prevention and therapy of ocular complications of diabetes will come only with a better understanding of the initial events which occur in the diabetic retina and other ocular tissues. The ophthalmologist must remain an important member of the interdisciplinary team, working closely

with the biochemist and pathologist as well as acting as a liaison between basic science and clinical application. Pragmatically speaking, this role can best be accomplished if financial resources are made available to enable interested research ophthalmologists to become specialists just in ocular complications of diabetes.

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17

RENAL DISEASE IN DIABETES MELLITUS

K. Lundbaek and R. Østerby

Diabetic renal disease occupies a central position in the general field of diabetic angiopathy. In the natural history of diabetes mellitus as it appears under current standard treatment, characteristic changes develop in the kidney, leading eventually to partial or complete loss of function. This development can be followed accurately with modern morphometric techniques and function tests. Recently biochemical methods have been added to the armamentarium of kidney research in diabetes.

In the present chapter a short presentation of the problems of renal abnormalities in diabetic patients will be given, emphasizing the important gaps in present information and understanding, and pointing out fields of research that should, in the opinion of the authors, be promoted.

A detailed discussion of the literature of the last decade dealing with renal disease in diabetes mellitus has been published recently (39).

DEFINITIONS AND ALTERNATIVES IN THE STUDY OF DIABETIC RENAL DISEASE

"Diabetic nephropathy" is and should be used as a *clinical term* to denote a chronic kidney disease with proteinuria and/or reduced renal function found in patients who have had diabetes mellitus for many years and supposed to be due to diabetic glomerulosclerosis, with or without chronic infection of the renal tissue.

The *clinical diagnosis* of diabetic nephropathy is always tentative and based on the assumed exclusion of other causes of renal disease. There are no specific clinical features. Late in the course of disease, an otherwise uncommon picture consisting of gross proteinuria, edema, and hypertension occurs, but only in a minority of cases.

"Diabetic glomerulosclerosis" and "diabetic glomerulopathy" are synonymous terms indicating the specific *morphologic abnormality* of the renal glomerulus. The first description of its nodular form was given by Kimmelstiel and Wilson in 1936. The diagnosis is made by microscopic study of kidney tissue, i.e., post mortem or on biopsy specimens.

Renal function is abnormal in diabetic patients, but this fact cannot be used as basis for a definition of diabetic renal disease. When functional tests are performed in patients with short-term diabetes, glomerular filtration rate and other functional parameters are seen to be *increased*. In late stages there is a generalized *decrease* of renal functions. Both patterns are quite nonspecific.

Recently a number of important papers have been published about the *biochemical* composition of the normal and the diabetic glomerular basement membrane and of the enzyme activities responsible for the synthesis of basement membrane glycoproteins. At the moment this field is rich in controversial problems. In human beings, biochemical anomalies can be studied only on autopsy specimens. Future technical developments may make it possible to introduce a biochemical

definition of diabetic renal disease.

Today the clinical definition--diabetic nephropathy-- and the morphological definition--diabetic glomerulosclerosis or glomerulopathy--are the only alternatives for the study of the natural history of diabetic renal disease, its intrinsic mechanisms, its relation to other diabetic phenomena, and its modification under various therapeutic or prophylactic conditions.

For research purposes the vague clinical term "diabetic nephropathy" can be sharpened by appropriate criteria for classification and made useful in some types of long-range, large-scale population studies.

However, diabetic glomerulopathy with its precise definition and practically speaking complete specificity is the only acceptable object for most types of detailed studies aiming at clarification of the pathogenesis of diabetic renal disease and the possible effect of treatment or prophylaxis.

It should be remembered, however, that the choice of diabetic glomerulopathy as a definition for research work in human subjects entails the use of kidney biopsy. This problem is a very general one and shall not be discussed in detail here. Both percutaneous and open kidney biopsy have a certain risk, albeit a very low one. In the hands of an experienced investigator and with the proper precautions, it can be a perfectly justified procedure in important and well-designed research projects.

DIABETIC NEPHROPATHY

The impact of renal disease per se as a cause of suffering, invalidity, and death seems obvious, but is not too well documented. It is not prominent in patients with short or moderately long duration of diabetes. Long-term diabetic patients may suffer for years from tiredness, some of them may have periods of low-grade fever and lumbar pain, but the incidence of such symptoms is hard to estimate. Once uremia sets in, the usual symptoms and signs appear, and finally complete renal insufficiency occurs.

The prevalence of various types and degrees of kidney disease in diabetics has been estimated in population studies, using some clinical definition of "diabetic nephropathy" (11) but much more could be done. For example, the prognostic significance of so-called intermittent proteinuria and proteinuria of various degrees need clarification. It might be studied in well-defined geographically stable diabetic populations. Another closely related problem is the time course of the development of proteinuria and of albuminuria. Still another important clinical problem--one that must be solved on large groups of patients--is the question about "ethnic" differences in the development of diabetic angiopathy. It seems as if there *are* such differences in the prevalence of various types of diabetes mellitus as defined by the degree of metabolic derangement (54), but there is still conflicting evidence about the prevalence of diabetic retinopathy or diabetic nephropathy among Caucasians, American Indians, East Asians, etc., with comparable degrees of blood glucose abnormality.

Editor's note: Investigations utilizing renal biopsies cannot be undertaken without full deliberation of the ethics of the proposed studies and the availability of alternate methods of assessment. There is a great need for noninvasive techniques for the evaluation of the earliest changes in parameters of renal morphology and function. Hopefully, such methods will be developed and validated in parallel with the morphometric approaches. Alternatively, it may prove feasible to secure adequate information about renal vasculopathy by the less hazardous technique of muscle biopsy.

Such clinical studies will be particularly valuable if they specify and quantify certain degrees of renal insufficiency, of proteinuria, albuminuria, hypertension, edema, etc., in men and women, in certain age groups, and certain groups of age at the onset of diabetes. Here, as in all other long-range studies of diabetic vascular disease, results are much more likely to be informative and interesting if performed on patients with acute onset diabetes, preferably diagnosed before the age of 30 or 35 years.

Diabetic nephropathy is a very common concurrent cause of death in long-term diabetic patients. However, it is well-known that the final stage of diabetic angiopathy is characterized by a combination of renal insuffiency, cardiac insuffiency, neuropathy (including diabetic encephalopathy) and evidence of diabetic macroangiopathy. It is often not possible to give a reasonable verdict as to the immediate cause of death in these patients. Moreover, the final picture of renal pathology itself is often a composite one. Ditscherlein (11) analyzed the histologic findings in 24 diabetics dying with the full-blown clinical picture of uremia. Glomerulosclerosis as the sole finding was observed in only eight cases. The other cases showed various combinations of diabetic glomerulosclerosis, chronic pyelonephritis, and nonspecific nephrosclerosis. Such studies are important and should be promoted when possible, but to be really significant they required a very high autopsy rate: ideally 100 percent.

DIABETIC GLOMERULOPATHY

Diabetic glomerulopathy appears at light microscopy as an increase in PAS-positive substance. In mild cases this material is seen diffusely spread out within the glomerulus. In late stages it often forms the highly specific "Kimmelstiel-Wilson nodules." At electron microscopy glomerulopathy appears as a thickening of the peripheral basement membrane and an increase of the basement membrane-like material in the mesangial regions. (At light microscopy the basement membrane cannot be clearly distinguished).

The basement membrane of the glomerular capillaries is analogous to that lining capillaries and larger blood vessels elsewhere in the body. A rather similar structure is found subjacent to other types of cells, e.g., epithelial cells, muscle cells, and interstitial cells.

Thickening of the glomerular basement membrane is the local representative of the general process of the basement membrane thickening that occurs in all types of capillaries throughout the body. Only little information is available about the state of the basement membrane in the larger vessels in diabetic macroangiopathy. The same holds true for possible changes of non-vascular basement membranes. They will not be considered in the present chapter.

The basement membrane of the renal glomerulus has been studied more intensively than that of any other vascular area. This is due especially to the fact that by its topography, it offers unique possibilities for exact morphological studies, and also that enough basement membrane glycoprotein is present in one kidney to allow isolation and biochemical studies.

In comparison, the vascular areas of other organs present many problems. The retinal and iridal vessels cannot be biopsied. Quantitative studies of muscular capillaries are difficult for a number of reasons. However, it seems established by now--especially on the basis of the extended and excellent studies of Williamson and co-workers--that the onset and further development of vascular changes in these vessels are fundamentally similar to those occurring in the renal glomerulus (30,67).

Local factors undoubtedly play a role, e.g., vascular proliferations apparently only occurring in the retina and on the iris, but in looking for a general trend or common denominator of microvascular disease in diabetes mellitus, the glomerular vessels must be accepted at the moment as the most appropriate paradigm.

THE GLOMERULAR BASEMENT MEMBRANE

The basement membrane material which is involved in diabetic glomerulopathy is partly situated in the wall of the capillaries, here termed "the peripheral basement membrane," and partly in the mesangial regions: "mesangial basement membrane-like material." Its shape, and its function as well differ at the two sites, but the ultrastructural appearance is practically identical. In contradistinction to earlier reports, it has now been shown that the development of abnormalities in diabetic patients occur at the same time at these two locations (72,73). The two separate morphological entities will therefore be considered together in the following.

It has been known for many years that in the final stages of diabetic glomerulopathy, the tuft is nearly filled up with basement membrane material. With increasing duration of disease, there is increasing chance that severe involvement of the glomerular basement membrane will be found.

By the use of quantitative electron microscopy, it has been possible to pinpoint exactly the earliest development of these abnormalities and, in particular, to date the relationship to the onset of the metabolic disease. This has been done on kidney biopsies from young patients with juvenile diabetes, i.e., patients in whom the time of onset is established with great certainty. It was shown that the glomerular basement membrane is normal at the acute onset of juvenile diabetes (69). Studying the first few years after the diagnosis of diabetes, it was demonstrated that the amounts of basement membrane are increased already after 2 years, and to a greater extent after 5 years (70,73).

These initial abnormalities cannot be demonstrated with the light microscope, and at electron microscopy only by means of extensive and rather cumbersome measurements. They represent the initial phases in a seemingly steadily progressive process, which finally leads to complete abolishment of glomerular function.

The fact that the basement membrane is normal at the onset of diabetes has great practical and theoretical implications. It rules out, for all practical reasons, that the basement membrane thickening is a genetically determined abnormality--a view which has been popular for some years. This also implies that it may be possible, by controlling metabolic factors, to prevent or postpone the development of diabetic microangiopathy.

Trials in which various clinical or therapeutic procedures are tested for the influence on the development of diabetic angiopathy are greatly needed, and as regards some specific questions, it is possible to carry them out. It has been documented that the thickness of the peripheral glomerular basement membrane is a useful parameter to evaluate the development when a precise quantitative technique is employed. The possibilities in this respect will be discussed later.

THE GLOMERULAR CELLS

What is the role and the fate of the glomerular cells in this development? This is an important question, since the synthesis, the maintenance, and breakdown of the basement membrane are the result of cellular activity. Also the glomerular function as a filtering unit may be

modified at the cellular level.

The established facts in this field are rather scanty. Most of the statements that have been put forward concern the number and differential distribution of the glomerular cells. Mesangial cell hyperplasia has been reported in light microscopic studies (27) and has been attributed a causative role in basement membrane overproduction. However, in a quantitative light-microscopic study of biopsies from patients with early diabetes (0-6 years' duration) cellular hyperplasia was not observed (74). The same conclusion was drawn from cell counts performed on electron microscopic photomontages of a smaller number of glomeruli from the same series of early diabetics. With this method, exact counts of individual cell types could be obtained, and it was found that the differential distribution of cells, as well as cell density, were normal (71). Cellular hyperplasia thus cannot account for the initial augmentation of basement membrane material.

Obviously, very late in the development when glomeruli are about to undergo complete obsolescence, the final outcome for the cells is total necrosis. What happens in between these two extremes of the development still needs to be studied with value quantitative techniques.

The cellular function with respect to basement membrane synthesis and breakdown is not clarified. From silver-labeling experiments in animals, there is indication of basement membrane synthesis by epithelial and mesangial cells (34,35).

It has not yet been possible to point out changes in cellular ultrastructure which could help to indicate the mechanism behind altered basement membrane metabolism in diabetes. However, cisternae of the endoplasmic reticulum of epithelial cells containing basement membrane-like material were observed with somewhat increased frequency in a series of young, short-term diabetics compared to normals, although statistical significance was not obtained (71). There is some evidence that this organelle is a site of basement membrane synthesis (1). This ultrastructural finding is of interest in connection with the finding of elevated levels of enzymes involved in basement membrane synthesis as observed in alloxan-diabetic rats (58). Further studies at the ultrastructural level are very desirable. Such studies, dealing with the smallest details at a sub-ultrastructural level, may at first sight seem to have only small chance to lead to conclusions that may have practical consequences for the diabetic patients. However, some clues as to how to interfere with the abnormal mechanisms might be gained if some of the steps involved were elucidated.

Possible ways to follow in an attempt to study cellular basement membrane synthesis at the ultrastructural level are: a) Quantitative determination on larger series of cases of cellular organelles involved in protein synthesis, i.e., the endoplasmatic reticulum with its cisternae, but also ribosomes, endoplasmic reticulum, Golgi apparatus. Such studies should preferentially run in parallel with biochemical determinations; b) Application of enzyme histochemistry--hopefully demonstrating enzymes which are specific in the basement membrane turnover, using autoradiographic techniques.

MORPHOLOGY AND FILTER FUNCTION

The functional consequences of basement membrane thickening are also unclarified.

Since basal proteinuria and albuminuria are very late phenomena, basement membrane thickening is present for many years before clinical signs of impaired filter function occur. Kidney

function as a whole depends on a complicated interplay between various segments of the nephron, including filtration, reabsorption, and secretion. Already immediately past the glomerular capillary wall, i.e., in the glomerular urinary space, the composition of the primary urine presumably is a result of a combined process of passive filtration over the basement membrane and reabsorption by glomerular epithelial cells. The basement membrane is believed to retain molecules larger than 100 Å in diameter in normal individuals. Studies of the passage of different enzymes indicated another barrier, the very fine membrane occluding the filtration slits between adjacent epithelial cells. This membrane was believed to stop somewhat smaller molecules with diameter down to about 65 Å (36). However, in a recent publication on glomerular permeability studied by means of dextrans of different molecular weights, the role of the filtration slit membrane in the filtration process was drawn into doubt (8). It has been shown that a surface coat made up of mucopolysaccharides covers the epithelial cell membrane and probably fills out the filtration slits (25), thereby binding some of the substances passing and facilitating their immediate reabsorption into the glomerular epithelial cell. In histochemical studies, a decrease in the glomerular sialic acid localized to this surface coat has been found in cases of proteinuria, both in human cases (4) and in animals with experimentally produced proteinuria (42). These findings might be related to a disappearance of the normal foot process structure. On the other hand, a thickening of the surface coat as measured in electron micrographs was reported in albuminuric rats (15). This coat of mucosubstances is not visualized in the electron micrographs when using conventional preparation of the tissue. It still remains to be clarified, with the application of special histochemical techniques, if abnormalities in these mucosubstances are present in diabetic patients and, if so, whether such abnormalities may account for alteration in the net result of the filtration process.

In late cases of diabetic glomerulopathy, when gross proteinuria is present, the basement membrane must be anticipated to be leaky as are the thickened basement membranes of muscular, retinal, and iridal vessels. Still, its fine structure does not deviate markedly from the normal, as far as we can determine with the presently available resolution. Since, however, it permits the passage of large quantities of protein molecules, the filter meshwork of these greatly thickened membranes must be altered in some way. Such sub-ultrastructural abnormalities could be evaluated with indirect techniques, studying the passage of tracer molecules of known sizes, which can be visualized on the electron micrographs. However, at present, such studies would have to be restricted to animal models, since techniques which could be used on kidney biopsies are not yet available.

BIOCHEMISTRY OF THE GLOMERULAR BASEMENT MEMBRANE

In the latter part of the 1960's, the foundation was laid for an understanding of the chemical nature of the basement membrane and its synthesis, by studies of material isolated from renal glomeruli. The results published in a long series of papers by Spiro and co-workers. Survey articles (56,58) may be briefly summarized as follows.

The glomerular basement membrane is made up of a complex glycoprotein with a carbohydrate content of about 9 percent. The peptide portion contains large amounts of glycine and substantial amounts of hydroxyproline and hydroxylysine.

There are two distinct types of carbohydrate units, an unusual type of disaccharide unit made up of glucose and galactose and a heteropolysaccharide unit occurring also in other glycoproteins--made up of galactose, mannose, hexosamine, sialic acid, and fucose. The small unit is bound by glycoside linkage to hydroxylysine. Two enzymes involved in the synthesis of the glucose-galactose hydroxylysine, a glucosyltransferase, and a galactosyltransferase, were prepared, purified, and characterized. Studying glomeruli from patients who had been diabetic for 6 to 20 years, Beisswenger and Spiro (3) found that the content of hydroxylysine and hydroxylysine-linked disaccharide was increased. They suggested that the increase in disaccharide binding could reduce hypothetical aldol cross linkage of the chains, thereby resulting in increased permeability. In alloxan diabetic rats, they found an increase in the activity of the two transferases which could be prevented by early insulin treatment.

Many of these findings have been confirmed in other laboratories but a number of them have been disputed.

There is controversy about the true nature of the normal basement membrane macromolecule, especially if real collagen is present in it. This is based, at least partly, on different opinions about how to separate the native subunits (29,55). More importantly in the present context, there is sharp controversy at the moment about possible differences between the basement membrane composition in normal and in diabetic subjects.

Westberg and Michael (65) did not find any increase in hydroxylysine or hydroxylysine-linked disaccharide of glomerular basement membrane from diabetics, including patients who had had diabetes for more than 20 years. On the other hand, they observed a decrease in cystine content not reported by Beisswenger and Spiro (3). Westberg (64) suggests that this may signify a loosening of the interchange stability, perhaps resulting in increased permeability. Another finding of possible importance was a decrease in sialic acid in diabetic basement membranes.

Kefalides (29) likewise found no difference in the amount of hydroxylysine linked disaccharide of the glomerular basement membrane from normal subjects and patients with more than 10 years' duration of diabetes. In his study the cystine and sialic acid from diabetic basement membranes was low, as in the studies by Westberg and Michael (65).

The high hopes that a biochemical explanation of basement membrane accumulation in diabetes mellitus was already at hand have somewhat abated. Some of the confusing differences may be due to the fact that the techniques employed for separation, purification, and identification of small and large components of the basement membrane macromolecules have been different.

Intensified efforts, if possible including close collaboration between individual laboratories, are highly desirable in order to solve some of the problems that have arisen in this potentially very significant field of diabetes research.

The limitation of biochemical basement membrane studies is that it does not provide information specifically about the metabolism of the glomerular basement membrane producing cells. An approximation to this aim is attained in the interesting study of Cohen (9), demonstrating *inter alia* an increased lysine incorporation in glomerular basement membrane and in non-dialyzable protein of subcellular fractions. However, combined morphological and biochemical studies are required to produce information about the metabolic processes in the individual cell types, especially in the epithelial cell.

Finally, although perhaps not to be expected at the moment, the possibility of biochemical studies on human biopsy material should be mentioned. However, this will require a degree of micromanipulation and microanalysis not available at the moment.

KIDNEY FUNCTION IN EARLY AND LONG-TERM DIABETES, AND PROTEINURIA

It has been shown many years ago and confirmed recently that renal plasma flow, glomerular filtration rate, and various tubular functions are reduced to about the same degree in long-term diabetes with pronounced morphological abnormalities of the renal tissues. See review article (39).

The fact, recently studied in much detail (12,45) that various functional parameters show high values in recent diabetes is surprising and cannot be fully explained today. The "hyperfunction" is not related directly to the momentary blood glucose level but is reversible in the sense that it can be normalized after some time of strict regulation of the metabolic state (45).

Macromolecular clearance studies of the effectiveness of the filtering structures failed to demonstrate any increase in functional pore size (43). Increase in filtration pressure may play a role since high filtration fraction has consistently been found in early juvenile diabetes.

Another possible explanation would be an increase in filtering area. It has been shown recently that the roentgenographic kidney size is increased to the same extent as is the GFR in early diabetes (46). After 3 months of strict insulin treatment, the kidney size and GFR had decreased to near normal values (47). Quantitative light-microscopical studies of individual glomeruli made it possible to demonstrate the the glomerular size is increased (74). It is of special interest that the volume of the capillary lumen is enlarged. This finding may well be the morphological counterpart of the increased GFR, and it may be due to increased filtration surface. For technical reasons the filtration surface could not be evaluated in the kidney biopsy material available for this study.

The mechanism of this enlargement may involve the high level of plasma growth hormone characterizing diabetes mellitus when not exceedingly well controlled (18,19).

In animal models it may be possible to correlate such morphological parameters with the degree of metabolic disturbance in the untreated state as well as after some time of insulin treatment.

The clinical importance, if any, of all this is not clear. Looking at the development during the whole life span of the diabetic patients, the situation can be described metaphorically as a conflict between a metabolically determined tendency towards high function and an angiologic tendency towards low function, the angiologic one finally winning the upper hand. It has been suggested that the hyperfunction of the early period may play a role in modulating the transition to low function in the late phase (12).

Proteinuria is often seen in long-term diabetes and heavy proteinuria occurs in the final stage in some cases.

Editor's note: Methodological difficulties have restricted studies of the normal biology and biochemistry of the specific cell types affected in diabetic nephropathy. Recent reports that human umbilical vein endothelial cells can be maintained and studied in tissue culture (Jaffe, E. A., R. L. Nachman, C. G. Becker, and C. R. Minick: Culture of Human Endothelial Cell Derived from Umbilical Veins. J. Clin. Invest. 52:2745, 1973) give hope that similar studies can be performed with renal mesangial cells. If successful they may alter the opportunities for progress in this field.

This phenomenon is usually regarded as an expression of leakage of the filtering structures (it might also be due, partly or totally to decreased tubular reabsorption). There is no contradiction between the finding of a generally thickened basement membrane and the idea of an increased passage of protein through it. A loosening of the cross-linking of the long-chain molecule structure of the thickened basement membrane is quite possible, although it has not been demonstrated with current electronmicroscopic techniques. Functionally it has been shown that there is an increased permeability of the thickened basement membrane of muscular capillaries (60) and also of retinal (23) and iridal vessens (2,24).

One interesting new finding that needs to be further investigated is the seeming difference between proteinuria, as determined with the Lowry method and albuminuria, determined with radioimmunological techniques. Protein excretion is reported to increase steadily during the years (52). The excretion of albumin is normal and remains normal in reasonably well controlled diabetics till after many years of diabetes when suddenly, so it appears, it begins to rise (44).

However, using physical exercise as a provocative test (work load kpm/min for 20 minutes) a clearcut abnormality in albumin excretion emerges in diabetics with a duration of diabetes of only a few years--i.e., at the point of time when morphological changes are known to be present but basal albumin excretion is still normal. This work load results in a pronounced rise of albumin excretion in the diabetics, while normal subjects are not affected (48,49).

PYELONEPHRITIS AND BACTERIAL INFECTION OF THE RENAL TISSUE

It was thought for many years that clinical symptoms of infectious kidney disease were common in diabetic patients. Based on histologic findings and autopsy, it was also accepted that chronic pyelonephritis was more common in diabetics than in nondiabetics (26). There is no doubt, of course, that what has been described for half a century as "chronic pyelonephritis" is a histologic reality, but recent critical studies have failed to establish that this picture is caused by chronic bacterial infection (32). First, it was pointed out that the histological picture of "chronic pyelonephritis" could just as well be caused by glomerulosclerosis and/or by impaired blood supply due to the more or less well-defined changes of large and medium size renal blood vessels supposedly more common in diabetics than in nondiabetics. Second, attempts to cultivate bacteria from biopsy-specimens obtained in cases of so-called chronic pyelonephritis have usually been unsuccessful (6,17).

These problems are not limited to chronic pyelonephritis in diabetes mellitus. They are more provocative, however, in diabetes, because there is no doubt that urinary tract infection, as defined below, is more common in diabetics than in nondiabetics.

It is not easy to indicate ways out of the present crisis of "chronic pyelonephritis" as a chronic renal tissue infection. The first point mentioned above could be studied again in greater detail and in larger series of diabetics and nondiabetic cases meeting the former admittedly rather vague histological criteria of "chronic pyelonephritis." The elucidation of the second point is hampered by the fact that this abnormality, whatever its nature, is certainly mostly found as small irregularly distributed focal processes. Continued efforts of finding bacteria in biopsy specimens is hardly an attractive suggestion. Recent experimental and clinical studies have indicated, however, that raised titres to urinary tract bacterial antigens are found in a certain number of upper urinary tract infections (22,53). It may be worthwhile to apply these techniques to the problem of renal tissue infection in diabetic patients.

Considering acute pyelonephritis the histologic diagnosis is much more clear-cut. It has been found in large autopsy series that signs of acute infections in the kidney tissue do occur more frequently in diabetics than in controls (11). In this connection the rather high frequency of papillary necrosis in diabetics should also be mentioned (37). Although the etiology of this syndrome is not entirely clarified, it seems clear that both infection and impaired blood supply to the renal medulla are important factors in some cases.

The clinical study of urinary tract infection was greatly promoted by the introduction of quantitative techniques for the evaluation of bacteriuria distinguishing between contamination and significant bacteriuria (i.e., $> 10^5$ bacteria per ml urine). Applying this method to diabetic and nondiabetic populations, results were at first presented which contradicted the statement of increased frequency of urinary tract infections in diabetics (51). However, in later studies a higher frequency of significant bacteriuria in diabetic patients was clearly demonstrated, at least in females (61,68).

Significant bacteriuria was shown, moreover, to be positively correlated with the duration of diabetes and with the occurrence of retinopathy--thus by implication also with the presence of diabetic glomerulopathy (62). If this coexistence is an expression of decreased resistance to infections in a kidney which is the seat of generalized vascular involvement, or if recidivating infection may accelerate the development of diabetic angiopathy in the kidney is not clear, although the first-mentioned possibility is more likely to be correct.

At any rate, it is clear that acute, recurrent infections within the kidney tissue may be contributory to the destruction of renal parenchyma, and may thereby be a significant cofactor in the development of renal insufficiency. Therefore, there is a great need for further studies in this field.

HYPERTENSION IN DIABETES

It is often thought that arterial hypertension in diabetes mellitus, or in some diabetics, is in some way connected with diabetic kidney disease. It may be so but there is no strong evidence for it. Moreover, there is no unanimity as to the simple question about the prevalence of arterial hypertension in diabetes. Partly, at least, this confusion is due to the fact that many investigators have neglected the two aspects known to be significant in most areas of diabetological angiology, viz. metabolic state and duration of diabetes.

Experimental studies have shown that blood pressure is slightly but demonstrably elevated in resting and exercising diabetic patients in poor state of control as compared to well-controlled diabetics or normals (7,16). The 5-10 mm higher average blood pressure found in a group of children after less than 15 years of diabetes, when compared with nondiabetic children, may have been due to incomplete normalization of the metabolic state (50).

A much quoted study of a large population showed that there was no statistically significant difference between the blood pressure in diabetics and in nondiabetics when age and sex were taken into account (14). Unfortunately, the relationship to the duration of diabetes was not analyzed. It has been shown, however, that the blood pressure of juvenile diabetics increases

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Editor's note: The difficulties in differentiating between bacterial involvement of the upper and lower urinary tract have long plagued the clinician. The recent application of immunofluroescent techniques to localize the source of urinary bacteria on the basis of their coating with antibody has facilitated diagnostic discrimination (Jones, S. R., J. W. Smith, and J. P. Sanford: New Engl. J. Med. 190:591, 1974; Sanford, J. P.: Ann. Rev. Med. 26:485, 1975).

with the duration of diabetes (66). In an unselected and representative series of long-term diabetics of all ages (duration of diabetes 15-25 years), arterial hypertension (systolic blood pressure > 150, diastolic > 100) was found in 18 percent of patients aged less than 40. In men and women more than 50 years old, it occurred in 84 percent (38). These values are obviously higher than those occurring in the general population and may well be connected with renal disease. In the study quoted there was a statistically significant correlation between hypertension and abnormal urine sediment.

The clinical importance of hypertension per se in diabetes mellitus is doubtful. Recent studies, contradicting earlier opinions, have indicated that the frequency of acute cerebrovascular accidents is higher in diabetics than in nondiabetics (13). It is not clear, however, how much of this pathology is due to arterial hypertension and how much to large or small vessel abnormalities independent of blood pressure.

In the initial phase of clinical diabetic nephropathy, i.e., when the patients begin to develop proteinuria, the blood pressure is often somewhat elevated. In such patients glomerular filtration rate decreases gradually in the course of years and the rate of this fall is correlated to the diastolic blood pressure. Antihypertensive treatment may therefore be of value at this stage. It has been shown that correction of the mild hypertension in such patients is followed by a decrease of the albumin excretion (47). Further studies will clarify whether antihypertensive treatment can also postpone the development of renal insufficiency in patients with diabetic nephropathy.

Severe arterial hypertension is uncommon in diabetes. Even in the series of long-term diabetics mentioned above, a diastolic pressure above 120 was seen in only 6 percent of the cases. This statement requires, however, one modification: it is general clinical experience that blood pressure is often considerably elevated in the last weeks or months of life of patients succumbing to diabetic nephropathy and other manifestations of diabetic angiopathy. This fact is well documented in the study of Watkins et al (63).

THERAPY AND PROPHYLAXIS

Diabetic glomerulopathy is an element of diabetic angiopathy in general. This has been clear for many years from the resemblance between light microscopic changes in the glomerulus and in other organs of the body, as well as from the well established statistical connections between the prevalence and degree of vascular changes in the kidney, the eye, the heart, etc. (38,59).

The problems of therapy and prophylaxis of diabetic glomerulopathy are therefore partly identical with the problems of therapy and prophylaxis of diabetic angiopathy in general. These general problems will not be discussed in detail here, but the usefulness of the results obtained from kidney studies deserve to be mentioned.

The recent developments in quantitative electronmicroscopic analysis of diabetic glomerulopathy described above have opened up new possibilities for estimating the effect of old as well as new therapeutic/prophylactic measures in diabetic renal disease as an expression of diabetic angiopathy in general.

Earlier studies were hampered by the many years it takes for diabetic vascular disease to appear clinically and to develop to severe forms, and also by the crude means of estimating appearance and development of vascular changes.

The fact that thickening of glomerular basement membrane can be detected after only 2-3 years makes it reasonable to envisage controlled clinical trials to determine the effect of any therapeutic or prophylactic measure, all the way from "good control" to new types of insulin, or, hopefully, pharmacological suppression of the overproduction of growth hormone in diabetic patients.

Trials could be constructed in which groups of young diabetic patients were allocated to one of two alternative treatments such as "good control" versus "very good control," ordinary insulin versus monocomponent insulin, etc.

Growth hormone having been proposed as a causal factor in the development of diabetic vascular disease (40,41), a trial of the effect of growth hormone suppression is indicated. The newly discovered hypothalamic growth hormone suppressor, somatostatin, provides the means of pharmacological and reversible suppression of growth hormone (5,20). At the present time, however, such a test is not feasible because somatostatin is an extremely short-acting compound and also because of the many as yet not fully understood actions of this preparation on various pituitary and extra-pituitary hormones (21). The manufacture of a long-acting somatostatin analog with action confined more exclusively to growth hormones will be necessary before controlled clinical trials can be initiated to test its effectiveness in inhibiting the development of diabetic renal disease and other vascular abnormalities in diabetic patients.

It should be possible and ethically acceptable to ask a reasonable number of informed young patients and their families to participate in controlled clinical trials covering a period of only 2-3 years. The group of patients turning out in the end to have had the relatively unfavorable treatment could then be switched over to the favourable regimen at a point of time when vascular changes were minimal, i.e., only just recognizable with the electronmicroscopic technique described above.

There is no specific prophylaxis or treatment of diabetic nephropathy as such. The treatment of chronic renal infection or pyelonephritis in diabetes does not differ from that in nondiabetics. It is equally unsatisfactory. The same was true for renal insufficiency until recently.

Hemodialysis and renal transplantation are now being performed on diabetic patients in certain centers (33). This raises many problems. First of all, there is a lack of information to elucidate the number of patients per year in a given area or country who could be considered as candidates for these procedures. In places where cases of uremia are registered, it should be possible to find the uremic diabetics in order to ascertain the incidence and degree of ocular disease, heart disease, and nervous system disease. In this way an estimate may be obtained of the benefit that would accrue from a systematic introduction of modern therapy in long-term diabetics with renal insufficiency. It should be noted, however, that long-term results are not yet available elucidating the fate of a successfully transplanted kidney in the body of a patient with severe diabetic angiopathy, especially if and when the diabetic glomerulopathy will appear in it.

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18

DIABETIC PERIPHERAL NEUROPATHY

J. A. Moorhouse

INTRODUCTION

Peripheral neuropathy is the earliest and most universal recognizable complication of diabetes mellitus. Morphologic (68), electrophysiologic (44, 46) and sensory-perceptive (12) abnormalities are present within days to weeks of its onset. Even this early neuropathy is not limited to sensation in the lower extremities. The special senses, finger-tip perception, and motor innervation are affected (12,52).

These and other observations appear to resolve the question of whether neuropathy is a complication or a separate genetic concomitant (31, 33) of the diabetic state. Electrophysiologic and sensory-perception abnormalities are not present in the nondiabetic immediate relatives of diabetic persons (12, Moorhouse, unpublished observations). Abnormalities in individuals with mild carbohydrate intolerance are absent or slight (15, 35). The morphologic (49, 76) and electrophysiologic (28, 40) manifestations of diabetic neuropathy regularly occur in nongenetic experimental diabetes. The electrophysiologic manifestations of diabetic neuropathy are readily corrected by early insulin treatment (41,45,46). These findings make it implausible that diabetic neuropathy has a primary genetic basis rather than being the consequence of disordered metabolism.

An overall question which does require resolution is whether primary damage to the nervous system in diabetes is multicentric, or whether a single target site can be defined. There is nothing in the existing literature which is inconsistent with the view that peripheral satellite cells of Schwann are the primary targets for damage in diabetes, and that the subsequent degenerative sequence is secondary to this primary lesion.

PATHOLOGY AND PATHOGENESIS

Diabetic peripheral neuropathy is characterized structurally by segmental demyelination (14, 82). Occlusive lesions of the vasa nervorum usually are minimal even in advanced neuropathy. Axons are normal to light microscopy until the disease is well advanced. Early nonspecific axonal abnormalities are visible to electron microscopy (3,7,8). The possibility that they are primary needs to be excluded.

Segmental demyelination is due to damage to the supporting cells of Schwann which surround a all peripheral nerve fibers. The myelin sheath is derived embryologically from the plasma membrane of the Schwann cell (5) and continues to be dependent upon that cell for preservation and support. In diabetes early degenerative changes within the Schwann cell (3,7) indicate it to be a primary target for damage.

Myelin Sheath

Splitting and separation of the myelin lamellae at paranodal regions is the earliest recognizable change (3,82). The degenerative process may advance to complete segmental loss, or may at any stage be followed by healing. Insulin replacement promotes healing (44, 45), but even without insulin the changes have a natural cyclicity which is of interest both in relation to

diabetes and to Schwann-cell physiology. Myelin regeneration tends to be disorderly and irregular (14).

Schwann Cell

Electron microscopy of the Schwann cell reveals lamellar lipoprotein inclusions and other amorphous inclusions which reflect a disturbance of its normal synthesizing function (3,7). Other changes are abnormal glycogen granules and thickening and reduplication of the cell basement membrane. These changes are present in nerve biopsy specimens from all diabetic patients from early in the disease. In a given biopsy specimen, however, their occurrence is markedly nonuniform. Only a minority of Schwann cells show abnormalities, and these vary from slight to severe. The factor(s) causing these intercell differences is unknown.

Light-microscopic changes occur much later. There develops a nonspecific hypertrophy of Schwann cells, which eventually occur in the form of clusters (82). This picture is seen in any disorder which results in repeated degeneration and regeneration of the myelin sheath.

Axons

In early diabetes axons are normal in number and appearance to light microscopy (14), although with electron-microscopy they may show increased cytoplasmic dense bodies and disorderly arrangements of neurofilaments (7, 8). These changes are nonspecific and probably are secondary to damage to the satellite cells, although the possibility that they are primary needs to be excluded. The axon, extending from its cell body over great distances, is dependent upon its satellite cells for local metabolic energy and control (73, 77).

Nerve Roots

Studies in early diabetes are not available. In late cases the dorsal roots are often severely demyelinated (24). It is of interest that in contrast the anterior roots may be normal to light microscopy. This observation, which could be sought in early experimental diabetes, might provide investigators with considerable leverage. At this paraspinal site, motor and sensory fibers are conveniently separated. What ultramicroscopic or biochemical differences characterize those fibers susceptible and those less susceptible to metabolic damage in diabetes?

Dorsal-Root Sensory Ganglia

Lesions in the sensory ganglia are minor and variable until late in diabetes (9,24), and even then are nonspecific. There occurs proliferation of ganglion-capsule cells and of fine nonmyelinated nerve fibers, with compression-degeneration of the ganglion cell-bodies. These changes occur in many neuritides and even after posterior rhizotomy. Therefore they are not in conflict with a unitary, Schwann-cell hypothesis for the pathogenesis of diabetic neuropathy. By that hypothesis, they are tertiary to Schwann-cell damage, secondary to axonal degeneration.

Spinal Cord

It is remarkable that oligodendrocytes, the axon-satellite cells of the central nervous system which fulfill the same function as the Schwann cells in the peripheral nervous system, are immune to discernible damage at least until late in the course of diabetes (9,27,67). Normal central myelin is similar in ultramicroscopic apperance to peripheral myelin, although it differs in lipid and in aminoacid composition (20). What crucial difference in the properties of the Schwann cell and of the oligodendrocyte makes the one so vulnerable to damage in diabetes and the other so impervious?

To light microscopy the spinal cord frequently is normal in diabetes. In advanced diabetes, as well as infarcts, there may occur irregular swellings of axons and of myelin sheaths, and

varying degrees of axon loss, most commonly in the posterior columns of the lumbosacral region. These findings are nonspecific, and their lateness and distribution suggests that they are secondary to peripheral nerve damage. Similar changes may follow posterior rhizotomy. Therefore they are not in conflict with a unitary hypothesis for diabetic neuropathy.

Cranial Nerves

There are no observations in early diabetes. In advanced diabetic neuropathy the peripheral portions of cranial nerves may show severe demyelination (70). Again it is arresting that in such cases the proximal portions, where oligodendrocytes replace Schwann cells as axon satellites, showed only some swelling and inequality of the myelin sheaths. This juxtaposition of Schwann cells and oligodendrocytes along the same fibers might enable an ultramicroscopic and biochemical analysis of the differences which affect their susceptibility to damage in diabetes.

Brain

Frequently the brain is normal to light microscopy in diabetes (9,24,70). In late cases variable degenerative changes may occur both in grey and white matter. It is not clear whether these changes are to any extent characteristic of diabetes, or whether they are only the results of hypertension and of atherosclerosis occurring at an earlier age than in nondiabetic subjects. Even if they are to some extent characteristic, they do not appear to be inconsistent with a unitary hypothesis, with late transynaptic extension of damage to neuronal systems having close functional links with those primarily affected.

Autonomic System

Nonmyelinated axons in peripheral nerves are closely invested by Schwann cells. The changes in diabetes seen by electron microscopy are similar to those in myelinated fibers (3, 8). Indeed in early diabetes the nonmyelinated fibers are quantitatively the more affected. As the disorder progresses, nonmyelinated axons become depleted in peripheral nerves (24,82).

Studies of sympathetic ganglia and trunks in diabetes are scarce and limited to light microscopy. Ill-defined degenerative changes are described within ganglion cells and in the rami communicantes (1,9,48). Further morphologic and functional studies are needed both in clinical and in experimental diabetes.

Changes in the vagus nerve are said not to be present to light microscopy of clinical material (48), but this is belied by the occurrence of esophageal hyperresponsiveness to stretch stimuli even in early diabetes (50), and by impaired gastric secretory responses to hypoglycemia in more advanced neuropathy (25). There is no information about the appearance of gastric intramural ganglia and plexuses (86). there are said to be no definite changes in intestinal intramural ganglia and plexuses (24, 68). Degenerative changes are said frequently to be present in bladder intramural fibers and in hypogastric nerve fibers (34). The morphology and function of the parasympathetic system in diabetes requires much further study.

Sensory Nerve Endings

Quantitative studies of peripheral sensory nerve endings suggest that their number is diminished in various forms of neuropathy, including that of diabetes (22, 27). Confirmatory duration-specific studies are needed, as well as electron microscopic observations on sensory-ending ultrastructure.

Motor End Plates

Morphologic abnormalities of the terminal neuromuscular apparatus are uniformly present in

muscle biopsies (2,16,69). They occur within weeks of the onset both of clinical and of experimental (49) diabetes. They persist throughout the course of the disease in degeneration-regeneration cycles with degeneration eventually becoming ascendent in patients with severe neuropathy. Their etiology is unknown, but it is noteworthy that the terminal branches of motor nerves are covered with a thin sheath of Schwann cells right up to their end swelling (3). Electron microscopy of motor nerve terminals in diabetes has not been performed.

The histopathologic changes begin with the appearance of motor end plates which are irregular in size and shape. Whether or not degenerative changes in muscle fibers occur at this state is unclear (16, 68). This end-plate dystrophy is followed by the appearance of degenerative fragmentations and swellings along terminal nerve-fiber arborizations, and by regenerative branching to muscle fibers thus denervated either from terminal nerve fibers, or from the motor end plate itself. This latter "ultraterminal branching" is said to be a specific feature of diabetic neuropathy (2). When these branches reach muscle fibers they form new end plates. In early diabetes the new branches are well-formed and healthy, but in more advanced cases they are thin, finely beaded, with few and poorly developed end plates. Thus in advanced diabetes there may be severe degeneration of the terminal neuromuscular apparatus accompanied clinically by peripheral muscle wasting.

Skeletal Muscle

In coincidence with the early changes in the motor end plates, muscular abnormalities are present at the onset of juvenile-type diabetes. These vary from individual slender fibers to areas of clear-cut neurogenic atrophy (68). Whether or not such changes invariably remain after treatment, or occur early in milder diabetes, is unknown. They usually are not clinically manifest and do not show a strong predisposition to advance, presumably in part because of continuing regeneration of end plates.

Electron microscopy of muscle in early diabetes has not been done. Electron microscopy in patients with clinical neuropathy reveals wide separation of myofibrils, abnormal glycogen deposition, swelling of mitochondria, dilation of the sarcoplastic reticulum, and lysosome formation (2). The extent to which these changes are neurogenic and nonspecific, and to which they are diabetes specific, needs to be established.

Diabetic amyotrophy is a rare syndrome characterized by rapid onset of a profound proximal motor deficit and muscle wasting and a relatively good prognosis for recovery. This is in contrast to the usual late development of mild, chronic, peripheral muscle wasting in diabetic neuropathy. There is a general present belief that the muscle dystrophy is of neurogenic rather than myogenic origin (10). This belief needs to be reviewed. It is noteworthy that the mild disseminated muscle atrophy seen histologically may far from reflect the severity of the weakness, and that at the time of return of function the same biopsy changes may persist (57). It could be suspected, therefore, that these changes are nonspecific, preceded the amyotrophy, and that actually nothing is known about the morphologic counterparts of this remarkable clinical condition.

PATHOPHYSIOLOGY

Nerve Conduction Velocity

The most apparent electrophysiologic characteristic of diabetic peripheral neuropathy is an early slowing of nerve conduction velocity by approximately 30% from normal values, followed by further slowing as the disease progresses (28,52,56,81). Reduced conduction velocity results

from degenerative changes in the myelin sheath, with a functional effect of sorbitol accumulation perhaps also being implicated (40). Diabetic neuropathy shares this characteristic with other demyelinating disorders such as diphtheritic neuropathy and subacute combined degeneration, in contrast to axonal disorders such as alcoholic and uremic neuropathy in which there is little or no early change in conduction velocity.

The reason for the lessened conduction velocity in diabetes and other demyelinating disorders is probably a change in the electrical properties of the myelin sheath (21,54,79). Conduction velocity in myelinated nerves is proportional to fiber resistance. Resistance is proportional to myelin thickness, myelin lipids being the principal determinant. Thus resistance along the internodes is 10^3 times that of the nodes. Internodal resistance is diminished within days of the the onset of diabetes (30). The internodes act as passive core conductors, the membrane action potential being regenerated at each node (61). Conduction velocity depends principally upon the time taken at successive nodes for the current to depolarize the axon membrane to the critical level necessary for triggering an action potential. As the insulating property of the myelin diminishes, it is likely that increased leakage of current along the internodes reduces the current density at the nodes, delaying their excitation. The early drop in internodal resistance in diabetic neuropathy may be due in part to changes in myelin fatty-acid chain length, saturation or branching. It probably also is related to the early paranodal separation of myelin lamellae (3), perhaps due to interlamellar fluid accumulation (81), a process in which sorbitol concentration might play a part.

Impaired sensory perception is a functional expression of changes in conduction velocity. It has been demonstrated shortly after the clinical onset of diabetes in all areas and modalities that have been tested. Perception thresholds for light touch, two-point discrimination and vibration in the hands and feet, and for corneal touch, visual flicker, auditory flutter, and taste, are all diminished early in diabetes (12,44,74). The diminution in conduction velocity occurs unequally amongst nerve fibers, so that not only the rate, but the amplitude and shape of sensory-nerve action potentials become abnormal (52). Temporal dispersal of the action potential likely blurs acuity of definition at the cerebral cortex.

Electromyography

Whether or not there is a corresponding functional expression in the motor system of the early changes in conduction velocity and in the structure of motor end plates is less clear. In one study (16) electromyograms were normal in early diabetes. In another study (52), however, fibrillation potentials, motor unit loss, and abnormalities of the shape and duration of muscle action potentials had developed in some patients within a few months of onset, although these changes were more common in case of longer duration. Abnormalities in muscular performance accompanying these early electrophysiologic findings, analogous to the early deficits in sensory perception, have not been described. This may be because insufficient care and sophistication have been employed to demonstrate them.

An attempt (49) to explore the onset of electromyographic abnormalities in experimental diabetes seems to have been unsuccessful, perhaps because of the added factor of nutritional changes due to glycosuria. The morphologic and electrophysiologic changes did not correspond to those seen in diabetes in man.

A systematic electromyographic study in diabetic amyotrophy seems not to have been done. The

findings have suggested a primary nerve disease (13, 52), but as with the morphologic changes, may be mild in relation to clinical severity. One thus wonders again whether they were related to the myopathy, or whether they preexisted it. Amyotrophy may be a functional muscle disorder with presently unknown morphologic, electrical and biochemical characteristics.

Response to Ischemia

The response of nerve function to ischemia is abnormal in diabetes. Vibratory perception has been studied most fully (78,80). Following inflation of a limb cuff in normal subjects the vibratory perception threshold remains normal for 10 to 20 minutes, and then swiftly rises until vibration can no longer be felt. In diabetic subjects the threshold during ischemia remains normal for 30 minutes or longer. The response to ischemia can be restored to normal by careful insulin therapy. More recently this phenomenon in diabetes has been shown to include motor-nerve conduction velocity, sensory-nerve action potentials, and touch perception thresholds (45,80). Pain- and heat-perception thresholds do not diminish during ischemia even in health. The specificity of the phenomenon has not been defined. It occurs in some healthy aged individuals (23) and in uremia (15), and is said to occur in pernicious anemia (80). Preservation of function during anoxia occurs in isolated diabetic nerve, so that the phenomenon is a property of the nerve fibers themselves rather than of the metabolites released by surrounding ischemic tissues (75). Its cause is unknown. Suggestions are a greater ability to maintain energy synthesis by anaerobic glycolysis, increased potassium diffusion away from nerve through a damaged Schwann-cell basement membrane, and facilitation of nerve conduction by focal areas of demyelination acting as points of depolarization.

Autonomic Nervous System

Systematic neurophysiologic studies are lacking. Heightened esophageal responsiveness to stretch (50), impaired papillary responsiveness to light (39), and cholecystic distension (42) in early diabetes suggest a generalized disorder of autonomic function well before the appearance of clinical neuropathy.

BIOCHEMISTRY

Structural Analysis

Peripheral myelin is an elaboration of Schwann-cell plasma membrane (5). Its biochemical analysis is at a relatively primitive stage. It consists of alternating protein and lipid layers. Compared to central myelin it has fewer sedimentary subfractions, a differing amino-acid composition, and differing proportions of cholesterol, triglyceride, galactolipids, phospholipids and polyunsaturated fatty acids (20,65,66,85).

The sedimentary and electrophoretic subfractions of peripheral myelin in diabetes differ from normal (65). It is not known whether these structural differences are synthetic or degradative in nature.

The breakdown of myelin in disease has been studied in several experimental disorders but not in diabetic neuropathy (47). The early lamellar splitting in myelin breakdown is accompanied by a progressive rise in several proteinases originating from Schwann-cell lysosomes and surface membranes.

Metabolic Abnormalities of Diabetic Nerve and Effects of Insulin

Glucose uptake, glucose oxidation, and glucose-derived lipogenesis in peripheral nerve are insulin responsive (36, 37). It is curious that glucose uptake is not insulin dependent, being actually higher in diabetic than in normal nerve. The action of insulin or glucose uptake is a

sterospecific cell-membrane phenomenon. Insulin also influences the intracellular disposition of glucose. Normal glycogen stores are absent and lipogenesis is depressed in diabetic nerve, while lipogenesis is increased in normal nerve even in a glucose-free medium. Sorbitol and fructose accumulate in diabetic nerve in the presence of depressed insulin-dependent pathways. More comprehensive, structure-specific (axon vs satellite cell) studies are needed to identify the relationship of these various observations to the morphologic and functional changes present in diabetic nerve.

Insulin markedly enhances the total incorporation of 14C-acetate into normal-nerve lipid, and alters its fractional incorporation into lipid components. Diabetes likewise alters these incorporations (29,37,66). Details within and amongst studies are confusing and do not lead to a comprehensible picture, perhaps because of an influence of cachexia as well as of diabetes, because the observations are not structure specific, and because pool sizes and specific activities were not measured.

The Sorbitol Pathway

The permeability of peripheral nerve to glucose is not insulin dependent, and hence its intracellular glucose concentrations is at the extracellular level. Its intracellular glucose disposal to glycogen and to lipids is insulin dependent, whereas disposal via the sorbitol pathway [glucose (aldose reductase)⟶ sorbitol (sorbitol dehydrogenase)⟶fructose] is not, so that at any given blood glucose level diabetic nerve has an even greater tendency to form sorbitol and fructose than does normal nerve (40, 41). These sugars traverse cell membranes poorly, and are metabolized only slowly, so that once formed they are trapped intracellulary. Nerve from diabetic patients contains sufficient sorbitol and fructose to have osmotic significance (10-80 mM/g). Furthermore, aldose reductase is located within the Schwann cells and sorbitol dehydrogenase mainly within the axon, so that local concentrations may be even higher than is apparent from whole tissue levels.

In diabetic rat nerve the defect in conduction velocity is concomitant with and proportional to the elevation of sorbitol and fructose (40, 41). Control of the blood glucose level with insulin restores both abnormalities to normal. The problem of establishing causality between them was approached by using galactose-fed rats. Galacticol, the analogue of sorbitol, accumulates in the nerves of these rats, coincident with which there is an increase in water content and a decrease in conduction velocity. Removal of galactose from the diet or administration of an aldose-reductase inhibitor corrects both abnormalities. The necessary critical experiments to establish the analogous causal link in diabetes have not been performed. It would be desirable that such observations be coupled with studies of myelin ultrastructure, composition, and electrical resistance.

Other Biochemical Properties of Nerve

Other properties of peripheral nerve could merit consideration in relation to the etiology or pathogenesis of diabetic peripheral neuropathy.

Nerve Growth Factor: Differentiation, growth and maintenance of the sympathetic nervous system in animals is dependent upon a specific protein having a structure and metabolic effect similar to proinsulin and to insulin respectively (38,55). The significance, if any, of these observations to health and disease has not been determined.

Axoplasmic Transport in Nerve: A slow and a fast transport mechanism carry protoplasmic materials from the cell body out into the axon (53,63,64). Slow transport is by means of an unexplained convection of whole axoplasm. Fast transport is by a sliding-filament mechanism. It is dependent

upon ATP generated by local oxidative metabolism, probably within the Schwann cell. The influence of diabetes upon these transport mechanisms has not been examined.

Role of Cyclic AMP in Nerve Impulse Transmission: Cyclic AMP has a role in the release of neurotransmitters from synapses and from motor end plates which is analogous to its regulation of the secretion of subcellular vesicles from endocrine glands (11,43,60,71). Study of the mechanism is incomplete. Cyclic AMP promotes glycolytic metabolism in nerve endings which may trigger the calcium-dependent release mechanism, or it may have a direct effect on calcium distribution. The effect of diabetes on this system has not been explored.

TREATMENT

The clinical literature and textbooks on diabetes mellitus convey an impression that the occurrence and progress of significant diabetic neuropathy are unrelated to the quality of diabetes management. This is untrue. The greatest need in the study of diabetic neuropathy now is a careful prospective clinical study which readily would dramatize this palpable fact. The results of such a study, and of similar ones related to the peripheral angiopathic complications of diabetes, could crystallize agreement amongst physicians that the principles and the details of currently-available diabetes treatment, primitive though it is, are effective, and could be used to motivate physicians and patients to learn and to apply them. Such a new era in diabetes management, pending the development of new insulin-delivery systems, is available now.

The preventibility of diabetes complications is in fact most clear for those disorders in which a satellite cell is the target for damage: the axon-supporting Schwann cell in neuropathy, and the capillary-supporting pericyte (17) in retinopathy. Quantitative studies (19) of the relationship of the severity of damage to the quality of treatment support one's own experience that severe, crippling neuropathy and retinopathy occur only in those individuals whose treatment has been negligent.

The principal present management of diabetic neuropathy is its prevention, or its arrest if it already is present. An acute reversal to normal by insulin of the early functional abnormalities in nerve conduction velocity and in the axon response to ischemia is demonstrable both in experimental and in clinical diabetes (40,45,46,80). Chronic diabetic neuropathy, however, tends to be intractable. It is not impervious to treatment effects, particularly those of meticulous, insulin-based diabetes control. However reports of its improvement with any regimen need to be evaluated against the fact that its symptoms are characterized by spontaneous remissions and exacerbations (59). Thus, since attention is likely to be sought by a patient during an exacerbation, improvement is likely to occur contemporaneously with treatment. In particular, symptomatic relief with the neurodepressors diphenylhydantoin (32) and carbamazepine (72), and the lipid-lowering agent chlorophenoxyisobutyrate (6,26) need confirmation. Thiamine chloride (18) and cyanocobalamin (4) also often are used, although firm evidence that they change the course of the disease likewise is lacking. It is interesting that latent vitamin B12 deficiency coexists with diabetes, possibly on an autoimmune basis, in about 3 percent of cases (84) and in about 9 percent of cases with peripheral neuropathy (51). The neuropathic signs in many of these individuals, originally attributed to diabetes, were responsive to vitamin B12. This observation is of therapeutic relevance in itself, but also it leads one to wonder whether in diabetic nerve the prevailing disorders of fatty acid and of pyruvate (62,83) metabolism might somehow lower the

thresholds for the manifestations of cofactor depletion, and whether the administration of cyanocobalamin and of thiamin may in fact have a useful therapeutic place on this basis. One retains a clinical impression that they are helpful, and investigations of this point seem appropriate.

At the level of applied research, the need in diabetic neuropathy as in other diabetes complications, is the development of transplantable or electronic insulin- and possibly glucagon-delivery systems which have sensing and input characteristics more closely resembling those which naturally occur.

At the level of basic research, the greatest need in diabetic neuropathy is definition of which enzyme reaction(s) in the Schwann cell is so critically insulin dependent that morphologic and functional disturbances develop in it from the moment of onset of diabetes. A comparative study of central and of peripheral satellite cells might assist in this search.

SUMMARY

Neuropathy is the earliest and the most universal structural complication of diabetes, coexisting in fact with the disease from its onset. It probably is caused by a specific biochemical lesion within the axon-satellite cell of Schwann, and all of its manifestations probably are secondary to this primary defect. It is the complication of diabetes most supressible even with present modes of management. Specific definition of its etiology and pathogenesis and development of improved modes of management leading to full preventibility are probably within the grasp of present technology.

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19

EFFECT OF DIABETES MELLITUS ON FETAL GROWTH AND DEVELOPMENT[1]

Daniel H. Mintz and Ronald A. Chez

BACKGROUND

Prior to the introduction of exongenous insulin therapy, few women with diabetes mellitus were able to become pregnant. Those who did faced a further threat to their existence, since pregnancy superimposed unique and unmet metabolic requirements on an already compromised nutritional state. Without insulin therapy, ketoacidosis was an inevitable terminating event for both mother and her unborn child.

Following the introduction of insulin therapy, more women reached the childbearing ages; fertility was unimpaired, and the spontaneous abortion rate reverted to that encountered in nondiabetic women. The dramatic reduction in maternal mortality then served to uncover a relentless and unremitting perinatal morbidity and mortality which has characterized pregnancies complicated by diabetes mellitus in the last five decades.

Diabetes mellitus is recognized increasingly as an accompaniment of pregancy. It is presently conservatively estimated that one out of each 100 pregnancies is complicated by diabetes (61). In the decades ahead this prevalence rate will undoubtedly increase, ultimately placing diabetes mellitus as a leading medical complication of pregnancy.

Considerable progress has been made in the last two decades in providing a clearer understanding of substrate and hormone dependence and independence in the placental fetal maternal unit. This report will briefly summarize the current extent of our information regarding fetal maternal carbohydrate metabolism in normal gestation. Limited insights which are now available, concerning the pathophysiologic alterations imposed by diabetes mellitus, will also be reviewed. Several reports can be consulted for more detailed treatment (9,27,38,46,53).

MATERNAL METABOLISM

As normal gestation progresses a lowering in the concentration of blood glucose occurs in the fasted state (22). These changes are accompanied by an elevation in plasma free fatty acids and ketone bodies and a decrease in plasma amino acids, particularly gluconeogenic precursors. Freinkel (21) has emphasized that these changes mimic the alterations observed in prolonged starvation in the nongravid state. He postulated that pregnancy was manifestly a state of accelerated starvation, with the conceptus serving as an obligate parasite. Pregnancy is also accompanied by an elevation in fasting immunoreactive insulin, enhanced glucose mediated beta cell responsiveness despite diminished tolerance to orally administered glucose, an increase in the fractional turnover for maternal insulin, decreased peripheral insulin sensitivity, diminished growth hormone responses to provocative stimuli in late gestation and a progressive plasma elevation of human placental lactogen (HPL), a peptide secreted by the placenta into the maternal circulation at a rate proportional to the trophoblastic mass. Placental lactogen appears to play a direct role in some, but

[1]Editorial comment: Various other aspects of the problem of diabetes in pregnancy are considered in chapters 2 and 8.

clearly not all, of the changes described above (6, 32). Moreover, it is unlikely that these multiple alterations will ever submit to a solitary signal that then initiates a sequential physiologic cascade. For example, the enhanced hepatic fractional turnover rate of gluconeogenic precursors may be related to a placental factor (42) other than placental lactogen; the increased beta cell responses to insulinogenic stimuli may relate to steroid hormones derived from the placenta (5) or augmentation in insular blood flow (43), and the alteration in the pituitary growth hormone releasing mechanism may be due to time-dependent opposing effects of estrogens and HPL derived from the placenta (48). In this emerging hypothesis the conceptus, more specifically its placental secretory products, is the critical regulator of metabolic events in the maternal compartment; its transport function, to the contrary, may play only a passive role in these changes.

The net result of these alterations in the pregnant female is to preserve glucose homeostasis. The enhanced insulin secretory capacity of the maternal pancreatic beta cell is critical to the adaptive response. Thus in the normal pregnant subject, food intake is associated with an augmented insulin response and only minimal and transient elevations in maternal plasma glucose level. The fetus, in turn, is supplied with a more or less constant nonperturbating source of nutrients from the mother across the placenta, despite the exigencies of maternal food consumption. To the extent that the maternal islet fails to compensate for the metabolic stress, the diabetogenic effect of pregnancy is manifested as: (a) gestational diabetes, (b) asymptomatic chemical diabetes, and (c) an intensification of the insulin dependent state in a previously recognized diabetic patient. When the efficiency of the maternal beta cell is reduced, the fetus must then develop its own adaptive mechanism to offset the effects of a fluctuating source of nutrition.

FETAL METABOLISM

A. Development and function of the normal human endocrine pancreas.

Alpha and beta cells can be identified in the human pancreas at 50 days of post-conception life (55). Our present knowledge about the morphogenesis and development of the organ has been summarized elsewhere (33,44). It would appear that a positive correlation exists between the number of alpha cells and beta cells and the amount of extractable immunoreactive glucogan and insulin; the percentage of either cell per total pancreatic cell mass is higher in the fetus than in the adult as is the insulin and glucagon content per gram weight of tissue. Furthermore the alpha:beta cell ratio of five in early pregnancy is one at birth and then approaches adult levels of 0.2 to 0.1 in childhood.

The precise role(s) served by the relative excess of alpha cells to beta cells in early life is unknown. Although the earliest appearance of glucagon in fetal plasma has not been precisely determined, it does appear that fetal pancreatic and plasma glucagon (3) may be present at a stage of gestation that precedes the development of glucagon receptors (13) or enzymatic pathways responsive to the hormone (28). Whether fetal pancreatic glucagon in early gestation has a role in beta cell ontogenesis has yet to be determined. Moreover, it has not yet been determined which stimuli, if any, affect changes in fetal plasma concentrations. In early reports, neither neonatal hyperglycemia nor maternal diabetes mellitus has been associated with changes in the plasma glucagon levels of the conceptus (7, 41). It does appear that perinatal hypoxia (29), intrauterine growth retardation (7) and neonatal hypoglycemia may influence plasma glucagon concentrations (7, 41).

Insulin containing granules can be identified by 60 days of gestation (55). The presence of insulin in fetal plasma has been observed at 84 days of gestation (1). The basal level of fetal

insulin, once achieved, remains relatively constant in a range similar to those observed in adult life.

The nature of the driving force to maintain this basal level has not been determined. Amino acids, glucagon, and theophylline, when administered intravascularly are associated with a rise in fetal or neonatal plasma insulin (26, 36). Therefore essential components of the insulin releasing mechanism are present in the fetal and neonatal pancreatic beta cell. A recent review (8) analyzed the human fetal and newborn pancreatic beta cell responsiveness to glucose. In almost all studies, the administration of glucose is associated with either the absence of or the delayed release of insulin. The mechanism responsible for the attenuated fetal response to a glycemic challenge, and the factor(s) influencing the gradual shift to an adult beta cell response pattern in early neonatal life are unknown. Whether a glucoreceptor or the intrinsic metabolic pathways of the beta cell required for glucose mediated insulin release are absent, inhibited, or not fully developed in the fetus is not clear. The absence of glucose mediated insulin responses in the fetus could be likened to islet cell function of the juvenile diabetic, whereas the delayed insulin responsiveness of the 1- to 3-day-old neonate resembles the pattern of release observed in maturity onset diabetes. Further in vitro study of islets from this transitional period in islet cell function may offer important insights into the mechanism(s) of control of the alpha and beta cells in both individuals and diabetic patients.

B. Effect of diabetes mellitus on the fetal endocrine pancreas

The transfer of glucose across the placenta is secondary to the driving force of the concentration gradient between mother and fetus. A direct relationship exists between maternal, fetal, and amniotic fluid concentrations of glucose (11). Thus in the patient with either fasting or postprandial hyperglycemia, the maternal glucose increment is proportionately reflected in the fetal blood compartment. Placental saturation kinetics for maternal to fetal glucose transfer have been postulated in man (4), but its presence has not been confirmed in studies in the subhuman primate (10).

Pedersen (52) proposed that prolonged exposure of the fetus in a diabetic pregnancy to hyperglycemia stimulated the fetal pancreas to produce excessive insulin. Fetal hyperglycemia and hyperinsulinemia together promoted glucose uptake in fetal adipose tissue leading to the obesity (20) characteristic of infants of diabetic mothers. The complication of neonatal hypoglycemia (14) could then be related to the persistence of pancreatic beta cell hyperresponsiveness into the neonatal period. The absence of the embryopathy characteristic of infants of diabetic mothers when maternal hyperglycemia was moderately controlled provided apparent confirmation of this pathophysiologic sequence (34,50,52). Moreover, increased fetal newborn basal insulin levels and the plasma insulin responses to a glycemic stimulus in newborns of pregnancies associated with diabetes mellitus were also in accord with this hypothesis (34,45,49).

There are, however, certain persuasive observations which suggest that fetal hyperglycemia/hyperinsulinemia may not be a sufficient reason for the embryopathy in infants of a diabetic mother. First, neonatal hyperinsulinemia has not been a consistent finding in all studies of infants of hyperglycemic mothers (35). Second, the structural integrity of the fetal neurohypophysis is required for the emergence of fetal hyperinsulinemia even in the presence of fetal hyperglycemia (15). Third, the classical appearances of these fetuses are not noticeable before a gestational age of about 34 weeks in spite of the presence of maternal/fetal hyperglycemia since

conception (19). Fourth, fetal hyperinsulinemia also accompanies erythroblastosis (17) and other fetal hemolytic disorders (60), but fetal obesity does not. Last, in humans (59) and in induced hyperglycemia in subhuman primate pregnancies (47), fetal basal hyperinsulinemia can occur in normoglycemic fetuses. These observations indicate factors other than fetal hyperglycemia may influence the fetal endocrine pancreas. The role and interrelationships of other hormone and substrate insulinogenic substances needs still to be elucidated.

A fundamental question of urgent clinical dimension concerns the precise relationship of fetal hyperglycemia and hyperinsulinemia to perinatal morbidity and mortality. Empirical clinical observations (34,50,52) demonstrate that strict control of maternal hyperglycemia prevents the embryopathy and improves the salvage rate of viable fetuses. Perinatal morbidity and mortality, however, even under these conditions, is still formidable. Even though considerable progress has been made in developing sophisticated laboratory aids (2,30,31,54,56), which help to signal fetus distress, the abnormalities uncovered still represent the trappings of a much more fundamental morbid process. The critical question of the mechanism of death in utero in these pregnancies is still to be elucidated.

FETAL THERAPEUTICS

The pathophysiologic sequence outlined above does provide a rationale for the management of pregnancy in the diabetic patient. The goal of fetal therapy is to prevent ketoacidosis with its attendent immediate and potential long-termed complications (12), and to minimize excessive placental transfer of glucose to the fetus. In order to achieve this, maternal hyperglycemia is avoided by administering insulin in a manner calculated to simulate the demand response of the normal pancreas. To avoid postprandial surges in plasma glucose concentration, multiple dosages of intermediary and short-acting insulin alone or in combination through the day are required.

The gradual evolution of effective treatment for the gravid diabetic has provided three important lessons in fetal therapeutics. Simulation of the normal homeostatic adjustments of gestation may be the key to successful medical care. To reach this goal in the care of a woman with diabetes mellitus in pregnancy, the critical factor is careful composite chemical control (34). The criteria for optimum medical care in the gravid are different from those needed in the nongravid state. It is essential to understand the special exigencies and delicate balance of the diabetic who is pregnant, in order to practice optimal fetal therapeutics. An important derivative of this experience is the realization that total care of the mother and conceptus requires a team approach. Long and meticulous care of the maternal diabetic state, diagnostic amnicentesis, delivery and its timing, and definitive neonatal care require a large medical team composed of a knowledgeable referring physician, a diabetologist, obstetrician, neonatologist, trained biochemist, radiologist, nurses, social workers, and anesthesiologists. Survival is a function of the combined inputs of these professionals.

The unfavorable gestational milieu accompanying diabetes mellitus represents a coersive influence on judgements concerning optimum timing of delivery (66). Despite considerable investigational efforts in this direction, no laboratory aid has yet emerged which can serve as an immediate sensitive and specific prognosticator of fetal distress or fetal well-being. Many clinics have developed normograms of estriol levels (2,56,57), placental lactogen levels (30,56), and other direct or indirect indices of fetal function which are alleged to be effective in this critical assessment

process. Opinion, however, remains divergent as to their ultimate utility, and the need for continued investigation in this area of fetal diagnosis is still highlighted (19).

The major cause of death in the newborn of a diabetic mother is the respiratory distress syndrome. It now appears that prediction and possibly prevention of this disease are imminent.

The phospholipid lecithin is the major component of the surface active alveolar lining layer which determines alveolar stability in newborn life. The concentration of phospholipids in amniotic fluid is predictive of the potential for extrauterine alveolar stability. Gluck et al. (23, 24) correlated the lecithin sphingomyelin ratio in amniotic fluid with the subsequent incidence of respiratory distress syndrome. The relative lung maturity of the newborn may now be predicted by biochemical examination of a sample of amniotic fluid.

In some circumstances, premature labor is imminent or must be induced because of overwhelming maternal or fetal risk even when the ratio of lecithin to sphingomyelin indicates a high probability of functional immaturity in the newborn lung. DeLemos et al. (16) and Kotas et al. (37) in animal models, and subsequently Liggins et al. (40) in human premature infants, have presented evidence that fetal lung maturation can be therapeutically accelerated and prophylaxis thereby accomplished. In humans, glucocorticoids administered to the mother prior to 32-weeks gestation can increase the lecithin sphingomyelin ratio (58) and result in a significant reduction in the occurence of respiratory distress syndrome (40). This current experience opens the possibility for intrauterine fetal treatment prior to forced premature delivery in complicated diabetic and other high-risk pregnancies. Both short- and long-term side effects of this fetal therapy require prospective evaluation.

Advances in fetal diagnosis and therapeutics are derivatives of the successful application of present knowledge. Continued advancement requires new information about the normal and pathological processes of pregnancy, the degree of control that the fetus-placenta exercises in regulating metabolic processes of the mother, the interdependence as well as independence of the fetal and maternal endocrine systems, and the time-related specificity of fetal metabolic pathways. Moreover, since fetal therapeutics, at its current state of development, is neither enzyme nor process specific, appraisal of the steps taken by the physician managing the fetus and its environment will require a focus more refined than the relatively gross standard of perinatal survival or mere absence of disease.

CONCLUSION

Considerable progress has been made in the last decade relative to our understanding of factor(s) influencing fetal carbohydrate metabolism. Medical care which the pregnant patient with diabetes mellitus receives today should be a derivative of these physiological insights. The actual level of care, however, is frequently determined by factors other than the availability of relevant new physiological information. The patient's geography, the family socioeconomic status and level of education of the family unit, the psychodynamics of the pregnant patient with diabetes mellitus (39), and the proximity to centers devoted to special care are major limiting influences. In order to provide the optimal environment for the unborn child, the full scope of public health must be utilized. To do so at this time may require a reorientation of individual and national medical care and educational priorities.

It was emphasized that ideal care for the individual pregnant, diabetic woman and her unborn child requires a consortium of health professionals. It is axiomatic that a physician responsible for only a few such patients in any single year will not be able to accumulate an essential clinical experience that matures his medical judgment. Centers of special care should be generally available to the physician for continuing education and to the patient for the expertise in clinical management that is often required. The predictable need of highly specialized neonatal care in some pregnancies makes mandatory predelivery referral to such centers if perinatal mortality/morbidity is ever to be significantly reduced.

A basic requirement for advancement in the emerging field of fetal therapeutics is new information about the normal and pathologic processes of pregnancy. The use of man as an experimental model is proscribed by ethical considerations, hence the search for animal models by investigators should receive continuing support. A parallel need of equal urgency is for support funds for training physicians as perinatal scientists.

The last two decades have witnessed a significant decline in perinatal morbidity and mortality in pregnancies affected by maternal diabetes mellitus. Further improvement should result as increased effort is directed at both fetal and neonatal therapy. To accomplish this, however, will require expanded support for fundamental and applied research in fetal growth and development.

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NEW APPROACHES TO CONTROL AND PREVENTION

20

DIABETES MELLITUS
A BIOENGINEERING APPROACH--AN IMPLANTABLE GLUCOSE SENSOR[1]

J. Stuart Soeldner, Kuo Wei Chang, Sol Aisenberg, John M. Hiebert, and Richard H. Egdahl

ABSTRACT

There is increasing concern focused upon diabetes mellitus and its complications. Current evidence strongly suggests that a desired endpoint is the normalization of blood glucose levels. Available therapeutic programs rarely allow the diabetic to achieve normal glucose levels due to the inability of a program to either frequently monitor blood glucose levels or to mimic the normal dynamic function of the beta cells of the Islets of Langerhans in the pancreas. A project has been initiated which focuses on the construction of a small implantable glucose electrode which can be incorporated into an implantable "Glucose Monitor" and which could provide the diabetic patient with frequent, instantaneous information on blood glucose control. It would produce an audible and/or visual alarm if blood glucose rose above or below a desired pre-set level. After further development, the glucose electrode could be incorporated into an implanted "Artificial Beta Cell" which would be a demand insulin release system programmed for normalization of blood sugar.

INTRODUCTION

Diabetes Mellitus is a disease which presents many problems and many challenges. Although it is the fifth leading cause of death, it is thought by many that the death rate from diabetes is underestimated. The major reason for this is that the complications of diabetes, particularly those that affect the cardiovascular system are often considered as the primary cause of death while diabetes itself is ignored or listed as a minor cause. Another important consideration concerning diabetes is the fact that at least 4 percent of the population of the United States have the disease in an overt or occult form.

The major complication of diabetes is coronary heart disease which in diabetics causes 3 to 4 times as many deaths than in the nondiabetic general population. In addition, it is recognized that death due to heart complications in diabetics occurs at a younger age than in the nondiabetic. Other complications include renal failure which is nearly 20 times more common in the diabetic patient than in the nondiabetic. The second leading cause of blindness is diabetes. Numerous other complications including gangrene, neuropathy, infections, etc., also serve to reduce the quality and duration of life in the diabetic.

CURRENT CONCEPTS OF THE COMPLICATIONS

Much evidence suggests that the frequency of the complications is reduced in patients with good control of blood sugar (glucose) (13). More current evidence supports the concept that

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elevated blood glucose levels may by itself induce metabolic and structural changes (particularly in small and large blood vessels) which underlie the broad spectrum of diabetic complications. Although the precise mechanisms have not yet been clarified, greater focus has been placed upon systems and techniques designed to truly normalize blood glucose levels in the diabetic patient.

CURRENT DIABETIC MANAGEMENT

The keystones of a diabetic treatment program today are essentially diet, exercise, and specific antidiabetic therapy (i.e., insulin injections for the more severe and diet alone or combined with oral antidiabetic agents in the milder forms of the disease).

Currently available control monitoring systems are crude considering the complexity and the dynamics of blood glucose homeostasis. Sporadic blood glucose determinations (perhaps only once a month) provide the diabetic patient and his physician with only a fleeting retrospective view of the degree of control. Urine glucose determinations are simpler than blood glucose determinations, can easily be employed by the patient, and can be as frequent as urination. However, certain features of the physiology of urine excretion of glucose make this determination gross at best. The main problems focus on the fact that there is a "threshold" of blood glucose concentration below which no glucose can be detected in the urine. Usually this threshold is 160 mg per 100 ml of blood glucose, but this tends to vary from person to person and increases with age. In addition, this determination of urine glucose is an integrated value, only crudely correlating with blood glucose and not indicating whether the elevated blood glucose is rising, stable, or falling. Finally, the urine test is negative for glucose at blood glucose values below threshold. These apparently negative values could be higher or lower than the range of blood glucose seen in healthy nondiabetic subjects (60 to 140 mg per 100 ml).

A GLUCOSE ELECTRODE

It appears conceivable that a small, inert, nontoxic, biocompatible, glucose sensing device which requires low power would have many practical applications in relation to diabetes control and could be the critical component of a mechanical device focused upon improving blood sugar control in diabetics.

Updike and Hicks (16) and Hicks and Updike (12) described a "glucose sensor" constructed around a Clark (7) oxygen probe. They introduced glucose oxidase enzyme in a film of hydrogel (polyacrylamide) positioned directly over the teflon membrane covering the platinum cathode. Glucose added to solutions in which the device was inserted was oxidized and the residual PO_2 activating the oxygen electrode reduced. The current output of the electrode fell in a nonlinear fashion proportional to the glucose concentration. A similar type of electrode had been proposed by Clark and Lyons (8) and Clark and Sachs (9). Irreversible loss of enzyme activity in these systems rendered them unsuitable for long-term use.

Bessman and Schultz (2) extended these studies. In their version, the glucose oxidase enzyme is covalently bound to a rayon acetate cloth and a special spiral type of silver cathode, lead anode type oxygen electrode was developed. Two electrodes are used, both electrode faces are covered by a membrane, onto one of which the enzyme oxidase has been conjugated. This arrangement allows for the oxidation of glucose in the area of one of the oxygen electrodes and consequently a lowering of the PO_2 relative to the other oxygen electrode. The difference in PO_2 between the electrodes is related to the glucose concentration.

Additional studies by Bessman and Schultz (1) had focused upon a regeneratable noble metal catalyst as a substitute for glucose oxidase. These studies demonstrated catalyst poisoning that was partially circumvented by an electric pulsing system.

A survey by the Joslin Research Laboratory group of methods and techniques used for measurement of glucose indicated that a new and novel method would have to be devised for long-term implantable use (4). An intriguing possibility was found in the area of glucose electrochemistry since glucose oxidation can be catalyzed in the presence of certain noble metals (3,14). The general scheme of postulated reactions that take place at the cathode and anode of a glucose sensor is:

$$\text{Anode: } 2C_6H_{12}O_6 + 2H_2O \xrightarrow{\text{Pt*}} 2C_6H_{12}O_7 + 4H^+ + 4e$$

$$\text{Cathode: } O_2 + 4H^+ + 4e \xrightarrow{\text{Pt*}} 4OH^-$$

$$\text{Overall: } O_2 + 2C_6H_{12}O_6 \xrightarrow{\text{Pt*}} 2C_6H_{12}O_7$$

*Platinum electrode catalyst

A program for the construction of a glucose electrode evolved which led to the fabrication of the device shown in Fig. 1.

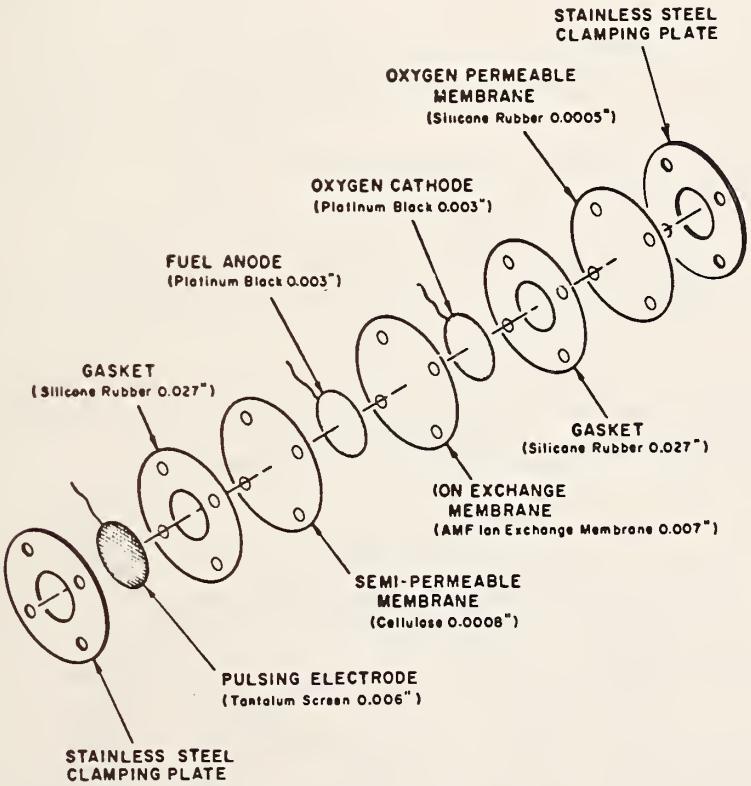

FIGURE 1. A schematic of the glucose sensor.

The membrane covering the fuel anode compartment was specially selected and constructed not only to shield the electrode from high molecular weight species, but to allow diffusion of glucose, oxygen, and water. The membrane over the oxygen cathode was selected on the basis of allowing O_2 and H_2O vapor diffusion but excluding glucose and other nonvolatile, water-soluble species. The ion exchange membrane placed between the two electrodes serves as a solid-state ion shuttle.

ELECTRODE PERFORMANCE

Prototype and modified electrodes have been studied extensively in vitro and in vivo (5,15, 6). The in vitro studies demonstrated that the device was stable and showed an increase in current as a function of glucose concentration (Figs. 2 and 3).

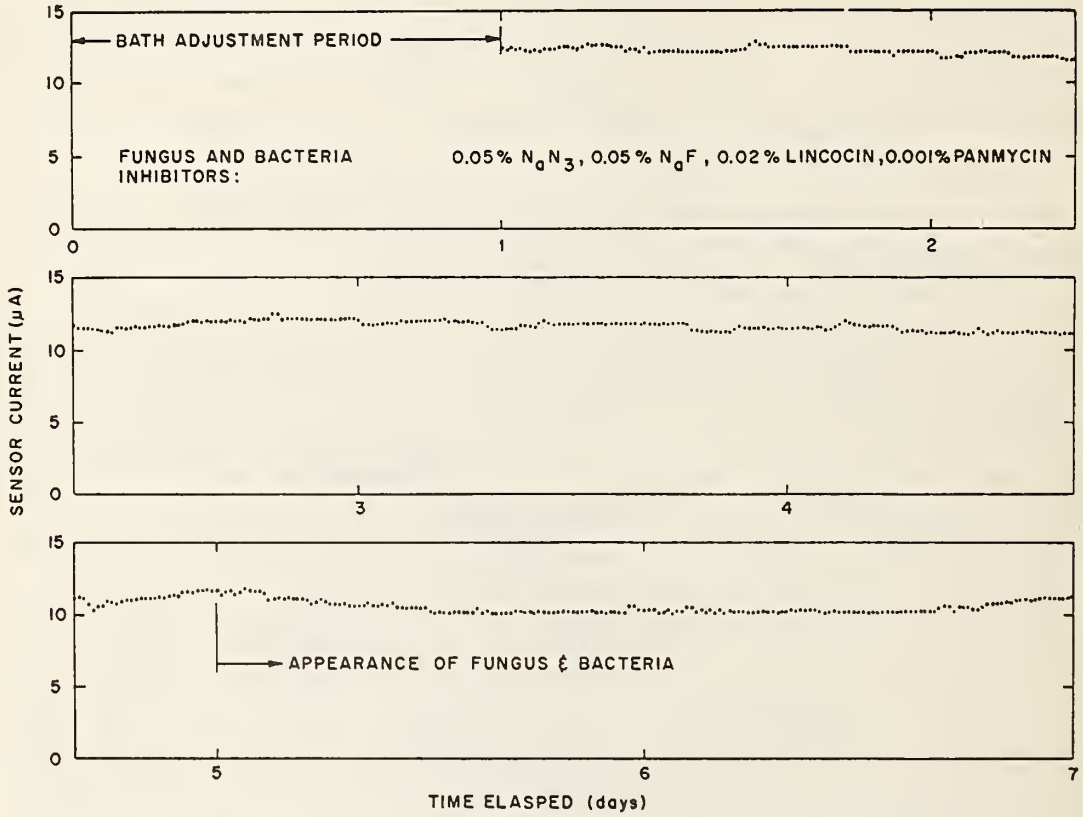

FIGURE 2. Results of a 7-day study in which a glucose sensor was incubated in a Krebs-Ringer buffer containing glucose (100 mg per dl) and a mixture of antibiotics. Sensor readings were recorded every 15 minutes. The overall coefficient of correlation was 6.6 percent (5).

In addition, it had a high specificity for glucose compared to other compounds found in biological fluids and could be constructed to respond rapidly to abrupt changes of glucose concentration.

Preliminary in vivo studies were performed in a variety of animals (monkeys, rabbits, and dogs). In these studies, the glucose electrode was attached via wires to an external power supply, amplifier, and recording unit (Fig. 4). The glucose electrode was implanted into subcutaneous tissues (abdomen or back) and in studies to date has survived for up to 117 days and produced a signal which correlates significantly with corresponding blood sugar levels following intravenous glucose administration (Fig. 5). In the majority of these trials, fracture of wires and infection associated with the skin area in which the wires emerged was the cause of the termination of the study. Current efforts are being focused upon the development of a totally implantable unit containing the glucose electrode, power supply, and a miniature radio transmitter with a suitable external receiver-recorder system (Fig. 6). This modification will be employed for more prolonged (6 months plus) studies of the performance characteristics of the electrodes.

Following the development of a satisfactory totally implantable system and documentation of the accuracy, reliability, and reproducibility of the glucose electrode signal in animals, trials in diabetic patients will be started.

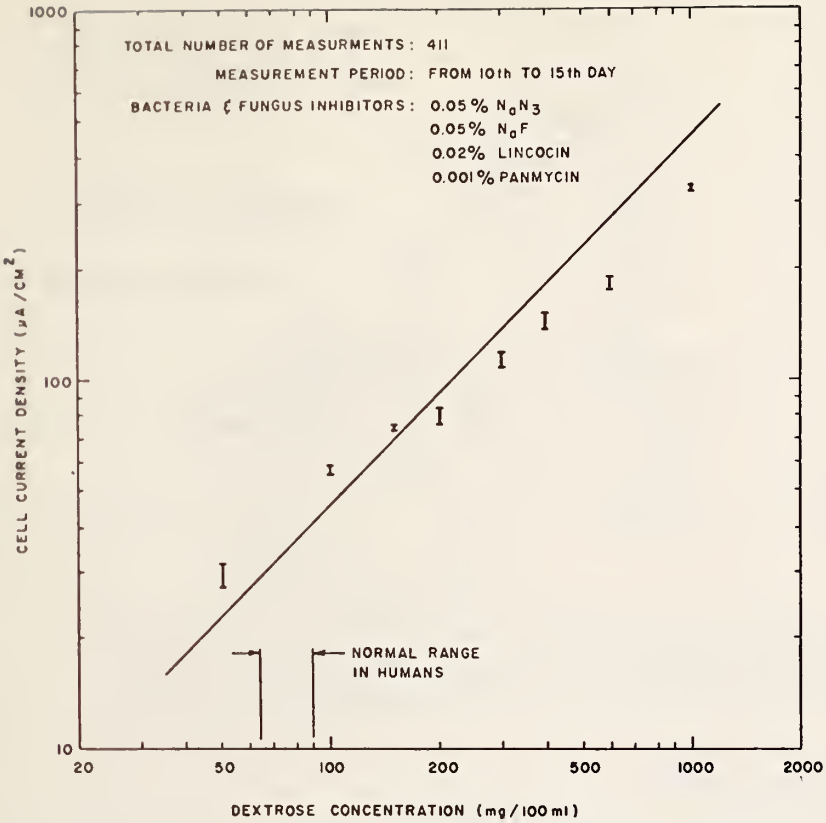

FIGURE 3. The dose response pattern of a glucose sensor incubated in Krebs-Ringer buffer at glucose concentrations ranging from 50 to 1000 mg per dl.

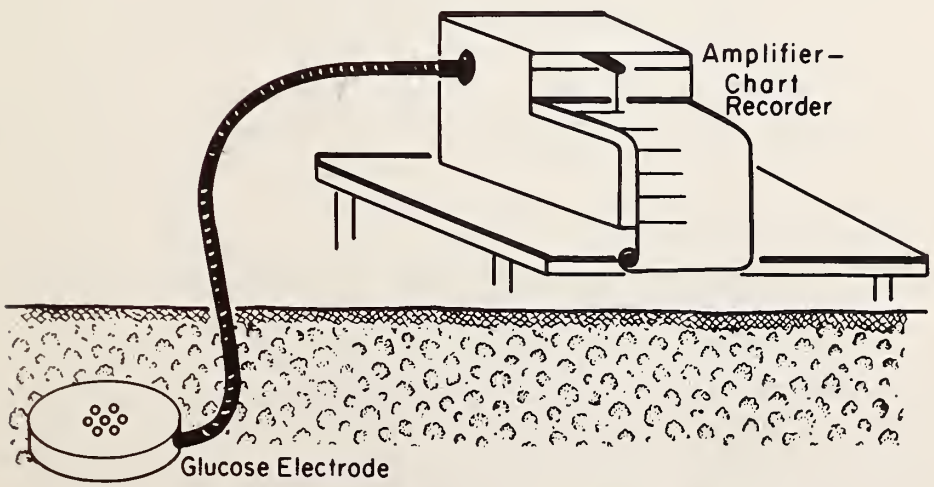

FIGURE 4. This shows the arrangement employed for the initial animal studies.

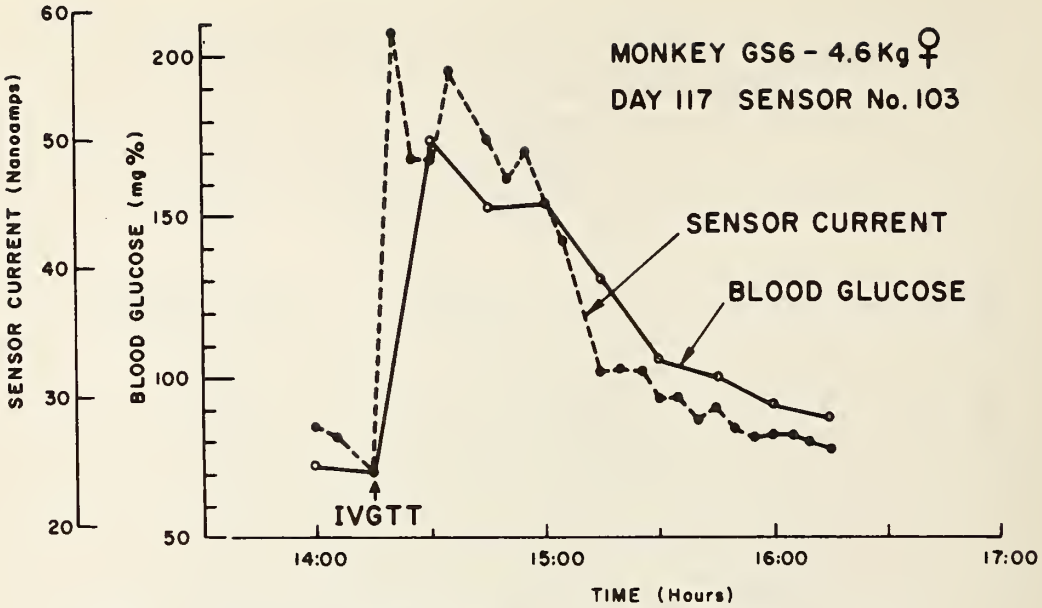

FIGURE 5. This shows a comparison of the glucose sensor current and the blood glucose levels obtained simultaneously during an intravenous glucose tolerance test in a female Rhesus monkey after 117 days of implantation (6).

APPROACHES TO BETTER DIABETES CONTROL

A general plan has been formulated by this group and a series of stages have been developed.

Stage I - A Glucose Monitor

It is plausible that the great majority of diabetic patients could be educated and trained to alter diet, exercise, and antidiabetic medication to produce better blood glucose control *if* they had constantly available information as to their body's glucose level. We propose initially to construct a device suitable for implantation into body tissue which could sense the glucose level and transmit this signal externally via an appropriate telemetry system to a suitable external receiver-recording device (Fig. 6). Experience to date has been confined to implantation in extravascular sites and the extent to which they reflect instantaneously changes in blood sugar remains to be clarified. This reading would be available to the diabetic patient on a moment-to-moment basis and could guide in the proper selection or modification of his regime. Also, the external receiver device could be constructed to produce an appropriate audible and/or visible signal if glucose levels rose above or fell below preset limits. The latter mode of operation would be particularly valuable for the unstable juvenile type of diabetic who is prone to develop serious episodes of insulin reaction (low blood glucose) at night while sleeping. Thus alerted, the diabetic could consume an appropriate amount of carbohydrate and avert the reaction.

Stage II - The Artificial Implantable Beta Cell

This stage will be difficult, but the validation of the implanted glucose electrode required for Stage I will obviously solve one of the problems.

A second component of the artificial beta cell will be a miniature computer which can translate the signal from the electrode to an appropriate amount of insulin release (Fig. 7).

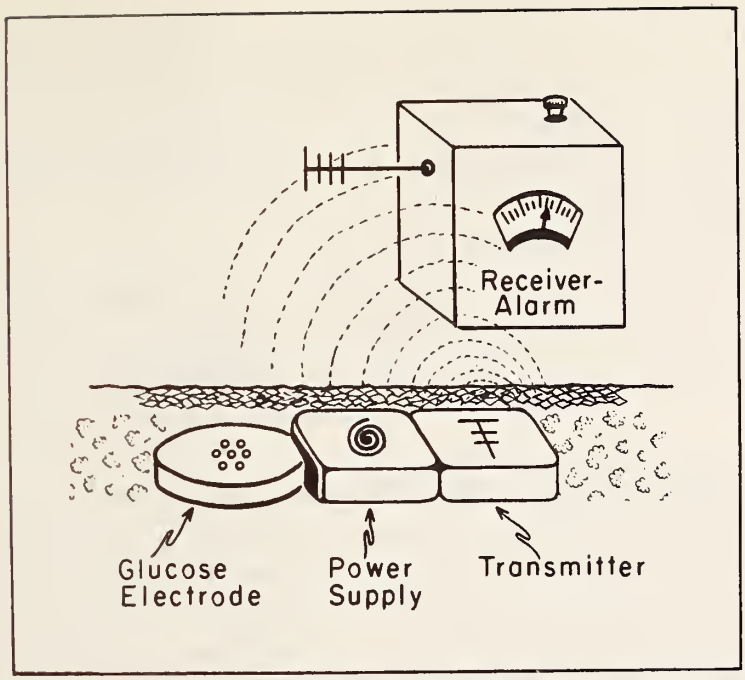

FIGURE 6. This depicts the major components of a totally implantable "Glucose Monitor" (15). Reprinted with permission from Plenum Press.

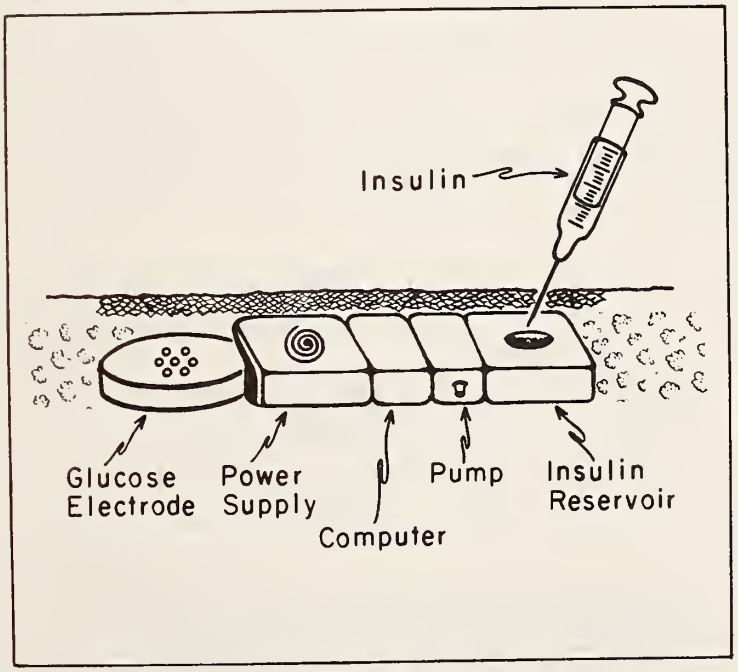

FIGURE 7. This depicts the major components of a totally implantable "Artificial Beta-Cell" (15). Reprinted with permission from Plenum Press.

Preliminary studies have been done in modeling glucose homeostasis in man with particular emphasis upon the temporal dynamics of glucose stimulated insulin release. It appears that the Systems Dynamics Model of R. O. Foster (10) as well as the model proposed by Grodsky (11) can be implemented in terms of a small computer component of the artificial beta cell.

An insulin reservoir will be an important component. Currently, this is conceived as a solid tank except for one surface which would be constructed of a self-sealing membrane. During implantation, the reservoir would be positioned such that the membrane would face the skin. To refill, the patient would fill a syringe with insulin; then after skin cleansing, he would pass the needle through the skin, subcutaneous tissue, and the self-sealing membrane until the tip was positioned inside the tank prior to injecting the insulin.

Power requirements for both the Stage I Glucose Monitor and the Stage II Artificial Beta Cell could be in the form of batteries currently being used in cardiac pacemakers. Recently, atomic power supplies have been introduced into pacemakers as have externally rechargeable batteries. It is also conceivable that a "glucose fuel cell" could be utilized (17). Here, design would allow for a constant current at all glucose levels to provide power for the unit (Fig. 8).

GENERAL DISCUSSION

It is difficult to chart a time table for both Stage I and II. Few precedents in biomedical devices are available to act as guidelines. Currently (June 1973) prototype "Glucose Monitors" are being constructed for prolonged evaluation totally implanted in animals. Figure 9 shows the critical path toward the Glucose Monitor. Success in this phase will be achieved when at least 18 or 20 of the devices perform satisfactorily for 6 or more months. At that point, it will become feasible to undertake clinical trials to evaluate whether normalization of blood sugar can materially reduce the incidence of diabetic complications.

The timetable for the "Artificial Beta Cell" is obviously more complex. Figure 10 shows the critical path toward an Artificial Implantable Beta Cell. It is anticipated that the validation of the performance of the glucose electrode will have been completed, and the major focus during this stage will be placed upon the insulin reservoir, pump, and computer interface. This will involve considerable skills in design, engineering, and physiology.

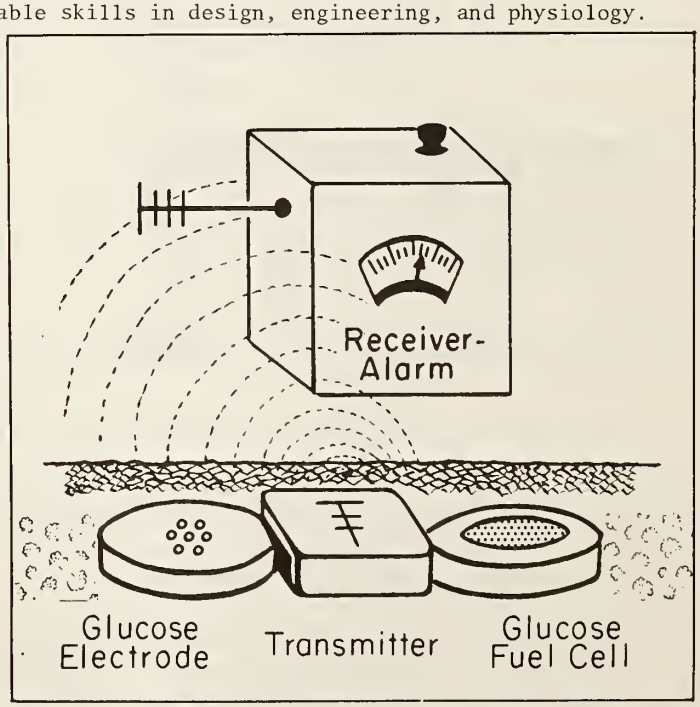

FIGURE 8. This depicts the manner in which a glucose fuel cell could provide power for an implanted "Glucose Monitor."

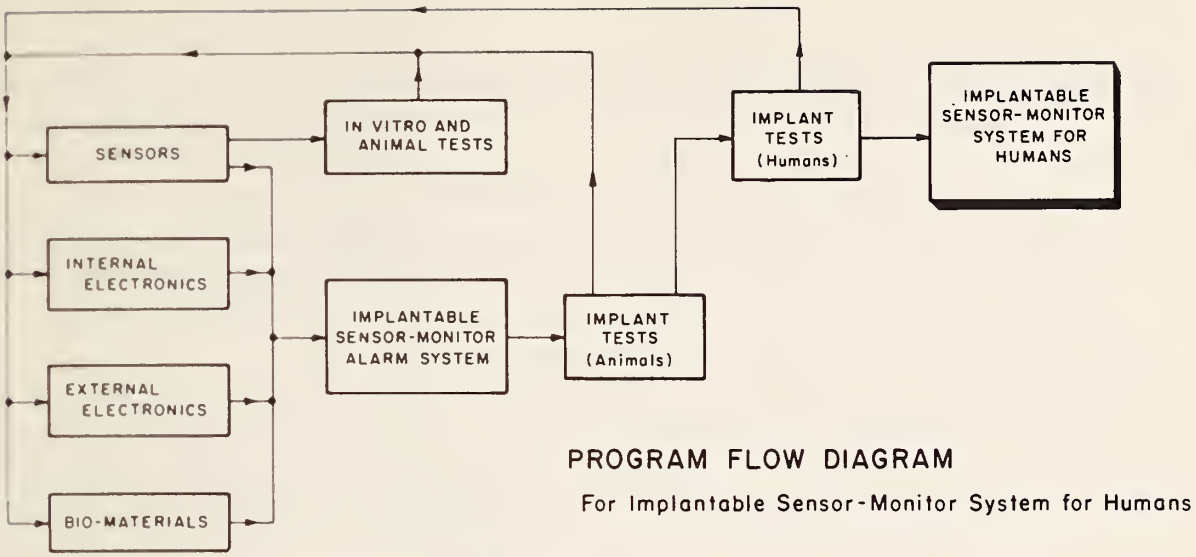

FIGURE 9. The Program Flow Diagram for the implantable "Glucose Monitor" for humans.

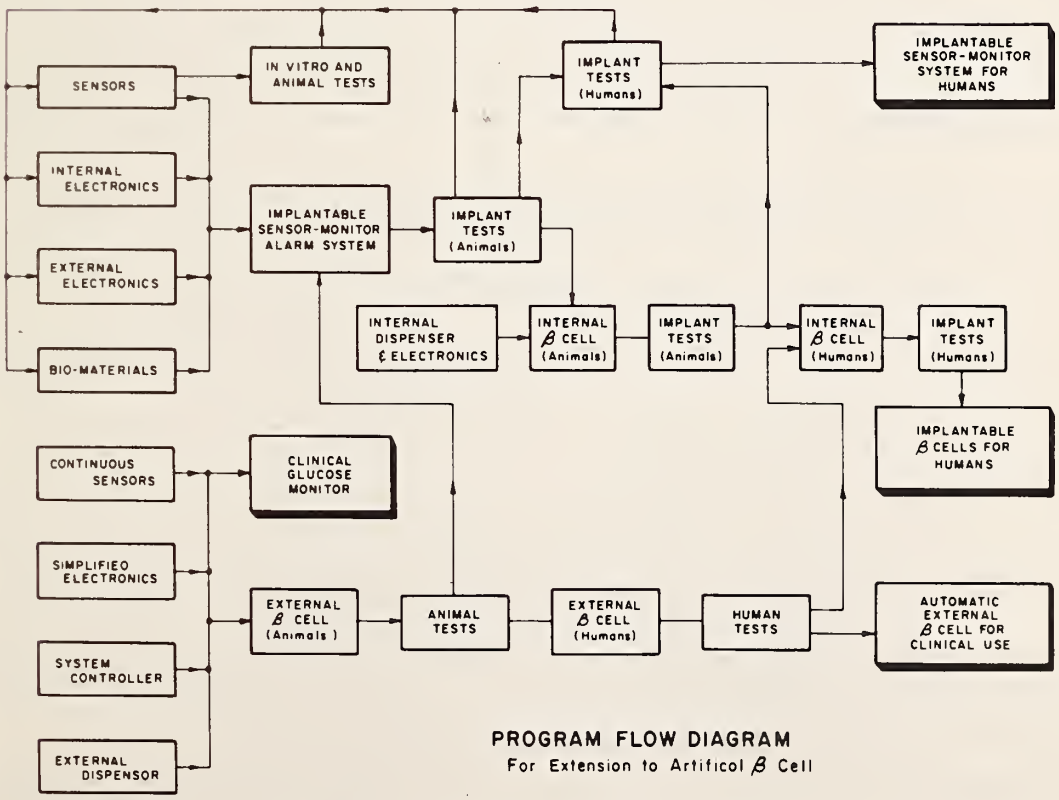

FIGURE 10. The Program Flow Diagram for the "Artificial Beta-Cell" in humans.

FINAL PRACTICAL CONSIDERATIONS

The greatest question that still remains to be answered is: Does better control or indeed normalization of blood glucose dynamics in the diabetic reduce or eliminate the so-called diabetic complications? It is evident that solid scientific proof is still lacking. It also appears that until therapeutic systems are available for treating diabetes that are much superior to any current method, then a sound broad-based prospective study could not be performed.

A second question that arises (if lack of blood glucose control does relate to the complications) would address the problem of establishing some criteria whereby if a diabetic's degree of control was not "satisfactory" with conventional measures, then the employment of a device (i.e., Glucose Monitor or Artificial Beta Cell) would be warranted.

It is apparent that there are certain diabetics who would qualify even with the current uncertainty concerning the long-term consequences of control. These types of diabetics would include those with frequent episodes of ketoacidosis, frequent hypoglycemic reactions especially without warning symptoms, the brittle-unstable diabetic, and probably those with a high renal threshold.

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21

TRANSPLANTATION OF INSULIN SECRETING TISSUES

Richard C. Karl, David W. Scharp, Paul E. Lacy, and Walter F. Ballinger

There are between 3 and 5 million individuals in the United States suffering from diabetes mellitus, and it is estimated that another 5 million, now alive, will develop the disease during their lifetime. Diabetes is probably the most common metabolic disorder in the western world today. The discovery of insulin in 1921 and its extraction and preparation for clinical use shortly thereafter was one of the most dramatic therapeutic breakthroughs achieved by modern biological science. It has been estimated that in 1916, 64 percent of the deaths of diabetic patients were due to diabetic coma. At that time, a child diagnosed as a juvenile diabetic was expected to be dead within two years. Today diabetic coma accounts for less than 1 percent of diabetic deaths and juvenile diabetics are still setting longevity records.

Yet, the increased longevity of both adult and juvenile onset diabetics made possible by insulin therapy and the advent of antimicrobials has revealed a host of complications thought to be secondary to the metabolic disorder. Both macro and microvascular disease is accelerated in the diabetic and these processes affect the brain, heart, kidneys, eyes, and the extremeties. The dismaying impotence of accepted therapeutic approaches to alter the increased morbidity and mortality caused by this secondary vascular disease has recently been quantitated by the University Group Diabetes Program (2). Although there are some legitimate objections to this study (3), it is universally accepted that insulin and the oral hypoglycemic agents have not rendered the diabetic patient normal. Other chapters of this monograph discuss in greater detail the complications of diabetes and the currently employed therapeutic agents.

The question of whether the vascular changes seen in diabetes are secondary to the metabolic derangement, or are instead manifestations of an entirely independent disorder which happens to be co-inherited with diabetes, is not yet fully resolved. Nonetheless, persuasive evidence has been put forward that indicates that the complications seen in diabetics are consequences of the disease. Williamson has quantitated changes in capillary basement membrane thickness in normals and diabetics (4,5,6). He has found that in both normals and diabetics this sign of microvascular disease is increased with age. Observations in diabetics indicate that these patients probably had normal capillary basement membrane thickness prior to the onset of their disease. Patients with the complications of retinopathy or nephropathy have a 90 percent incidence to capillary basement membrane thickening. Patients suffering from the disease for 20 years or longer have a 93 percent incidence of such thickening. These data suggest that the metabolic derangement precedes the abnormality of the capillary basement membrane. Furthermore, the occurrence of retinopathy and nephropathy in patients suffering from nonhereditary forms of diabetes such as hemochromatosis (7) or pancreatic damage (8) is suggestive of a causal relationship between diabetes and vascular disease.

Although great controversy has been elicited about the exact biochemical lesion in diabetes, it is reasonably well accepted that at least one basic defect resides in the β cell of the islets of Langerhans (9). Thus, all forms of diabetes are characterized by a "relative" deficiency of

circulating insulin (10). Detailed studies of insulin secretion in normal and diabetic subjects indicate that the cells are sluggish in their response to glucose stimulation (9).

The failure of exogenous agents (e.g., insulin and the oral hypoglycemics) to effect a rigorous control of blood sugar from minute to minute may explain the failure of such agents to prevent the complications of diabetes. As the basic defect in diabetes lies in the β cells, it is postulated that successful transplantation of normal β cells into the diabetic patient may prevent the development of these crippling complications.

MODELS FOR TRANSPLANTATION

Experiments designed to test the feasibility of transplanting functioning insulin secreting tissue capable of "curing" diabetes all have a common requirement: the recipient animal must be diabetic. Although a few experiments have been done involving spontaneously diabetic animals, almost all investigations have employed animals rendered diabetic by the administration of a specific cell poison, alloxan, or streptozotocin. The biochemical mode of action of both these agents is obscure. Alloxan is thought to be the less reliable of the two, as it is labile at room temperature and late reversion of alloxan diabetic animals to normal has been reported. Nonetheless both these agents have been very important tools in the evaluation of the efficacy of β cell transplantation.

TRANSPLANTATION OF THE PANCREAS

The role of pancreatic islets in the pathogenesis of diabetes was under active investigation by the late nineteenth century. In 1889 Von Mering and Minkowski first demonstrated that hyperglycemia followed pancreatectomy (11). In 1892 Hendon reported a technique of placing a vascularized autograft of a segment of the pancreas in the subcutaneous tissues of dogs (12). When the remaining nontransplanted pancreas tissue was excised, the dogs did not develop hyperglycemia. In light of such observations Ssobolew first proposed pancreatic transplantation as a cure for diabetes in 1902 (13).

Attempts at pancreatic transplantation for the cure of diabetes have involved the use of the whole gland or portions of the pancreas, with or without direct vascular anastomosis between the graft and the recipient's vascular system. In addition, pancreas fragments and isolated islets of Langerhans have been transplanted.

TRANSPLANTATION OF THE PANCREAS WITH VASCULAR ANASTOMOSES IN EXPERIMENTAL ANIMALS

Transplantation of the entire pancreas or portions of the pancreas with vascular anastomoses to the recipient arterial and venous system have been described for many years. The recipient animals were usually rendered diabetic by pancreatectomy or the administration of alloxan. Most investigators described only a modest survival rate in homotransplant recipients. Some of those animals surviving transplant have demonstrated endocrine function of the graft for varying short periods of time. In 1966 Largiader reported transplantation of the entire pancreas into unrelated dogs (14). The pancreas and duodenum were transplanted enbloc and the duodenum was drained into the gallbladder. Approximatley 50 percent of the animals succumbed immediately postoperatively; the remainder survived only four to nine days. These short survivors maintained normoglycemia until death. Unfortunately, no circulating insulin levels were measured. In a series reported by Merkel, one-half of surviving canine whole pancreas recipients had evidence of

functioning endocrine tissue. This was indicated by near normal serum glucose levels and elevated circulating insulin levels when compared to diabetic controls (15). In another set of experiments, 33 percent of those diabetic dogs receiving pancreatic allografts were normoglycemic for a period up to 32 days (16). Some of these dogs demonstrated significant increases in insulin secretion rate when challenged with a glucose tolerance test.

These homotransplantation studies in dogs unfortunately combine problems of tissue rejection with the technical challenges posed by such surgery. A study of Aquino et al., using an auto transplant model (thereby eliminating tissue incompatibility phenomena), demonstrated that the surgical problems caused a significant percentage of failures (17). Only 2 of 12 dogs survived longer than 17 days. Mortality of the remaining dogs was due to vascular thrombosis, anastomotic leaks, and intercurrent infection (see below). Studies in inbred strains of rats have also been designed to circumvent tissue incompatibility complications. The microsurgery necessitated by the caliber of rat blood vessels is difficult, and a 48-hour postoperative mortality rate of 60 percent has been described for pancreas transplantation (18). A few inbred rat pancreas recipients did survive and were normoglycemic. Resection of the transplanted graft was followed by hyperglycemia. In a recent study Lee et al. described isologous pancreato-duodenal transplants in inbred Lewis rats using a new microsurgical technique (20). None of the diabetic rat isograft recipients developed mean blood glucose levels greater than 200 mg percent. Diabetic control levels were 300 to 400 mg percent; normal mean values were less than 150 mg percent. However, no survival statistics were reported.

It is evident that such statistics are presently unacceptable if human transplantation is to be considered. There are a number of complications attendant to the transplantation of the pancreas in vascular continuity with the recipient which contribute to these disappointing results:

A. Exocrine Function. The exocrine portion of the pancreas comprises well over 90 percent of the gland by weight. Although the acinar tissue does not contribute factors thought to influence significantly the course of diabetes in the recipient, secretions from the exocrine pancreas pose formidable technical problems. Early attempts to tie off the pancreatic duct at the time of transplantation led to a high incidence of pancreatitis in the graft (15,20). The exocrine secretion problem has been handled in a variety of ways:

1. Transplantation of the pancreas with a segment of duodenum as a drainage condut (14). The duodenum is anastomosed with the recipient small bowel or brought to the skin. Such procedures have reduced the incidence of pancreatitis although the duodenum has proved to be particularly susceptible to rejection, and disruption of the duodenal stump is frequently described.
2. DL Ethionine has been employed in an attempt to suppress acinar activity of the graft without significant salutory effect (18).
3. Donor pancreatic duct ligation six weeks prior to grafting has been shown to cause fibrosis of the acinar tissue and result in a decrease in exocrine secretion (18). Using this technique Reemtsma et al. found that they could increase the percentage of canine grafts which function but could not increase the length of graft survival.
4. Four hundred to 5,000 rad roentgen therapy will cause a decrease in the exocrine function of the gland as a result of selective injury to the acinar tissue. Use of this technic

in conjunction with previous duct ligation was felt to reduce the incidence of complications secondary to exocrine pancreas function in dogs (15).

B. Tissue Incompatiblity-Rejection. Various immunosuppression regimen have been employed in an effort to increase the duration of transplanted allograft pancreatic function. Antilymphocyte serum had a modest effect in one series, increasing average graft survival from 10 to 15 days in dogs (15). Sixmercaptopurine was ineffective in increasing functional survival of transplants in dogs. Azothiaprine increased functional survival from 5 to 15 days in dogs (18). Azothiaprine and postoperative radiation together increased survival from 5 to 20 days. To date, no immunosuppressive regimen has been discovered which consistently provides long-term protection of pancreatic allografts in experimental animals.

C. Thrombosis. One of the most common causes of pancreatic graft failure has been vascular thrombosis (16,17,22,23). Although various anastomotic tricks have been proposed, even recent reports of pancreas transplantation have emphasized this continuing problem. Merkel et al. did, however, demonstrate a decreased incidence of both arterial and venous thrombosis when the pancreatic graft was interposed into the iliac circulation so that blood going to and from the leg must pass through the graft (15).

D. Miscellaneous. Small bowel obstruction, intusseception, and infection (commonly pulmonary or operative site) accounted for other postoperative deaths in pancreatic transplant recipients (24).

Considerable emphasis has been made concerning the manner in which the pancreatic arterial and venous connections have been made. Physiologically, the normal pancreas is perfused with arterial blood from the aorta, and its venous drainage contributes to the portal vein. Thus, pancreatic vascular effluent first passes through the liver before reaching the systemic circulation. The circulating arterial level of insulin secretagogues (glucose, amino acids, fatty acids, etc.) determine insulin release from the cells. Released insulin first reaches the liver, where a large percentage of the hormone is usually cleared. The physiological significance of hepatic insulin clearance and the effect of insulin on the liver is not clearly understood.

Pancreatic grafts may be inserted either heterotopically or orthotopically. In the heterotopic graft pancreatic venous blood drains into a systemic vein and therefore by-passes the liver. One would anticipate such a circulatory arrangement might yield abnormal systemic insulin and glucagon levels. Orthotopic grafts drain into the portal system. Hence the delivery of insulin and glucagon to the liver is physiologic. In an interesting study in pigs, Sells et al. (24) compared some metabolic effects of orthotopic and heterotopic transplants. Orthotopic graft recipients had normal glucose tolerance curves and raised plasma insulin levels. The glucose tolerance curves of the heterotopic graft recipients demonstrated significant hypoglycemia and marked hyperinsulinemia. The hyperinsulinemia in heterotopic transplants may be due to diversion of pancreatic venous effluent from the liver. The more modest increase in insulin levels in the orthotopic transplant may reflect pancreatic denervation.

In any case, one might argue a priori, that orthotopic pancreatic transplantation much more closely approximates the normal physiologic arrangement in animals. It has been stated that one major drawback to the administration of exogenous insulin in clinical settings is that the hormone is delivered into the systemic circulation, which does not mimic the normal physiologic situation.

TRANSPLANTATION OF PANCREAS IN MAN

Despite the relatively disappointing results of animal experiments, a small number of pancreas transplants have been attempted in man. Lillehei's group in Minneapolis first transplanted two patients in 1966. The gland with associated duodenum was placed in the iliac fossa and the vascular anastomoses were heterotopic in nature. The first patient graft survived six days, the second functioned for five months (25). In all, 36 human pancreas allografts have been done in the world. Four of these patients went 10, 12, 22, and 30 months with functioning grafts (26). One patient who received a cadaver pancreas in June 1972 remains free of the need for exogenous insulin. In addition, one patient died of accidental causes ten months after transplantation with a functioning gland and no histological evidence of rejection at necropsy (29). Indications for pancreas grafting in man have been severe diabetic complications such as terminal nephropathy, retinopathy, neuropathy, and coronary artery disease. Contributing to graft failure were factors similar to those reported in experimental animals. They have been handled in the following manner:

A. Exocrine Function

1. Duct ligation. Leakage from the pancreas for which no exocrine drainage route had been provided has necessitated reoperation in the two patients receiving such a graft (27). Gliedman concluded that duct ligation is ill-advised.
2. Drainage of exocrine secretion by duodenal conduit. Drainage of exocrine secretion by anastomosing the duodenum to the recipient's small intestine in a Roux en Y fashion has been advocated. Unfortunately, both stump leaks and duodenal rejection have been commonly described (28). It appears that the duodenum is more sensitive to rejection than the pancreas (28). In at least two instances grafts had to be removed because of duodenal rejection even though the pancreas was functioning and appeared normal.
3. Pancreatic duct anastomosis to ureter. Gleidmen et al. have advocated a different pancreatic drainage technique in order to obviate those complications attendant to pancreato-duodenal transplantation (22). The utilization of direct uretero-pancreatic duct anastomosis has provided exocrine drainage, eliminated the need for co-transplantation of the duodenum and allowed the procedure to be performed outside the peritoneal cavity. In addition, pancreatic exocrine status can be assayed directly by measuring the urinary amylase concentration. In a small series of patients, this approach seems to have decreased the incidence of complications attributable to exocrine function of the pancreas.

B. Rejection. Many of the patients receiving pancreas transplants have had histories of renal failure secondary to diabetic nephropathy. For this reason most pancreas transplant recipients have received coincident renal allografts. Although the clincal experience is very limited and the numbers involved are small, concern that the pancreatic graft in some way elicits a more potent rejection (either to itself or to the renal allograft) has recently been voiced (27). Accordingly, it has been proposed that renal and pancreatic allografting be performed asynchronously, i.e., pancreas first. Using this procedure, Gleidman has improved results in terms of both number of grafts surviving and length of survival. The small number of patients in this series makes any conclusion about this phenomenon tenuous.

Immunosuppression regimen commonly employed included azothiaprine, antilympocyte globulin, and prednisone. The use of large doses of steroids in the pancreatic graft recipient considerably

obscures the relationship between graft function (i.e., insulin secretion) and blood glucose levels. So-called "steroid diabetes" has been reported in a number of patients.

C. Vascular Complications. Many graft recipients have been reoperated upon for sudden disruption of the vascular anastomoses resulting in serious bleeding (28).

In summary, then, only a small number of patients have been subjected to pancreatic transplantation. No patients have survived longer than three years; few have had prolonged reduction in their daily insulin requirement. Little data have been collected concerning the course of diabetic vascular disease in these patients.

TRANSPLANTATION OF PANCREAS FRAGMENTS OR FETAL PANCREAS IN EXPERIMENTAL ANIMALS

Various attempts have been made to avoid the complications caused by the vascular anastomoses and pancreatic exocrine secretion in whole pancreas transplants. An early approach was the transplantation of fetal rat pancreas fragments. Two observations made by Gonet and Renold (31) were encouraging. First, the exocrine portion of the transplanted pancreas was noted to degenerate when the minced fetal rat pancreas was placed in the testes. Second, approximately 20 percent of the homografts (using inbred Wistar rats) demonstrated histologic evidence of endocrine proliferation. Furthermore, immunoreactive insulin could be extracted from the grafts. These testicular grafts were thought to represent a greater tissue volume and hence the delivery of a greater endocrine cell dose to the recipient than previously described grafts which had been placed in the hamster cheek pouch or in the anterior chamber of the eye (32,33). In approximately 20 percent of the grafts to alloxan diabetic recipient rats, Gonet and Renold (31) noted a prolonged decrease in glycosuria and blood glucose. Interestingly, these same animals had the best preserved islet population when examined histologically. Further description of transplanted fetal pancreas histology was reported by Hegre et al. Portions of pancreas from fetal rats with normal or alloxan diabetic mothers were grown on organ culture for four days prior to transplantation to one of two different sites in the mother, into the anterior chamber of the eye or under the capsule of the kidney. The grafts were examined four and ten days after transplantation. Following transplantation into normal mothers, the β cells were noted to be granulated in both recipient sites. The tissue transplanted into diabetic mothers exhibited degranulation of the β cells. These data were interpreted as demonstrating that β cells from the fetal rat pancreas were capable of surviving for four days in organ culture and were able to maintain their functional integrity. Degranulation of the cells when transplanted into the diabetic mother, indicated that the cells were responding to hyperglycemia.

Another interesting histological observation has been reported by Coupland (35). Not only did transplanted fetal pancreas fragments undergo a degeneration of exocrine cells, but activation of the duct epithelial cells occurs. These cells show mitosis and form new islets of Langerhans. A similar observation has been made by Hultquist (36) when fragments from rat pancreas with a previously ligated pancreatic duct was transplanted into the anterior chamber of the eye.

Despite numerous reports detailing the histological evidence of the function of pancreas fragment grafts to the eye, cheek pouch, or beneath the kidney capsule, few authors have addressed themselves to assessing the physiologic response of a diabetic animal to such a graft. Brown et al. (37), however, have recently examined the success achieved in transplanting fetal

rat pancreata into diabetic syngenic recipients. Using a streptozotocin-induced diabetic recipient Lewis rat, this group placed two to three pancreata of fetal age 15 to 18½ days beneath the kidney capsule. The rats were treated with insulin for eight days post-transplant. Success of the graft was evaluated by urine glucose, urine volume, and serum glucose after insulin therapy had been discontinued. Sixty-four percent of the grafts resulted in complete or partial ameloriation of the diabetic state for up to 165 days. When the transplants were removed at 42 days from one group of rats, urine glucose and volumes rapidly increased, as did serum glucose. Interestingly, in 10 to 20 percent of the animals, diabetes did not return when the graft was excised, emphasizing the importance of adequate controls in judging the effects of "hormonal" transplants (38). Histological examination of the graft revealed a multilobed organ beneath the kidney capsule comprised of mostly adipose tissue. No exocrine tissue was seen, and β cells appeared normal on electron micrographs. Unfortunately, no evidence was presented which indicated the response of these animals to glucose tolerance testing. Information concerning circulating insulin or glucagon levels was not reported.

There have been only scattered reports of transplantation of fragments of pancreas in man. The most recent attempt was described in 1970 (39). A portion of a β cell tumor removed from a middle-aged woman was transplanted beneath the fascia lata in a 17-year-old juvenile diabetic. Preoperatively, the recipient had required approximately 300 units of insulin a day. One week postoperatively, the patient was aglycosuric for the first time. He was maintained on decreasing amounts of insulin, immuran, and a steroid preparation. At three months the patient required 200U of insulin daily. At that time, biopsy of the graft demonstrated granulated β cells. Nine months after grafting, a similar biopsy revealed no β cells. Despite this finding, the patient only required 125U of insulin per day. A decreasing insulin requirement in the face of histologic evidence that the graft was not functioning implies that insulin requirement is an imprecise parameter for estimating functioning β cell mass. Furthermore, it is difficult to conclude that this transplant effectively altered the course of the patient's diabetes.

ISOLATION OF ISLETS

The major stumbling blocks in the transplantation efforts described above are all related to an awkward anatomical reality: Pancreatic islets are distributed throughout a gland the volume of which is many times greater than that of the islets themselves. Most of the complications plaguing transplantation efforts are, then, direct corollaries of the fact that in order to transplant functioning endocrine tissue, which amounts to 2 percent of the pancreatic tissue mass, the remaining 98 percent, the troublesome and metabolically unimportant exocrine tissue, must likewise be foisted upon the recipient. Although minced fetal pancreas tissue had been employed in an attempt to increase the relative concentration of insulin secreting tissue in the graft, the obvious objective was to transplant islets which had been isolated from all acinar tissue. Furthermore, since islets had appeared relatively undamaged in rejected whole organs (40) it was hoped that they might be relatively protected from the ravages of tissue rejection.

The hope for isolating viable intact islets of Langerhans from the pancreas lay dormat until Moskalewski (41) described collagenase digestion of the pancreas, and Lacy and Kostianovsky perfected the method in 1967 (42). This more advanced technique entailed distention of the pancreas by the injection of Hanks solution under pressure into the common bile duct. The pancreas was then excised, finely minced, and digested with collagenase. Individual islets were then readily

visible in the dissecting microscope, and they were collected with a glass loop. Islets isolated in such a fashion appeared intact histologically and responded to insulin secretagogues.

A more sensitive method for in vitro assessment of islet functional integrity was reported by Lacy et al. in 1972 (43). This technique is commonly referred to as islet perifusion. The isolated islets are placed on a millipore filter and placed in a small millipore chamber. A balanced Krebs ringers bicarbonate solution is then perfused through the chamber by a Harvard infusion pump. The effluent from the chamber which has bathed the islets is then assayed for insulin. When high concentrations of glucose are added to the perifusion medium, a rapid release of insulin occurs. The release of insulin is characteristically biphasic, there being a sharp first peak of insulin released immediately and a prolonged second phase of release which may last for many hours if the islets are continually stimulated with high concentrations of glucose (Fig. 1). This technique has proved to be very sensitive and islet perifusion has been a critical test for assessment of islet viability. In addition, this method has become a potent basic science investigative tool. One further refinement of islet isolation has increased the yield of islets, decreased the time required to obtain them, and has led to a burgeoning of isolated islet transplantation efforts. This refinement consisted of using a density gradient to separate islets from acinar debris after collagenase digestion. Originally, a sucrose density gradient was proposed (42). Lindall et al. suggested a discontinuous gradient of different concentrations of ficoll, a polymer of sucrose (44). This technique was explored by Scharp et al. who found that increased yields of viable islets were obtained with the method (45). They emphasized the importance of dialysing the ficoll before exposing islets to the polymer. These techniques have allowed the isolation of functionally intact islets of Langerhans from rat pancreata in relatively large numbers.

Once the isolation of islets of Langerhans had been described and these islets had been demonstrated to be intact functionally, the first attempts at transplantation of isolated islet followed soon afterward. The subsequent flurry of transplantation projects has generated a great deal of excitement among researchers, clinicians, and the public alike.

TRANSPLANTATION OF ISOLATED ISLETS IN EXPERIMENTAL ANIMALS

In an early report describing the transplantation of isolated islets, Reemstsma took advantage of the relative ease with which piscine islets could be obtained free of acinar tissue (46). Thus, fish islets were transplanted into rats which had been made diabetic by the administration of streptozotocin. A variety of methods of islet implantation were employed: 1) islets in millipore chambers placed in the peritoneal cavity, 2) islets implanted intramuscularly, and 3) islets inserted into the anterior chamber of the eye. Although a decrease in blood sugar in the diabetic recipient rats was described, this effect was short-lived, generally not lasting for more than four days.

The first major attempt at transplantation of isolated islets from one animal to another of the same species was reported by Ballinger and Lacy in 1972 (47). Using inbred strains of rats, these experiments were not subject to the complications of tissue immunorejection. The recipient animals had been rendered diabetic with streptozotocin. Blood glucose, urine volume, urine glucose, and weight were monitored in both control and experimental animals. The transplantation of 400 to 600 isolated pancreatic islets into the peritoneal cavity or into the thigh muscle of the recipient resulted in a significant long-term reduction of hyperglycemia, polyuria, and glycosuria, and a restoration of weight gain.

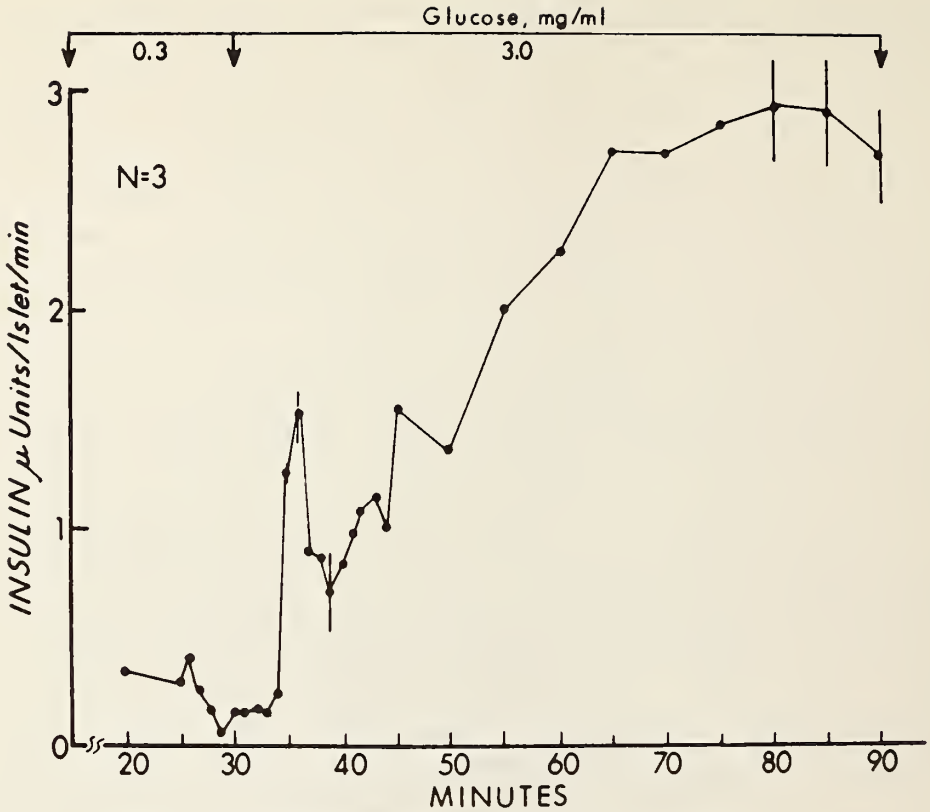

FIGURE 1. Biphasic pattern of insulin secretion following stimulation of perifused rat islets with glucose (3.0 mg/ml). Vertical lines represent S.E.M.. From Lacy, Walker, and Fink. Diabetes 21:987-98, 1972. Reprinted with permission from Diabetes the Journal of the American Diabetes Association.

Although the average urine glucose, urine volume and blood glucose levels were improved in the transplanted group, these values did not approach the normal control levels. Nonetheless, some individual animals did achieve normal control values for two months or more. Excision of islets which had been transplanted into the thigh muscle resulted in a rapid return to the diabetic state. Histologic examination of the excised islets revealed intact α and β cells with a marked degranulation of β cells; indicative of the physiological demand for insulin placed on these cells. This report also detailed preliminary allograft experiments. Islets were transplanted across a major histocompatibility barrier and the recipients received immunosuppression. The animals subjected to this protocol evidenced some amelioration of their diabetes.

These experiments were the first to demonstrate that isolated islets were capable of permanently reversing chemically induced diabetes in experimental animals, and they served to activate islet transplantation interest in other laboratories.

The obese hyperglycemic mouse was used as an experimental animal in a few early studies examining the effects of islet transplantation. Obese mice provide an interesting model, exhibiting a syndrome characterized by obesity, elevated blood glucose levels, and markedly high circulating insulin concentrations. Isolated islets were placed in a millipore diffusion chamber and the chamber was placed in the peritoneal cavity. A chamber pore size of 0.45μ presumably allows

ingress of insulin secretagogues and the egress of insulin and glucagon. When obese mice received normal mouse islets, weight gain stabilized, blood glucose levels fell, and interestingly, a dimunition of insulin levels followed (47). The mice reverted to pretransplant phenotypes when the chamber was removed. These data were interpreted as indicating that the obesity syndrome in the obese mouse was secondary to a defect in a factor elaborated by pancreatic islets which was capable of passing through the millipore diffusion chamber. Gates et al. (50) extended these studies, observing normalization of obese mouse blood glucose and insulin levels for up to 10 weeks. Oral glucose tolerance tests in transplanted animals approached the response seen in normals.

Leonard et al. have performed a series of transplants in rats, using neonatal pancreas which has been finely minced and digested with collagenase although the islets were not separated from remaining acinar tissue (53). Pancreata excised from neonatal rats on days two and a half to four and a half postpartum, had the lowest exocrine enzyme concentration and the highest relative insulin content. Tissue prepared from donors of that age was transplanted into the peritoneal cavity of either semi-inbred (homologous) or highly inbred (isologous) diabetic recipients. Ninety-six percent of the homologous rats had an amelioration of their diabetes for 3 to 13 days, with an average duration of 10 days. Moderately diabetic isologous recipients, on the other hand, all experienced normoglycemia, persisting in some up to five months.

SITE OF IMPLANTATION

There are several important theoretical and practical considerations involved in the selection of an appropriate site for islet administration in the diabetic animal, especially man. Ideally, the site should be accessible with only a minor operative procedure. The organ or tissue into which the islets are placed should be expendable so that should an untoward tissue reaction occur, the graft can be removed. Finally, although the significance of the peculiar vascular arrangement of the pancreas (arterial input, portal vein effluent) is not fully appreciated, it is likely that such a configuration is of physiologic import. The profound effects of insulin on the liver and the remarkable ability of that organ to clear the hormone, imply that the metabolic relationships between normal pancreatic islets and the liver are intimate.

The importance of the site of islet implantation was explored by Kemp et al. (53). They found that 600 to 850 islets placed subcutaneously had no significant effect on the urine glucose, urine volume, or blood sugar of diabetic isologous rat recipients. A similar number of islets transplanted into the peritoneal cavity resulted in amelioration of the diabetic state, but none of the parameters examined reverted to normal values. When an equal number of islets were injected into the portal vein, the results were more dramatic. The diabetic animals achieved normal urine volumes and blood glucose levels (Figs. 2 and 3). Histologic examination of the recipient liver revealed intact, vascularized islets lodged in the terminal portal tracts.

The selective ability of islets injected into the portal vein to reverse chemically induced diabetes in the rat in these studies is a provocative observation. It is clear that whatever factors operate in this experimental situation to allow islets lodged in the portal tract to revert the diabetic animal to normal must be investigated further. Questions deserving investigation include: 1) Do a greater percentage of islets survive in the liver than other sites? 2) Is β cell replication more pronounced in the liver? 3) Do portal tract administered islets release more insulin (less glucagon) than islets transplanted to the peritoneal cavity or subcutaneous tissues? 4) What, exactly, is the significance of insulin delivered in the portal

circulation versus that in the systemic circulation? 5) How does the liver respond to islets lodged in its parenchyma? 6) How, if at all, are islets innervated in the various transplantation sites? 7) What is the exact nature of the transplanted islet's blood supply once lodged in the liver? and 8) What effect does portal blood have on insulin secretion? Presumably portal blood should be rich in absorbed foodstuffs and thus present higher levels of insulin secretogogues to the islets lodged in the liver.

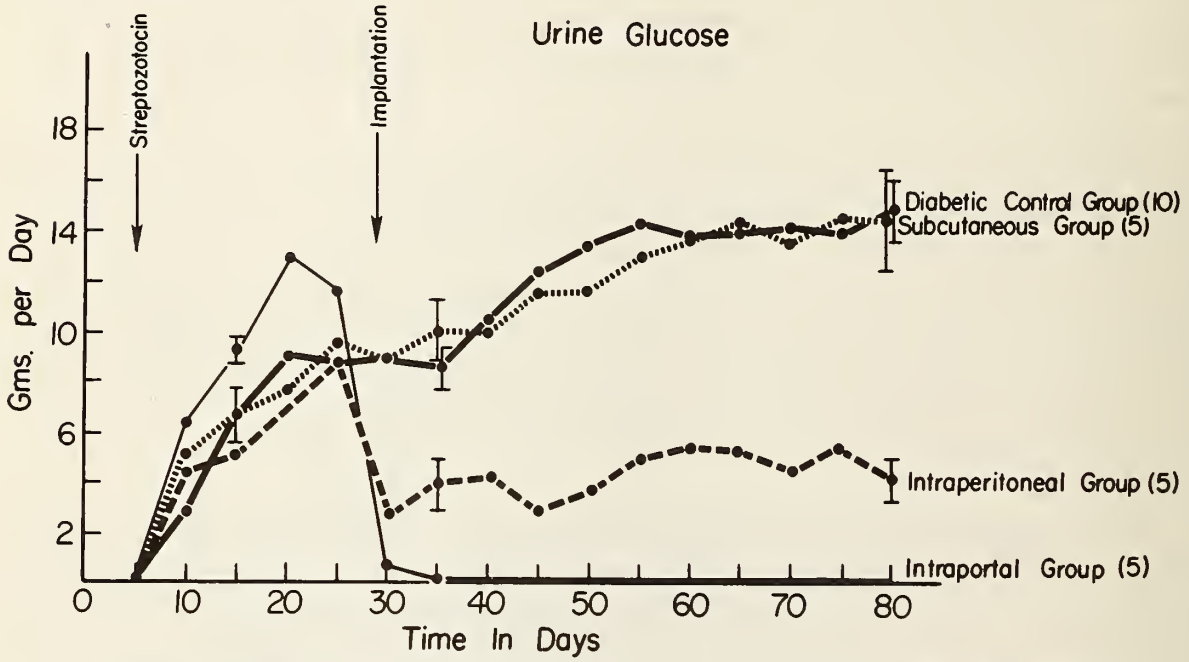

FIGURE 2. Effect of portal vein, subcutaneous, and intraperitoneal implantation of pancreatic islets on the urine glucose of diabetic rats. Diabetic controls. From Kemp, Knight, Scharp, Ballinger, and Lacy. Diabetologia 9:486-491, 1973. Reprinted with permission from Springer-Verlag.

IMMUNOSUPPRESSION

There have been conflicting reports on the relative ability of endocrine tissue to provoke transplantation immunorejection. Reckard et al. (51) examined the fate of isolated islets transplanted into rats which had a major histocompatability difference from the donors. Using a streptozotocon-induced diabetic recipient and administering 600 to 1200 islets into the peritoneal cavity, these investigators found that the islets did indeed provoke rejection. In fact, several other interesting observations were made:

1. Islet homografts functioned only one to three days when donor and recipient differed by a strong histocompatability (Fischer to ACI). The period of functional survival was not lengthened by a course of immunosuppression (antilymphocyteserum).
2. Islet homografts compatible at the AgB locus (ACI to DA) resulted in normoglycemia of the recipient for a mean of twelve days. Intriguingly, antilymplocyte serum had a pronounced effect on these animals, extending the median functional islet survival to 30.5 days.
3. To determine if the recipients of homologous islets had become sensitized to donor tissue, six Fischer rat recipients of 800 to 1200 AgB incompatible Lewis islets were challenged with donor skin grafts. These grafts were rejected in an accelerated manner, indicating that homologous islets did indeed stimulate the host immune system. By

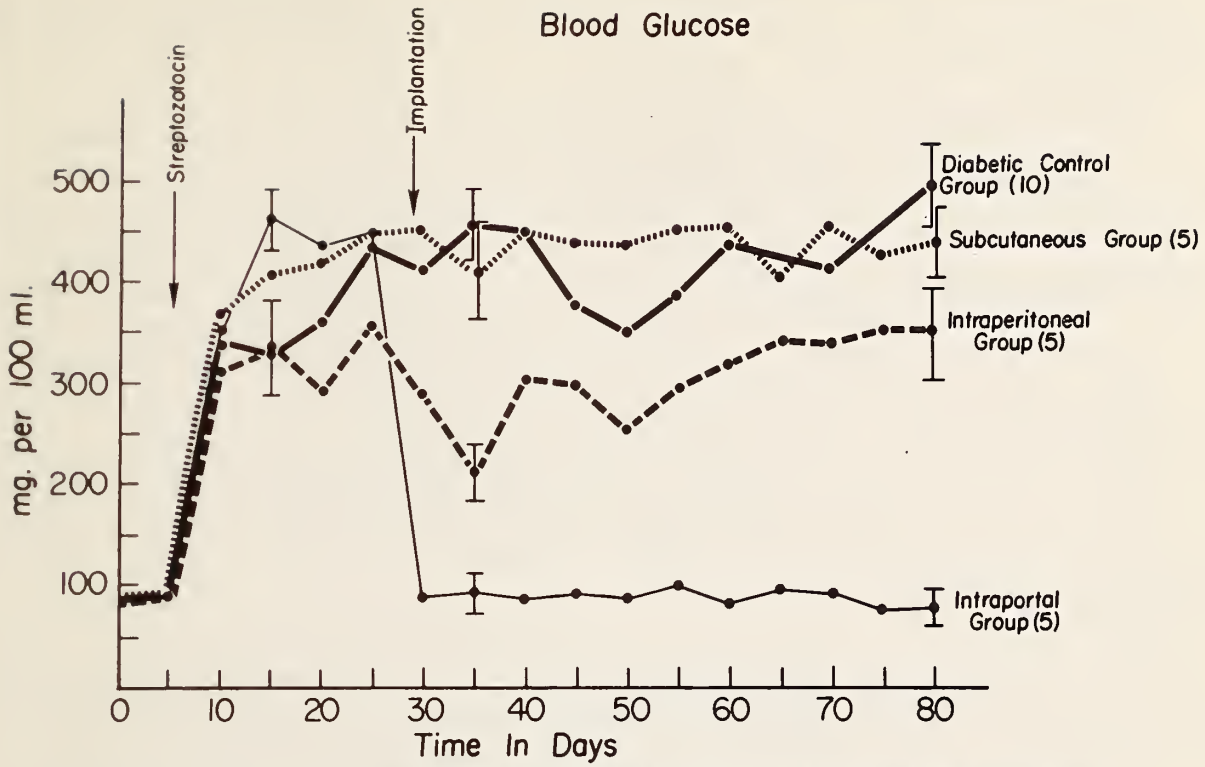

FIGURE 3. Effect of portal vein, subcutaneous, and intraperitoneal implantation of pancreatic islets on the blood glucose of diabetic rats. Diabetic controls. From Kemp, Knight, Scharp, Ballinger, and Lacy. Diabetologia 9:486-491, 1973. Reprinted with permission from Springer-Verlag.

contrasting the period required for tissue rejection to become manifest, the authors concluded that islet tissue is at least as antigenic as skin or heart. It should be noted, however, that there was no attempt to control the dose of islets administered in comparison to amount of skin or heart transplanted. Furthermore, the animals in the study were selected without regard to sex. It appears that there may indeed be a difference between males and females in regard to their ability to mount an immunorejection response. Finally, the authors have employed a physiologic end point to determine islet viability (i.e., the ability to secrete insulin) whereas they have used an anatomic end point to assess skin graft survival.

EFFECT OF ISLET TRANSPLANTATION ON THE COMPLICATIONS OF DIABETES

Some preliminary evidence concerning the effect of islet transplantation on the secondary complications of diabetes has been gathered. Sutherland et al. (54) have described renal glomerular lesions in rats six months after induction of diabetes characterized by immunoglubulin and complement deposition in the glomerular measangium. This is followed by mesangial matrix thickening. In diabetic rats which had received intra-peritoneal transplantation of isologous neonatal pancreatic islet tissue, plasma glucose levels were significantly lower and several serial biopsies showed progressive decrease in mesangial immunoflourescent staining for IgG, IgM, and B_1C four to nine weeks after transplant only traces of the above could be detected. Mesangial matrix thickening was arrested or actually was reduced. The relationship between mesangial deposition of complement and immoglobulin and streptozotocin induced diabetes is not

clear. The alteration of these lesions by islet transplantation, although intriguing, cannot be interpreted easily.

TRANSPLANTATION OF ISOLATED ISLETS IN OTHER ANIMALS

Islet transplantation in man will pose two problems not addressed by the model defined in the rat. The pancreas in the human is compact and fibrous as compared to the readily distensible gland in the rat. In addition, inbred strains of humans do not exist. To examine these problems, preliminary work has been reported in animal models thought to be more relevant to the problems posed by human islet transplantation. The isolation of islets and the transplantation of same in both pig and monkey have been described. Although the more compact pancreas of the pig and the monkey pose formidable islet isolation problems, some of the challenges have been met. Nonetheless, the in vitro assay of these isolated islets had not been as impressive as those described for rodents. A great deal of work remains to be done. Transplantation of these islet preparations has also been described. Again, the results are less impressive than the results described in the rat. Here, too, many more experiments will be required before these higher animal models of islet transplantation are fully developed.

TRANSPLANTATION OF ISOLATED ISLETS IN MAN

Human islets have been successfully isolated from recently expired donors or from operative specimens. The compact fibrous nature of the human pancreas makes this feat much more difficult than isolating islets from the diffuse, easily distended rat pancreas. Human isolated islets have been shown to release insulin when exposed to high concentrations of glucose in a perifusion system. Isolated islets have been transplanted into the peritoneal cavity of at least one diabetic patient by the Minnesota group (57). Unfortunately, no long-term salutory effect was documented in the one patient described.

It is clear that a great deal of intensive investigation needs to be done before routine islet transplantation in man is a clinical reality. A number of the questions remaining to be answered might well be worked out in animal models before further clinical experimentation takes place. A list of such questions might include:

1. What number of islets needs to be transplanted to achieve normalization of either spontaneous or chemically induced diabetes?
2. Which recipient site of transplantation maximizes the chance of islet survival and replication, yet minimizes the likelihood of tissue rejection?
3. What is the most appropriate assay of islet viability, such that only live functioning islets will be transplanted?
4. What various types of islet preservation will prove feasible? It is clear that islet tissue may become available at a site far removed from the prospective recipient. Methods of storage and preservation must be developed. Furthermore, pooled islets from more than one donor may be required to successfully transplant one recipient. Two types of preservation are now under active investigation:

a) Cryopreservation. The ability to store certain blood elements at very low temperatures has stimulated investigation of cold storage of isolated islets. Islets have been stored in Hanks solution at 4°C for up to 48 hours with some success (58). Knight has preserved islets in DMSO at -180°C for up to 14 days with maintenance of islet functional integrity as assayed

both by perifusion and transplantation. The appropriate storage media, methods of cooling and thawing must be further explored, as successful cryopreservation will most likely be a cornerstone of clinically applicable islet transplantation.

b) Tissue Culture. Tissue culture has been the other major approach to islet preservation. This is for several reasons. First, it is hoped that islets might be stored for long periods of time without loss of functional integrity or dimunition of numbers. Second, some optimism has been expressed that islets on tissue culture may replicate, hence increasing the original islet yield from a donor pancreas. Further, it had been postulated that tissue maintained in organ culture might lose those antigenic determinants which provoke host versus graft tissue rejection (the so-called Summerlin hypothesis).

Some preliminary data have been collected which bear upon the above postulates. Islets have been maintained in tissue culture successfully by many groups (38). They maintain their ability to synthesize proinsulin and insulin and will release the latter in response to glucose stimulation. Acinar contamination of isolated islets seems not to survive in tissue culture. Although in vitro culture of the whole fetal rat pancreas by Hegre et al. (38) demonstrates a relative increase in islet volume in comparison to exocrine tissue, conclusions about the ability of isolated islets to replicate in cell culture are difficult to make. Some of the most elegant work on culturing of islet tissue has been described by Chick (38). Monolayer cultures of dispersed cells from the neonatal rat pancreas have been carried out. Cells cultured in such a fashion respond to glucose by releasing insulin and they have maintained this ability for a period of one year. Furthermore, it is thought that these cells actively replicate, although this process is ultimately inhibited by fibroblast overgrowth.

5. What type of immunosuppression will be best suited for discouraging immunologic response to transplanted islets? Although a course of antilymphocytic globulin has proved to be the most efficacious immunosuppression regimen in preliminary allograft studies in rodents (51), little systematic evaluation of immunosuppression protocols has been described.

Although transplantation of insulin producing tissues as a treatment of diabetes mellitus was proposed almost three quarters of a century ago, the hint that such an approach might be clinically relevant has come within the past decade. Recently, experimental isolated islet transplantation has developed rapidly, making it the most likely approach to prove useful for human diabetics. Yet despite the rapid growth of accumulated information about isolated islet transplantation, there remains a great body of knowledge still to be collected. The scientific community has learned that premature emphasis of transplantation of certain organs ultimately retards progress and takes lives. The decision to intervene in the course of a diabetic patient will generate even greater agony than the decision to transplant for a failing heart. Furthermore, it seems apparent that insulin-producing tissue will be subject to all the manifestations of immunorejection seen with other transplanted organs.

Yet the notion of definitive treatment of the diabetic patient is a glamorous and very important goal. The rationale for the transplantation of insulin secreting tissues is to see whether functioning normal β cells will prevent or arrest the progression of the complications of diabetes. It is imperative that detailed studies be carried out on the tissue transplanted and the effect it has upon the recipients. Longitudinal studies designed to assess the efficacy of the transplant over a period of years will be central to our understanding of this approach to the treatment of

diabetes. A final benefit of exploring islet transplantation is the great bulk of basic science information generated. This information and experience may ultimately prove to be the most meaningful of all.

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22

DIET AND DIABETES MELLITUS

Ronald K. Kalkhoff

Elsewhere in this monograph a variety of experimental and clinical evidence has been assembled that stresses the importance of controlled metabolism in the diabetic patient. Obviously, a most important means of good clinical management is strict adherence to an acceptable dietary regimen. In the following discussion, the nutritional aspects of diabetes mellitus are related to its incidence, control, and potential complications. Controversies in various research areas are delineated, and suggestions are made regarding the need for additional studies. Finally, the practical problems of diet and diabetes that confront physician and patient are emphasized and possible solutions to them are offered.

THE IMPACT OF NUTRITION ON DIABETES PREVALENCE

There is abundant evidence suggesting that average caloric intakes of a given population have a substantial influence on the prevalence of diabetes mellitus. Thus, in underdeveloped countries where total caloric intake is reduced below the average of more affluent nations of the world, the incidence of the disease has been less in many instances (20).

Extremes of malnutrition provide greater support for this point. During the latter portions of World War II, countries devastated by war developed food shortages even to the point of death from starvation. While these events were very unfortunate, epidemiologists recognized a concomitant fall in the incidence of clinically symptomatic, diagnosable diabetes mellitus. In Japan, for example, newly discovered cases of diabetes in one major clinic were reduced in number nearly threefold (7). During the next 10 years after the war's conclusion, an increase in food supplies correlated well with a proportional increase in diabetes prevalence (Fig. 1).

At the other extreme of malnutrition, i.e., obesity, one sees the opposite effects on diabetes prevalence occurring. Approximately 50 percent of obese, middle-aged adults exhibit some form of carbohydrate intolerance, and about 40 percent of all adult-onset diabetic subject are obese. The higher incidence of diabetes among more affluent populations generally has been shown to relate best to the prevalence of overweight, obese states.

All of these observations suggest that overnutrition unmasks the diabetic syndrome, whereas normal or restricted food intake has some protective effect on diabetes-prone individuals. Unfortunately, these observations have gone unheeded, since the prevalence of obesity is steadily rising in the United States and other advanced countries each year (18).

INFLUENCE OF DIET COMPOSITION ON DIABETES PREVALENCE

Although it is generally agreed that caloric excess and obesity increase the prevalence of diabetes, there is much controversy about the influence of dietary composition on this parameter.

Himsworth (9) proposed that diets high in fat content were chiefly responsible for the higher incidence of diabetes in certain populations. However, the American Eskimo, who subsists on a high fat diet, has a low incidence of the disease. West and Kalbfleisch (20) have shown that in rural Uruguay, where a high fat-protein diet is common, the incidence of diabetes is quite low, whereas

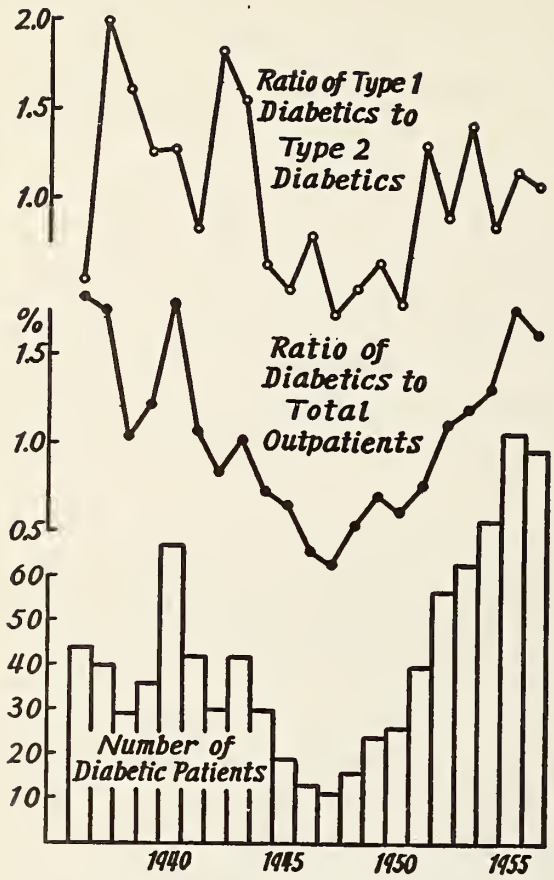

FIGURE 1. Fluctuation in number of diabetic patients, ratio of diabetics to total outpatients and ratio of type 1 diabetics (mild and obese) to type 2 diabetics (severe and thin) during 1936 through 1956. Pacific war: Dec. 1941 to Aug. 1945. From Goto et al. 1958. Reprinted with permission from *Diabetes*, the Journal of the American Diabetes Association.

in urban Uruguay, where composition of diets among relatives of the rural group is similar but obesity is more common, there is a higher frequency of the disease.

Nutritionists also have examined the possible relationship between carbohydrate consumption and diabetes. Yudkin (21) concluded that Himsworth's statistical association of diabetes and vascular disease with the degree of fat intake did not take into account concomitant increases in dietary consumption of refined sugar in these same populations (Fig. 2). His epidemiologic data for 22 countries demonstrated a highly significant relationship between the amount of sugar consumed and mortality rates due to diabetes. Campbell (4) in his analysis of various ethnic groups, reached similar conclusions and particularly stressed the increased use of refined sugar as having a causative role in the dietary aggravation of diabetes. A general epidemiologic study of diet and diabetes in Central and South American countries uncovered a similar trend between sugar intake and diabetes prevalence, but on a statistical basis, this could not be proven (20).

Not all published reports favor the sucrose-diabetes relationship. Among five geographic areas in Trinidad, the incidence of diabetes was lowest in those districts with the highest annual per capita consumption of refined sugar (13). Moreover, nutritional studies of Pima Indians in Arizona, whose prevalence of diabetes is 10 to 15 times greater than the general

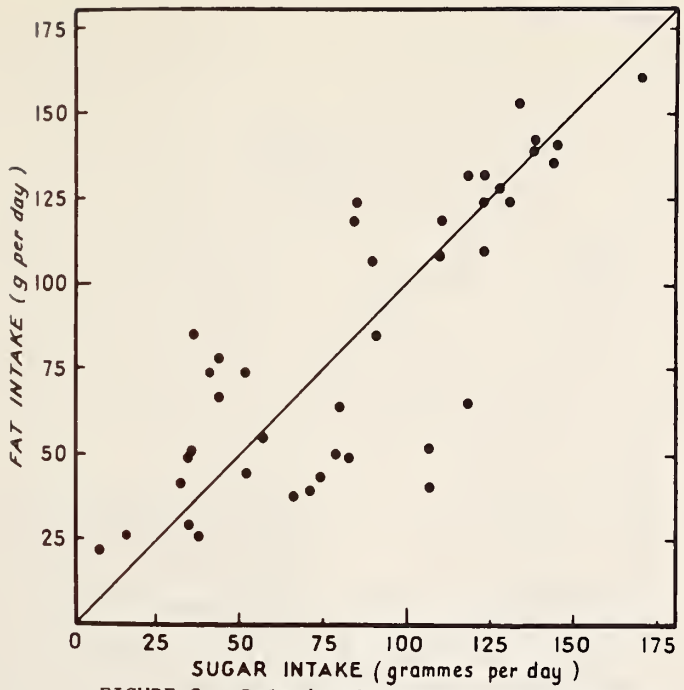

FIGURE 2. Relation between average fat intake and average sugar intake in 41 countries. From Yudkin 1964. Reprinted with permission from *Lancet*.

population in the United States, also do not support the view that selected nutrients profoundly influence the prevalence of this disease. Detailed dietary histories of 248 or over 80 percent of the young adult female population in this tribe compared food intake of 169 nondiabetic and 79 diabetic subjects. Although the diabetic women were significantly more obese, their total carbohydrate and sucrose intakes were significantly less than corresponding intakes of the nondiabetic group (14).

It would seem from these studies that the relationship between the distribution of carbohydrate, fat, and protein in a given diet and the prevalence of diabetes remains highly controversial and unsettled.

EFFECTS OF DIET ON PREEXISTING DIABETES IN THE OBESE

Clinical investigations have provided further insight into the greater prevalence of diabetes among overweight individuals. In both the fasting and postprandial state, the maturity-onset diabetic individual has higher plasma insulin concentrations than the nonobese, diabetic subject. This suggests that insulin is less effective in controlling glucose levels when obesity is superimposed on diabetes. There are several reports demonstrating that with weight loss, carbohydrate tolerance improves despite lower insulin concentrations in thinned obese, indicating an amelioration of the resistance to endogenous insulin and increased efficiency of hormonal action (10), (Fig. 3).

Disturbances in circulating lipids including triglyceride and cholesterol, also are frequently improved with weight reduction (6). It is also reported that at least a partial correlation between plasma insulin concentrations, serum glucose, and serum triglyceride levels exists in obesity (1). The restoration of insulin to normal levels after achieving ideal body weight may also serve to improve blood lipid as well as blood glucose abnormalities.

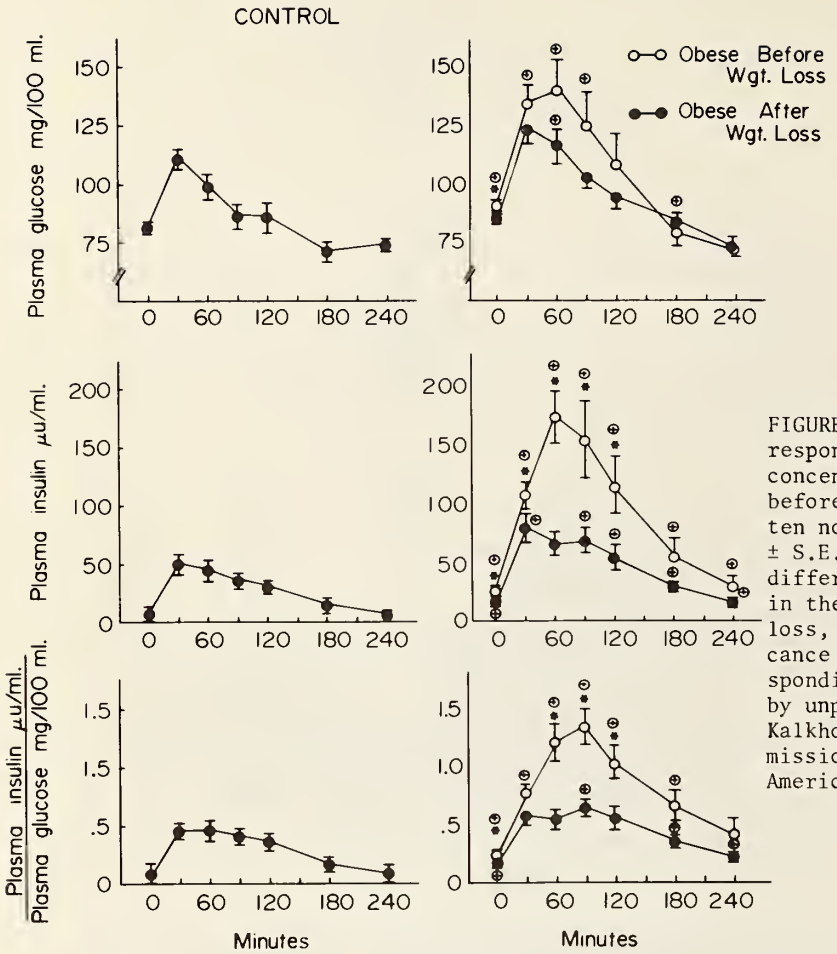

FIGURE 3. Plasma glucose and insulin responses and insulin-glucose (I/G) concentration ratios in six obese patients before and after weight reduction and in ten nonobese patients. Values are mean ± S.E.M. Asterisk: significance of the differences between corresponding means in the obese group before and after weight loss, $p < 0.05$. Plus sign (+): significance of the differences between corresponding means of obese and control subjects by unpaired data analysis, $p < 0.05$. From Kalkhoff et al. 1971. Reprinted with permission from *Diabetes*: The Journal of the American Diabetes Association.

It is also of interest that several animal models illustrate the antagonistic effect of obesity on diabetes. For example, in the New Zealand obese mouse, an animal that is genetically prone to obesity and carbohydrate intolerance, overeating and weight gain promote the development of diabetes, as well as elevated basal insulin concentrations in the blood. Caloric restrictions and avoidance of weight gain effectively obviate these complications.

Thus, a major principle in diabetes management is prevention of obesity and aggressively treating overweight diabetic subjects with reasonable caloric restriction until ideal body weight is achieved.

DIET AND THE NONOBESE DIABETIC SUBJECT

Concepts concerning the ideal diet for diabetic patients who are nonobese are constantly changing. Because a basic problem is control of high blood sugar concentrations, it was recommended for several years to restrict carbohydrate intake to a moderate extent.

The formulation recommended by the Council on Foods and Nutrition in 1958 included total calories of 30-35 calories per kilogram body weight for middle-aged, nonobese adults. Calories

derived from carbohydrate and fat were each 40 percent of total intake, concentrated sweets were restricted, and a substantial portion of fat calories were to be ingested in the polyunsaturated form. The remaining 20 percent of calories was to be derived from high quality protein foods to insure at least one gram of protein per kilogram body weight each day. Since carbohydrate constitutes 45 to 50 percent of calories consumed by Americans, this recommendation did not represent a serious departure from eating habits in the United States.

However, in several countries throughout the world, particularly where rice and other grains are the main food staple, daily carbohydrate intake may greatly exceed 50 percent of total consumed calories. Nevertheless, diabetic diets tailored to this type of eating pattern have shown no adverse effects on control of hyperglycemia or insulin requirements even when total daily calories as carbohydrate approached 70 percent. This experience has been shared by several investigators, including those in Europe and the United States and for periods of follow-up as long as 8 years. This has led a special committee of the American Diabetes Association (2) to modify recommendations concerning diabetic diets. In individual cases, this group stated, liberalization of carbohydrate intake does not appear to be contraindicated, providing appropriate caloric intake is not exceeded and other metabolic parameters of good diabetic control are not disturbed.

DIETARY CARBOHYDRATE IN DIABETES MANAGEMENT

There are additional observations suggesting beneficial effects of high carbohydrate diets on diabetic patients. This has been summarized recently by Brunzell and colleagues (3). In their studies, a change-over from a balanced regimen consisting of 45 percent carbohydrate, 40 percent fat, and 15 percent protein to an 8- to 10-day course of an 85 percent carbohydrate, 15 percent protein, 0 percent fat diet significantly lowered fasting and postprandial blood glucose concentrations during 100 gram oral glucose tolerance tests in mild, adult diabetic subjects (Fig. 4). Fasting insulin concentrations were also lowered significantly. The authors concluded that high carbohydrate diets render insulin more efficient in the control of blood glucose concentrations in diabetic patients.

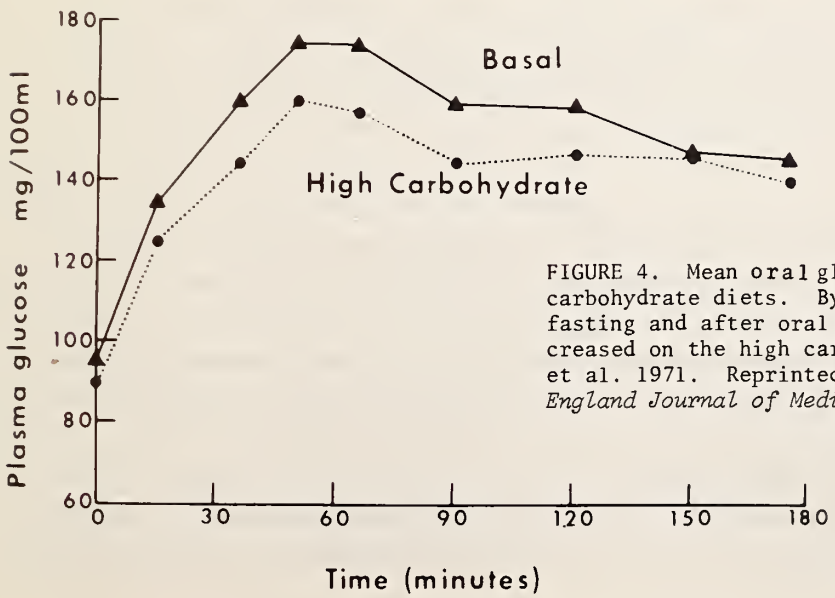

FIGURE 4. Mean oral glucose tolerance on basal and high carbohydrate diets. By paired comparisons, values both fasting and after oral glucose were significantly decreased on the high carbohydrate diet. From Brunzell et al. 1971. Reprinted with permission from the *New England Journal of Medicine*.

Others have reported improved carbohydrate tolerance in normal men following treatment with high sucrose diets on a short-term basis, but longer administration of this regimen resulted in no beneficial effect as compared to a more favorable outcome with high starch diets. Still others observe that while high carbohydrate diets may improve oral 100 gram glucose tolerance tests, they do not necessarily lower blood sugar or insulin responses to a more typical mixed meal; in fact, a worsening effect may be demonstrated under these conditions.

The foregoing observations lead to widely divergent conclusions concerning high carbohydrate diets in control of blood glucose homeostasis in diabetes. There are more uniform opinions regarding low carbohydrate diets, however. It is well known, for example, that restricted carbohydrate diets lead to impaired oral glucose tolerance. Basal insulin secretion is also decreased. More recently it has been reported that limited carbohydrate intake may promote deterioration of glucose tolerance by impairing peripheral utilization of this fuel in tissues such as skeletal muscle. Apart from diabetogenic effects, low carbohydrate diets severely restricted in calories, as in quick weight loss schemes for obese subjects, may promote demineralization of the skeleton and increased urinary losses of calcium and other minerals. It would appear that severe limits placed on carbohydrate intake (20 percent of total calories or less) have no important role in the dietary management of most diabetic patients today.

DIETARY COMPOSITION AND DIABETIC SERUM LIPIDS

Factors responsible for elevated blood lipid levels in diabetes are most complex and are reviewed in greater detail in other portions of this monograph. In brief, the vast majority of disturbances of triglycerides are due to overproduction of this moiety by the liver and increased entry into the systemic circulation. Defective removal of triglyceride by peripheral tissues also contributes to a greatly expanded blood triglyceride pool (6). Most of these abnormalities, as mentioned earlier, are corrected by reduction of obese patients to ideal body weight and optimum control of blood glucose levels by diet alone or in combination with oral agents and insulin. Nevertheless, a segment of the diabetic population will continue to manifest blood lipid abnormalities despite these measures.

Some of these individuals are unduly sensitive to carbohydrate; others may demonstrate sensitivity to both dietary fat and carbohydrate. These two groups have been classified into Types IV and V acquired lipid disturbances by Fredrickson and Levy (6), based on laboratory measurements of lipid-carrier proteins or lipoproteins in blood. However, to complicate this scheme further, additional research has recently shown that type IV lipoprotein disturbances are found in a heterogenous collection of patients whose sensitivity to carbohydrate and fat in the diet is highly variable and not readily predictable by lipoprotein classification. Some do require restriction of carbohydrate, others, limited fat intake, and some respond best to low fat and carbohydrate diets based on empirical observations. These data have particular relevance to the trend toward liberalizing carbohydrate intake in diabetic diets. While this manipulation may be of no consequence in the control of blood glucose concentrations, the undesirable side effect of triglyceride elevations may preclude its use in certain individuals.

Kaufmann and Stein (11), in their review of the subject, note that the induction of hyperlipemia by carbohydrate may be more contingent on the type of nutrients administered. Sucrose may have more profound effects than other types of sugars, and the triglyceride response may be

exaggerated to a greater degree when saturated, as opposed to unsaturated fats, are a part of the regimen. They also cited evidence that men and post-menopausal females more frequently manifest this phenomenon.

Refined sugar has been implicated in the causation of hyperlipemia by several authors, as reviewed by Roberts (16). He also showed that omission of sucrose from diets given to men with elevated serum triglyceride concentrations effectively improves the condition in most instances (Fig. 5).

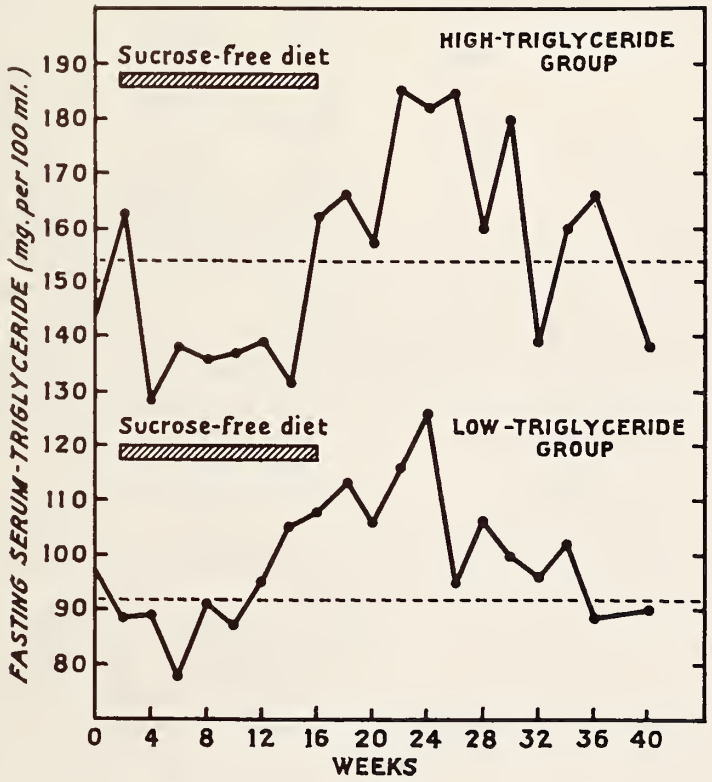

FIGURE 5. Mean fasting serum-triglyceride levels for the five men with the high basal serum triglyceride and thirteen men with normal basal serum triglyceride concentrations. The dotted lines represent the pre-dietary mean level for each group. From Roberts 1973. Reprinted with permission from *Lancet*.

EFFECT OF DIETARY COMPOSITION ON DIABETIC COMPLICATIONS

Other studies also have attempted to relate dietary patterns of a diabetic population with the types and frequency of complications occurring in them. In Rimoin's data on this subject (15), it was pointed out the clinical features of diabetes in various countries are difficult to correlate with dietary factors. Table 1 summarizes his findings. The frequency of juvenile-types of diabetes mellitus, as well as the general prevalence of vascular complications in chronic diabetes failed to incriminate relative dietary intakes of fat and carbohydrate in various ethnic groups. He suggested that it is not possible to separate dietary and other environmental factors from genetic heterogeneity of the disease sufficiently to draw specific conclusions about diet and diabetic complications on a broad, epidemiologic basis.

TABLE 1. Ethnic Differences in Diabetes Mellitus*

	Diet		Ketosis	Vascular Complications
	Fat	Carbohydrate		
European	High	High	Common	Common
Rhodesian Sephardic Jew	High	High	Uncommon	Common
Pima Indian	High	High	Rare	Common
Alabama-Coushatta Indian	High	High	Rare	Common
Seneca Indian	High	High	Rare	Common
Navajo Indian	High	High	Rare	Uncommon
Eskimo	High	Low	Rare	Rare
Japanese	Low	High	Rare	Uncommon
Ceylonese	Low	High	Rare	Uncommon
Indian	Low	High	Rare	Common
South African Indian	Low	High	Rare	Very Common
South African Zulu	Low	High	Common	Rare
Rhodesian African	Low	High	Common	Rare

From Rimoin 1971. Reprinted with permission from *Medical Clinics of North America.*

However, previous discussion in this monograph alludes to epidemiological surveys that take an opposing view about dietary influence on prevalence as well as complications of diabetes. Some relate sucrose intake to vascular disease; others the fat content of meal regimens. This controversy continues to be unresolved.

EFFECTS OF DIET ON CONTRA-INSULIN HORMONES

Theoretically, the ideal diabetic diet should promote efficient insulin action while avoiding stimulatory effects on other hormones known to oppose insulin. The latter include glucagon, another pancreatic islet hormone with potent hyperglycemic properties, adrenal cortisol, catecholamines, and pituitary growth hormone.

It is known that the proportion of carbohydrate in the diet may influence day-to-day basal secretion of insulin (8) (Fig. 6). High carbohydrate diets maintain a higher ratio of insulin to glucagon plasma concentrations in association with optimum glucose tolerance, whereas low carbohydrate diets have the opposite effect on this hormonal ratio and are attended by reduced tolerance to glucose loads (19) (Fig. 7). These physicians have also reported that plasma glucagon is inappropriately elevated relative to glucose concentrations in the diabetic subject.

Pure protein meals and certain amino acids stimulate secretion of insulin, glucagon, and growth hormone. Floyd and coworkers (5) have emphasized the synergistic effect between certain amino acids and glucagon on the pancreatic islet secretion of insulin, whereas Unger and coworkers (19) have reported inhibition of amino acid-induced glucagon release when glucose is administered concomitantly. In this same context, protein meals ingested before an oral glucose load improve glucose tolerance, but fat meals generally do not act in a synergistic fashion with glucose. High protein diets and very high sucrose-containing diets have been linked with elevated plasma levels of cortisol or increased adrenal cortisol secretory rates, which may explain the tendency toward impaired glucose tolerance reported in these situations.

Manipulating portions, mixtures, and sequence of administration of nutrients does influence the balance between insulin and contra-insulin hormones and, perhaps, their summative action. Long-term effects of these dietary alterations, particularly with regard to the practical management of the diabetic patient, remain to be defined.

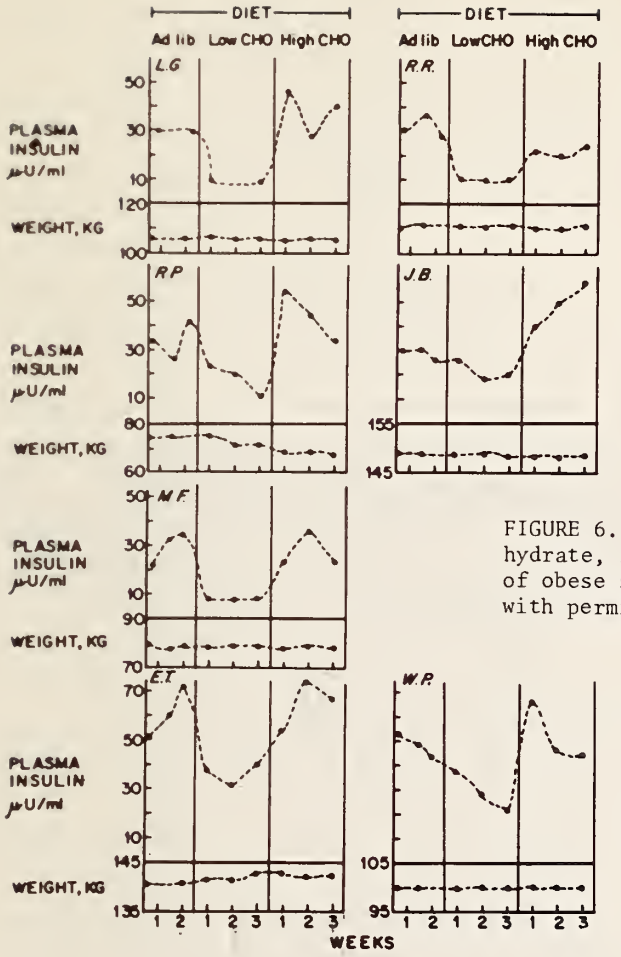

FIGURE 6. Effect of high-carbohydrate and low-carbohydrate, isocaloric diets on basal plasma hyperinsulinemia of obese subjects. From Grey and Kipnis 1971. Reprinted with permission from the *New England Journal of Medicine*.

WRONG DIET OR POOR ADHERENCE TO DIET?

The discussion up to this point has dealt with research aspects of diet and diabetes. However, certain other major aspects of this subject are of more practical importance, and, to some degree, demand even greater attention.

It is becoming more apparent than ever that another major deficiency of the dietary management of diabetic subjects relates directly to the failure of doctor and patient to ascertain whether or not the diet is actually being followed.

In West's excellent review (20) of this problem, a number of publications were cited which indicate that adherence to diet by diabetic subjects is generally poor. In a British study only one-third of diabetic patients were found to consume within 10 percent of their prescribed calories. Results of a national health survey in the United States in 1968 indicated that only 53 percent of diabetic subjects follow a physician's diet. The remainder were equally divided between those who were never given a meal plan or who simply had never adhered to one that was given to them. Even those who were ostensibly abiding by goals of good nutrition had a poor understanding of what sound dietary principles really are.

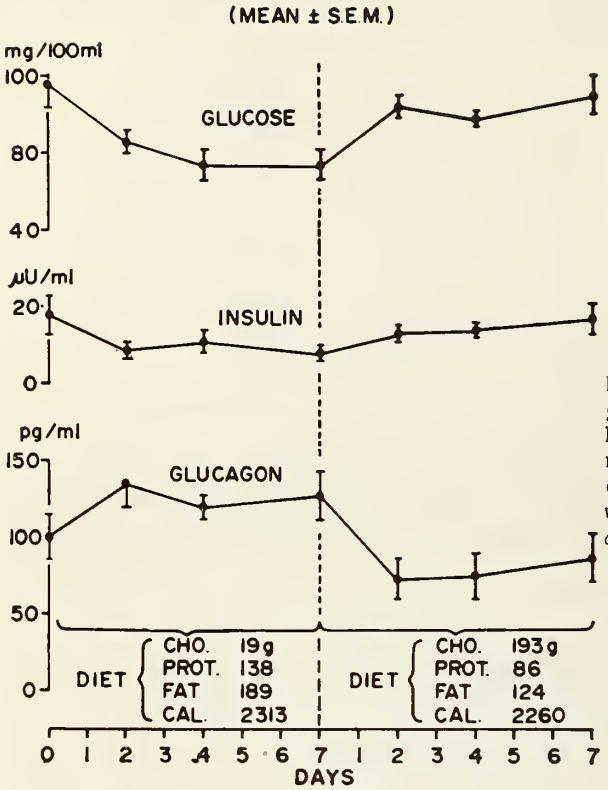

FIGURE 7. Fasting levels of glucose, insulin, and glucagon during one week of normocaloric, low carbohydrate diet and during one week of a more balanced normocaloric diet containing adequate quantities of carbohydrate. From Unger et al. 1971. Reprinted with permission from *Transactions of the Association of American Physicians*.

The failure of both physician and patient to achieve greater dietary precision in the control of diabetes was very apparent in the data provided by the University Group Diabetes Program. Over 800 diabetic subjects were followed and evaluated in twelve different university-affiliated clinics for periods exceeding 8 years. The majority were obese. Despite carefully planned dietary regimens and the availability of expert clinicians, dieticians, and other medical personnel, the vast majority of these subjects failed to register a significant weight loss and control of their diabetes at the conclusion of the program.

This rather sad commentary on the poor success of optimum medical care facilities for controlling diabetes can be extended to nondiabetic obesity as well. At best, similar clinics that are specifically devoted to weight control have failed to correct obesity in the majority of cases when reporting is honest and follow-up periods span several months (17).

The question is: Who is at fault, doctor, patient, or both?

FUTURE DIRECTION OF RESEARCH

The ideal diabetic diet, whether it is a single entity or a group of regimens fitted to various individual needs, has not yet been ascertained adequately enough by medical research. General epidemiological or retrospective studies of diet and diabetes to date prevent identifying the relative influence of genetic and environmental variables in sufficient detail to offer definitive conclusions. This applies equally well to detailed clinical and laboratory research

carried out on a short-term basis, because the ultimate effect of specific regimens on control and the prevention of complications of the disease cannot be predicted on this basis. Such studies, nevertheless, do offer valuable clues and should continue with additional refinements and in conjunction with long-term investigations.

One solution to this dilemma is a coordinated prospective study of diets of various types and their respective influence on carefully selected and monitored parameters. This requires a relatively large-scale, cooperative venture involving a number of patients and a number of medical facilities.

ORGANIZATION OF RESEARCH: PLANNING PHASE

Experts in the fields of diabetes mellitus, nutrition, epidemiology, biostatistics, and administration are invited by the National Institutes of Health to plan the organization and types of research to be initiated in this program. Several approaches might be adopted, but basically they should include:

1) The establishment of a central administrative agency to coordinate the effort.

2) Procurement of a central data processing center for pooling of information and analysis of study results.

3) Development of a central laboratory to perform all critical laboratory examinations or else provide guidelines and quality control for procedures performed in other centers.

4) Selection of participating medical centers throughout the country whose existing resources, facilities, and manpower would allow an in-depth investigation of these research problems. Selection might be based on competitive research, center, or contract grant systems.

5) Definition of patient selection, types of research, parameters to be monitored, etc. after appropriate exchanges with principal representatives of participating medical centers durin the planning phase.

ORGANIZATION OF RESEARCH: RESEARCH PHASE

Patients participating in this study are selected on a voluntary basis with each individual being made fully aware of the nature and purpose of the study and acknowledging all aspects of it through written, informed consent.

Selection of participants is one of the most critical factors in the study. It is the author's opinion that diabetic patients should be free of major complications of the disease and in relatively good health as determined by initial history, physical examinations, and laboratory screening procedures.

A portion of subjects in any one of the study groups serves as a control population and is given a standard diabetic diet consisting of 40 percent carbohydrate, 40 percent fat, and 20 percent protein and of sufficient calories to maintain ideal body weight. Obese diabetic subjects are reduced to ideal body weight before being considered for this study. Extreme care is exercised to insure maintenance of ideal weight throughout the investigation.

Other groups of patients are given diets to test the effects of varying distribution, composition, and sequencing of nutrients on a variety of pertinent metabolic profiles. For example, some studies would focus on diets relatively high in carbohydrate content; others might examine the outcome of low fat intake or high protein consumption. Types of nutrients also must be assessed such as regimens comparing different kinds of carbohydrate (e.g., starch, fruit

carbohydrate, and more refined sugars) or different types of fat (polyunsaturates *vs* saturated dietary lipid). Finally, the influence of timing and combinations of nutrients should be evaluated. One might examine the number or frequency of meals and the order of administration of specific foods. The latter would include comparisons of mixed meals with isolated intake of specific nutrients separately and in various sequences.

Patients report to designated outpatient centers at regular intervals. During this time appropriate entries into standardized records are performed and with reference to physical findings and laboratory data. Nutritional and medical counseling is an integral part of these visitations.

Parameters to be monitored in the laboratory include diabetic control as evidenced by basal blood glucose, cholesterol, and other lipid levels. These, in turn, might be correlated with plasma concentrations of other fuels including amino acids, ketone bodies, free fatty acids, etc. The influence of diets on hormonal profiles is also ascertained. Insulin dynamics are related to concentrations of circulating contra-insulin hormones such as growth hormone, epinephrine, glucagon, cortisol, etc. At various intervals patients are hospitalized for very brief periods to determine how various dietary regimens influence these parameters throughout a 24-hour period, both during and between meals.

One could envision initial prospective studies proceeding on a relatively short-term basis for a 6- to 12-month period. Pooled data analyses might dictate which regimens show greatest promise for optimum control as compared to more standard regimens in use today. Such regimens could be adopted for long-term studies (5-10 years) while continuing standard diets in control groups as a basis for reference.

In long-term studies one might scrutinize in greater detail physical evidence for development of diabetic complications, such as microvascular diseases of the eye and kidney, peripheral and cardiovascular disease, and neurologic changes, which are parameters that are more likely to change over more extended periods of time. These, in turn, are related statistically to laboratory data derived from analyses of blood tests.

The value of this research has relevance to health beyond that of diabetic patients. Nutritional information such as this very likely would apply to nondiabetic populations as well, since the high incidence of vascular disease in this country undoubtedly reflects dietary habits of citizens without diabetes as well. In this regard, long-term studies might very well involve additional control groups who do not have diabetes mellitus, but who might also be examined in a systematic way to evaluate diet and its effect on ultimate development of cerebral, cardiac and peripheral vascular disease, and other indices of morbidity and mortality.

Information derived from short-term studies could conceivably reduce the costs of similar long-term studies by as much as 50 percent annually, since many types of regimens could be eliminated and attention placed on dietary approaches of fewer number.

The final outcome of this research is the development of meal regimens that promote optimum control of blood glucose and lipids, minimal aggravation of metabolic and hormonal factors that oppose insulin action, and maximum suppression of events leading to vascular, renal, and neurological complications of diabetes mellitus.

FUTURE GOALS OF MEDICAL EDUCATION IN THE FIELD OF NUTRITION

It is unfortunate that very few physicians have a great understanding of nutrition generally and the dietary aspects of diabetes and obesity management. It is appropriate, then, to carefully examine present day curricula in our medical schools, other paramedical professional

schools, and postgraduate training programs in order to assess the seriousness of the deficiency. In this regard, groups whose charge is improvement of medical education might take specific steps to evaluate the situation.

In this same context, pilot programs in nutritional education might be initiated in selected universities and subsidized by the Federal government. The costs incurred would very likely approach the cost of supporting a small department or section within a medical school for personnel, teaching aids, and equipment.

The adequacy of these programs could be evaluated directly by comparing performances on board examinations of medical and other professional students who have been exposed to this type of education to board scores of those who have not been exposed. The value of this type of approach would be to improve the expertise of future practicing physicians in the nutritional management of patients with diabetes and related disorders, as well as in other forms of disease requiring similar dietary therapy.

FUTURE GOALS OF DIABETIC CARE FACILITIES

The education of patients with diabetes and/or obesity about nutrition is a team effort. In its broadest scope, it is a multi-institutional objective involving physicians, dietitians, nurses, hospitals, lay groups, news media, industry, labor unions, and public health agencies. At this point in time, however, dissemination of information about sound nutrition is often fragmented and duplicated, and its impact is not felt by the general public or else it is obsucred by more sensational types of food fads that receive greater publicity, despite their lack of acceptance by the medical profession.

The American Diabetes Association and TOPS Club, Inc. (Take Off Pounds Sensibly) are two nonprofit organizations devoted to the care and management of individuals with diabetes and obesity, respectively. Their combined membership totals several hundred thousand individuals. Their success and those of other self-help groups are already well known and often compare favorably with results of more expensive, less practical specialty clinics (17). They look to the medical profession for guidance and have already taken steps to improve communication along these lines. Their own publications reach virtually every state in the union and several countries throughout the world at the local chapter level.

Public health officials concerned about nutrition for the diabetic or the obese individual should take advantage of existing resources of these self-help groups. One major step forward would be to develop a more extensive flow of information derived from medical research and opinion or from guidelines and viewpoints expressed by public health agencies to these nonprofit organizations. In this regard an interlocking council could coordinate such an effort. The council might be composed of representatives from professions and lay societies and public health organizations whose specific responsibility is informing the public in the most efficient manner about nutritional principles for the control of diabetes and obesity.

The Federal government should also encourage the development of more comprehensive information centers within nonprofit lay societies who are already helping the diabetic and obese subject. Subsidization of activities concerned with release of information about medically accepted nutritional principles at meetings, through publications, other news media; the establishment of telephone "hotlines" for physician and patient needs; the institution of reference libraries to aid physicians, paramedical personnel, and the general public in their quest for specific nutri-

tional information are all means of bringing the public close to the facts at the lowest possible cost.

Ultimately, these activities may insure the development of more organized, well-informed, and effective programs in communities for purposes of weight control and diabetes management. Such preventive medicine will benefit public health to a great degree, since the number of individuals with either obesity, diabetes, or both has reached significant proportions and constitutes a major health hazard in this country today.

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23

INSULIN SYNTHESIS AND ANALOGS

Harold E. Lebovitz

INTRODUCTION

The major defect in diabetes mellitus is a lack of normal metabolic regulation by insulin. Insulin controls many vital cellular processes and acts either in concert with, or in opposition to, several other regulatory hormones (glucagon, growth hormone, catecholamines, and glucocorticoids). Research during the last two decades has provided significant insight into an understanding of insulin secretion and action. Utilizing this information, it is reasonable to develop a schema that envisions diabetes mellitus as not being caused by a single defect but resulting from any one of a variety of biochemical disturbances. Figure 1 illustrates at least eight or nine possible metabolic abnormalities that could result in the disturbance of carbohydrate and lipid metabolism that we call diabetes mellitus. Defect 3, which is an absolute absence of insulin synthesis in the beta cell, is the abnormality noted in the juvenile form of diabetes mellitus (63). Defects 1 and 2, either or both of which would result in an inadequate amount of insulin being released even though significant pancreatic stores are present, is the type of abnormality seen in patients with adult onset diabetes mellitus (28,31,64). Defect 4, an alternation of hepatic action of insulin, would be an inability of insulin to properly inhibit the enzymes of gluconeogenesis and stimulate those of glycolysis and glycogenesis, resulting in unabated hepatic glucose production even in the presence of insulin (41). Such a defect may account in part for the uncontrolled hyperglycemia seen in nonketotic hyperosmolar coma. Defect 5 is an alteration of the binding, or rate of destruction of insulin, in either passage through the liver or circulating in the blood. Defects 6, 7, and 8 are lack of insulin action because of either deficient insulin receptors, deficient production of an intracellular messenger, or inability of the messenger to affect the final biochemical intracellular effectors. While no definitive proof of these types of defects (6 through 8) exists, there are several animal (sand rat) (18) and human (lipoatrophic diabetes mellitus) (43) types of diabetes mellitus that could be explained by such mechanisms. Defect 9, caused by insulin antagonistic hormones, is probably mediated through mechanisms 6 to 8.

Since all forms of diabetes mellitus are ultimately associated with lack of insulin action, therapeutic endeavors naturally center upon developing agents which either exert insulin action or increase the release of endogenous insulin. It must be borne in mind, however, that other therapeutic agents which either facilitate endogenous insulin action or stimulate one or more of the biochemical effects of insulin, may be equally useful.

The present section is devoted to an analysis of the potential benefits of synthetic insulin peptides and chemically modified insulins in the treatment of diabetes mellitus and other metabolic disorders. In order to do this, it is necessary to first review the chemical nature of insulin, the spectrum of insulin's biologic activities, the relationship of insulin to other anabolic agents or growth factors, and the mechanism by which insulin exerts its actions. Since the availability of synthetic and modified insulins depends on peptide synthetic methodology, some attention must necessarily be focused on this area.

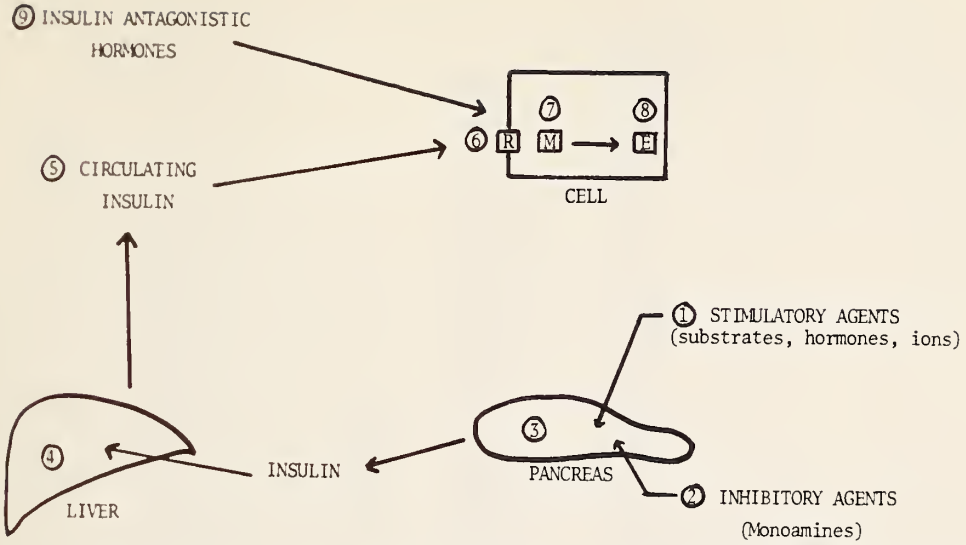

FIGURE 1. Biochemical defects that could cause the carbohydrate and lipid abnormalities associated with diabetes mellitus. Defects 1, 2, and 3 are associated with decreased release of insulin from the pancreas. In defect 1, insulin secretion is impaired because the stimulatory release mechanism is disturbed. In defect 2, release is impaired because of the presence of agents that inhibit the secretory process. Defect 3 is absent or abnormal insulin synthesis so that the pancreas does not contain any normal insulin to be secreted. Defect 4 is failure of the liver to respond to insulin. Defect 5 is excessive destruction of insulin either in passage through the liver or circulatory system. Defect 6 is an abnormality of the insulin receptor so that insulin does not bind. Defect 7 is failure to generate a secondary messenger after insulin attaches to the receptor. Defect 8 is failure of the generated intracellular messenger to effect the appropriate response inside the cell. Defect 9 is antagonism of insulin action by other hormones and is probably due to events caused at sites 6 to 8 by those hormones.

INSULIN STRUCTURE

Since insulin is a small, readily available, and biologically important protein, it has served as a model for the development of much of the methodology of modern protein chemistry. Insulin was the first protein to have its amino acid sequence completely determined (Ryle et al., 1955). The development of methods for determining N-terminal amino acids indicated that insulin consisted of two chains: one with an N-terminal glycine and one with an N-terminal phenylalanine (48). It contains three disulfide bridges which contribute to its three-dimensional structure. Table 1 depicts the primary amino acid sequence of beef insulin. The primary sequence for more than 20 vertebrate insulins have been completely determined, and the data indicate that 21 of the 51 amino acid residues (including the 6 cysteines) are invariant. Table 3, taken from Blundell et al. (3), summarizes these data.

A major achievement in opening new vistas into understanding the chemical basis of insulin action has been the elucidation of its three-dimensional structure (Fig. 2). X-ray crystallography has allowed delineation of the structure of the insulin monomer, diamer, and hexamer. Complete descriptions are available in several recent reviews (2,3,21). The most obvious stabilizing forces result from the disulfide bonds. The A7-B7 bond is on the outside of the molecule, while B19-A20 is more concealed but still somewhat accessible to the solvent. The A6-A11 disulfide bond is completely buried within the molecule and forms part of the nonpolar core. A second important feature of the monomer structure is the existence of a completely nonpolar core. This hydrophobic center consists

TABLE 1

AMINO ACID SEQUENCE OF BOVINE INSULIN

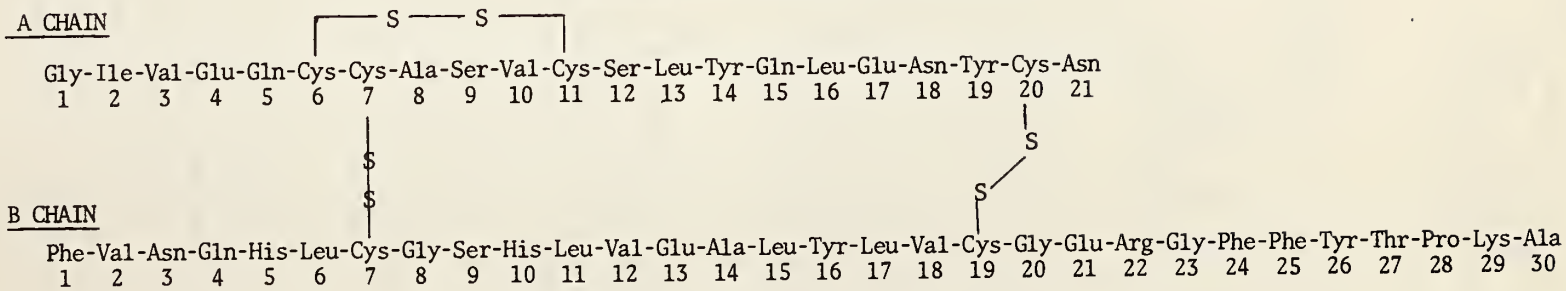

TABLE 2

THE KNOWN AMINO ACID SEQUENCES OF INSULIN

A CHAIN

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Mammals	Gly	Ile	Val	Glu Asp	Gln	Cys	Cys	Thr Ala	Ser Gly	Ile Thr Val	Cys	Ser	Leu	Tyr	Gln	Leu	Gln	Asn	Tyr	Cys	Asn
Birds								His	Asn	Thr											
Fish				Leu				His	His Lys Arg	Pro		Asn Asp	Lys Ile	Phe	Asp		Gln	Ser Asn			
Guinea Pig Coypu				Asp Asp				Ala Thr	Gln Asn	Thr Ile		Thr	Arg Arg	His Asn			Gln Met				Asp

B CHAIN

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Mammals		Phe	Val	Asn Lys	Gln	His	Leu	Cys	Gly	Ser Pro	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys Met	Ala Ser Thr
Birds		Ala	Ala																												
Fish	Met Ala Val	Ala	Ala Pro	Ala Pro										Asp								Asp						Asn	Ser	Lys -	-
Guinea pig				Ser	Arg						Asn				Thr			Ser			Gln	Asp	Asp					Ile		Lys	Asp
Coypu		Tyr		Ser		Arg					Gln			Asp	Thr			Ser			Arg	His	Arg			Tyr	Arg	Pro	Asn	Asp	-

of the residues B6, B11, B15, and A16 leucines; B18 valine; B24 phenylalanine, and the phenyl ring of B26 tyrosine; the A6-A11 and part of the A20-B19 cysteines. A third set of stabilizing forces helping to maintain the three-dimensional structure are ion pairs and hydrogen bonds. Evidence suggests that the C-terminal carboxyl group of A21 and the guanidinium group of B22 arginine, and the B29 lysine α amino group and A4 glutamate carboxyl group form ion pairs. The complete insulin molecule is very compact and only the carboxyl and amino terminals of the B chain extend out from the main structure. The surface of the molecule consists of two small nonpolar regions which are involved in diamer formation, but the major portion of the surface consists of polar (hydrophilic) residues. Zinc binding occurs through the B10 histidine and leads to the formation of hexameric crystals.

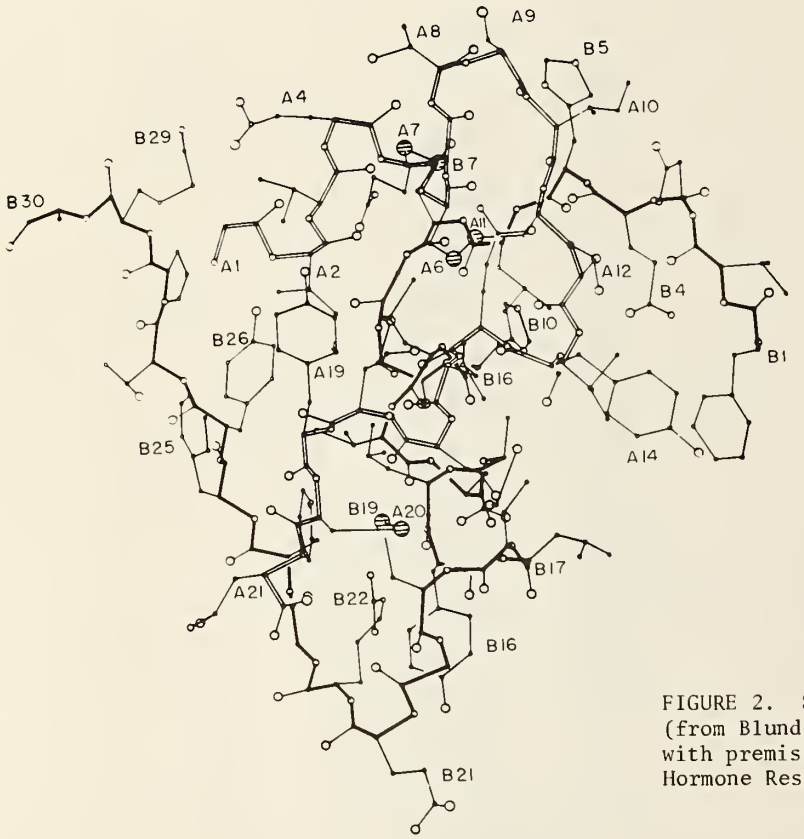

FIGURE 2. Structure of insulin monomer (from Blundell et al. 1971). Reprinted with premission from Recent Progress in Hormone Research.

From the three-dimensional model, it is of interest to note that the invariant amino acid residues of naturally occurring insulins (as noted from Table 2) may be grouped into three categories: (1) Those residues responsible for the backbone of the monomer structure, i.e., all three cystine groups--glycine B8 and B23, leucine B5, B11, B15, and A15, valine B18 and isoleucine A2; (2) those polar and nonpolar residues involved in diamer formation--serine B9, valine B12, tyrosine B16, and phenylalanine B24; and, (3) certain polar residues lying near one another on the surface of the A chain--glycine A1, glutamate A5, tyrosine A19, and asparagine A21.

STRUCTURE-ACTIVITY RELATIONSHIP OF THE INSULIN MOLECULE

The chemical approaches to defining the structure-activity relationship of the insulin molecule have involved chemical modifications, selective degradation by enzymes or chemical reagents, peptide synthesis, or a combination of these procedures. For structure-activity studies to be meaningful, several criteria must be met. The insulin derivative must be isolated and the alteration specifically characterized. The effect of the alteration on the three-dimensional structure of the insulin molecule must be determined. The most readily available procedures for determining whether the three-dimensional structure of the molecule has been altered is to measure whether the insulin derivative rotates polarized light in a pattern different from that of the native molecule. Two techniques which have been useful in measuring the optical properties of the insulin molecule and its derivatives are optical rotary dispersion and circular dichroism. Finally, the methods for measuring biological activity must be sensitive and precise and should assess the entire spectrum of biological activities.

Early studies on insulin structure-activity relationships involved chemical modifications in which the specific products were not characterized. These studies showed that limited acylation of the α and ε amino groups, esterification of the side chain hydroxyls, and, to some extent, blocking of guanido and imidazolyl groups have little or no effect on the measured biological activity. In contrast, extensive esterification of the free carboxyl groups, diazotization of the histidine and tyrosine residues, excessive iodination and reductive splitting of the disulfide bonds causes definitive loss of biological activity. Complete reviews of the studies of chemical modifications on insulin activity have recently been published (3, 23).

Structure-activity studies in the last 10 years have utilized the basic knowledge derived from the amino acid sequence and three-dimensional structure data and have relied heavily on very specific chemical modifications, selective enzyme degradation, resynthesis of insulin-like compounds from selectively degraded insulins, synthesis of insulin chains or analogs, and combination of these natural chains or synthesized chains (68). From the results of all these studies, several major concepts concerning the structural basis of insulin action can be made. Those modifications which cause the greatest change in three-dimensional structure (circular dichroism or optical rotary dispersion changes) are associated with the greatest loss of activity (3). It is not possible to define a specific active site of the insulin molecule (3, 23).

Specific aspects of structure-activity relationships that appear to be important are:

A. *Disulfide Groups:* Disruption of all three disulfide bonds leads to loss of all activity (26, 66). The least protected disulfide group (A7-B7) can be specifically reduced and either carboxymethylated or S-sulfonated (8). As noted in Table 3, this partially reduced insulin has significant biological activity. Weitzel and co-workers have synthesized an insulin analog in which cysteine A7 is replaced by alanine, and it is still active (23). The most protected disulfide bond A6-A11 is buried in the nonpolar core of the molecule. If both cysteines are replaced by alanine, the resultant synthetic analog is inactive (55, 56). If, however, the disulfide bridge is replaced by a trioether bridge (A11-A6 cystathione insulin), insulin activity is not lost and circular dichroism is unchanged, indicating little or no change in the conformation of the molecule (24, 25).

TABLE 3. Insulin Analogs with Alterations of Disulfide Groups[1]

	Percent Biological Activity			Percent
	Fat cell	Mouse Convulsion	Rat Hypoglycemia	Immunoreactivity
[D_1 (S-carboxymethyl cystein) $_{A7\ B7}$]		40	100	
[Di (S-sulfocysteine)$_{A7\ B7}$]		15	4 to 10	5.6 to 11.3
Carba A^6 Insulin (A 11-A6 cystathione insulin)		"Active"	"Active"	

[1]Zahn et al. 1972. Diabetes 21 (suppl 2) 468-475. Reprinted with permission from Diabetes, the Journal of the American Diabetes Assn.

B. *Alpha and Epsilon Amino Groups:* Many studies have been carried out with reagents interacting with the free amino groups of the molecule, and the results are somewhat dependent on the nature of the reacting group. Table 4 summarizes results obtained with a few derivatives (see (3) for complete list). Alterations in B1 phenylalanine do not alter the three-dimensional structure of the molecule, however, deletion or alteration of A1 glycine does change circular dichroism significantly. Alteration of the A1 glycine leads to a remarkable loss in activity, whereas loss or alteration of B1 phenylalanine has little effect on activity (4, 68). Elongating the A chain significantly decreases activity (68). Additional synthetic studies by Weitzel and his group have shown that the sequence A1-3 Gly, Ile, Val is necessary for activity as replacement of Gly A1 by Ala or βAla, of Ile A2 by Ala or Leu, and of Val A3 by Leu or Ile, lead to an almost complete loss of activity (22, 53, 56).

CARBOXYLIC ACID SIDE CHAINS AND TERMINAL RESIDUES

The six carboxylate groups in insulin lie on the surface of the monomer. The A4 glutamate and A21 carboxylate probably form salt bridges with B29 lysine and B22 arginine, respectively (3). Total esterification of all the carboxyl groups results in conformational change in the molecule and loss of biological activity (32). Table 5 lists the data that indicate that a carboxyl at A21 is very important in maintaining structure and activity. The B terminal alanine and several adjacent residues play little or no role in structure or activity, but the region B23 to B27 is very important for molecular activity.

B22 ARGININE SIDE CHAIN

With the exception of guinea pig insulin, all insulins have an arginine at B22. Weitzel et al. (59) synthesized B chain analogs using the Merrifield solid state synthesis technique and recombined them with natural A chain. Replacement of B9 and B27 by alanine yielded an analog with 75 percent activity as assessed by the mouse convulsion assay. When insulin analogs were made which had alanine at B9 and B27 and either histidine or alanine at B22, their activities were less than 0.5 percent that of native insulin. On the other hand, replacement of arginine B12 by ornithine gives a derivative with 16 percent of the activity of the synthetic analog with arginine at B22. These results are consistent with the presence of a B22 guanidinium group, or other positive ion, salt bridge with A21 carboxylate, which is important in maintaining the structure of the molecule. Unfortunately, studies of the conformation of the B22 modified insulins are not available.

TABLE 4. Insulin Derivatives and Analogs with Alterations in the N Terminus and B29 εAmino Lysine[1]

	Percent Biological Activity			Percent Immunoreactivity
	Fat Cell	Mouse Convulsion	Rat Hypoglycemia	
Des Amino A1	15			40
Des Gly A1	2 to 10		10	20 to 30
Des Phe B1			90	103
Acetyl A1	40		100	
Acetyl B29	75		100	
Diacetyl B1, B29	85		100	
Arg Gly A1	68	59		
Lys Arg Gly A1	20			
[Gly B1] εacetyl B29		35		25
Des Gly A1 des Phe B1	1.6		7	15
Des (Gly A1 Ileu A2)	0		0	
Des (Phe B1 Val B2)	0.2			2

[1]Zahn et al, 1972, Diabetes 21 (suppl 2) 468-475. Reprinted with permission from Diabetes, the Journal of the American Diabetes Assn.

TABLE 5. Insulin Derivatives with Modifications at the Carboxyl Terminus

	Percent Biological Activity		Percent Immunoreactivity	Conformational Change
	Fat Pad	Mouse Convulsion		
Desoctapeptide Insulin[1] (B23 to B30 deleted)	0.7	<1	3	Marked
Des Ala B30[2]		100		None
Des Ala B30 des Asn A21[2]	5.5	4	5	Marked
Methylated Insulin[3]	<3.0			Marked
Des Amido A21[2]		100		
[Glu 5, Ala A12, Phe A19, Ala A21][4] Insulin		100		
Des Ala B30 des Lys B29 des Pro B28][5] Insulin		84		

[1]Rager et al. 1969 (44)
[2]Carpenter 1966 (10)
[3]Levy and Carpenter 1970 (32)
[4]Weitzel et al. 1968 (56)
[5]Katsoyannis 1969 (26)

TABLE 6. Actions of Insulin

A. Nucleic Acid Metabolism
   1. Increases uridine and cytidine transport or phosphorylation in bone cells (42)
   2. Increases DNA synthesis (49)
   3. Promotes cell division (49)
   4. Facilitates differentiation of muscle cells (13)
   5. Increases synthesis of t-RNA (36)

B. Protein Metabolism
   1. Increases amino acid transport (45)
   2. Increases amino acid incorporation in protein (37,60,62)
   3. Increases protein synthesis by ribosomes (61)
   4. Decreases protein catabolism (38)

C. Glucose Metabolism
   1. Increases glucose uptake by muscle, adipose tissue, and liver (45)
   2. Increases UDPG-α glucan transglucosylase activity (30)
   3. Decreases gluconeogenesis (35)
   4. Increases glycolysis

D. Lipid Metabolism
   1. Increases synthesis of free fatty acids (33)
   2. Decreases lipolysis (15)
   3. Decreases ketone formation (17)
   4. Increases esterification of free fatty acids (1)

E. Increases Transport of Potassium into Cells

F. Decreases Cyclic AMP in Some Tissues (9)

OTHER SIDE CHAINS AND AMINO ACID RESIDUES

Many studies of the role of the tyrosine residues on the biological activity of insulin have been done but the data, for the most part, are conflicting. The A14 and A19 tyrosine residues are on the outside of the monomer, diamer, and hexamer. The B26 tyrosine is on the surface of the monomer, but is buried on the inside of the diamer and hexamer. The B16 tyrosine is fully exposed in the monomer, but partially buried in the diamer (3). The properties of several of the tyrosines are anomalous, probably due to their specific environments. For nitration reactions at pH 7 to pH 3, the reactivity is: Tyr A14 > Tyr A19 > Tyr B16 > Tyr B26. The mononitro (A14 or A19) and dinitro (A19 and A14) insulins have 104 ± 10 percent and 74 ± 6 percent activity, as compared to insulin as measured in blood glucose depression in chronically diabetic mice, but only 53 ± 10.8 percent and 25 percent in the mouse confulsion test (39). Tetranitrotyrosine insulin is reported to have 50 percent activity in the mouse convulsion test (3). These data, which indicate that the tyrosines are probably not necessary for activity, have been confirmed by the synthesis of insulin analogs in which some tyrosine residues have been replaced and biological activity maintained [Tyr A14 can be replaced by Phe or Ala (55, 56); Tyr A19 can be replaced by Phe but not Ala (56)]. Iodination of the insulin molecule gives a variety of products, many of which have markedly reduced activity. While the B10 histidine does not appear to be essential for insulin activity, there is some data which indicate that His B5 may be important for biological activity (58) but additional studies are necessary to substantiate this thesis.

The aliphatic hydroxyl groups Thr A8, Ser A9, Ser A12, Ser B9, and Thr B27 are on the surface of the diamer. Semisynthetic analogs [Glu A5, Ala A12, Ala A18, Ala A23] insulin (56) and [Ala B9, Ala B27, desAla B30, desLys B29, desPro B28] insulins (57) are reported to have 75 percent the activity of native insulin. These data indicate that Ser A12, Ser B9, and Thr B27 are not important for biological activity.

BIOLOGICAL ACTIONS OF INSULIN

Since the early 1950's, there has been an extensive inquiry into the nature and mechanism of insulin actions. These studies have led to the realization that insulin exerts numerous actions at many different biochemical loci. The net effect of all these actions is to facilitate the storage and utilization of substrates and to promote growth and differentiation. Table 6 lists the actions of insulin. This list is not exhaustive, but does include most of the actions that have been reasonably well studied.

Many of these actions of insulin are interrelated. For instance, insulin facilitates the entry of glucose into muscle and liver cells where it is either metabolized via glycolysis, stored as glycogen, or converted to free fatty acids. As insulin acts on the liver to increase glucose uptake and metabolism, it also blocks gluconeogenesis and ketogenesis. The insulin facilitated entry of glucose into adipose tissue cells causes increased fatty acid synthesis and esterification of fatty acids to triglycerides. Likewise, many of the anabolic actions are interrelated.

There is much evidence to indicate that the different actions of insulin are not mediated through the same mechanism. Some of its actions involve transport across the cell membrane (glucose uptake, amino acid transport, uridine and cytidine uptake), while others involve alterations in intracellular processes (increased DNA and RNA synthesis, increased protein synthesis by ribosomes, enzyme induction). Some insulin actions are thought to be mediated by a decrease in

tissue cyclic AMP (effects on isolated rat liver; antilipolytic effects) but this is not unequivocally proved, and there are many actions for which this mechanism is not likely (glucose transport, enzyme induction, protein synthesis). Insulin has been shown to alter the activity of a number of enzymes (29). At least two mechanisms have been described by which insulin alters enzyme activity. One involves the dephosphorylation of a protein enzyme (glycogen transferase, adipose tissue lipase). The other involves de novo synthesis of the enzyme (glucokinase, hexokinase II of adipose tissue, tyrosine aminotransferase of hepatoma cells in tissue culture). Insulin stimulation of protein synthesis can occur either by stimulation of transcription (glucokinase, hexokinase II) or translation (tyrosine aminotransferase of heptoma cells in culture).

RELATIONSHIP OF INSULIN TO OTHER ANABOLIC HORMONES

Insulin is essential for anabolism to occur in mammalian systems. As noted in the preceding section, insulin stimulates many processes that are involved in tissue growth and development. There have been described in the last few years a number of other anabolic peptide hormones which seem to be related to insulin.

Growth hormone, which is secreted from the anterior pituitary gland and seems to play the major role in controlling linear growth, has been shown to exert some, if not perhaps all, of its anabolic activity through a second circulating protein whose synthesis it seems to control (12). This second hormone has been named sulfation factor, or more recently, somatomedin. Somatomedin is responsible for the stimulation of cartilage macromolecular synthesis and growth. Somatomedin has not been completely purified and characterized. Partially purified preparations, however, have some insulin-like activity on adipose tissue [antilipolytic (51) and increase ^{14}C, glucose oxidation to $^{14}CO_2$ (19)], and according to Hintz et al. (20) interact with the insulin receptors of adipose tissue, liver cells, and chondrocytes. Of additional interest are the observations that very high concentrations of insulin will mimic the effects of somatomedin on cartilage macromolecular synthesis in vitro (47). These data suggest that insulin and somatomedin may be related.

The growth of autonomic nerves has been shown to be stimulated by a factor which is present in the salivary glands of male mice. This factor, which is called nerve growth factor, has been purified and its entire amino acid sequence determined (16). Bradshaw and co-workers have suggested that nerve growth factor is structurally related to proinsulin (16). Nerve growth factor has been shown to facilitate growth of autonomic ganglion and sensory nerves in embryos and in tissue culture. It has not been shown to have insulin-like actions, but insulin in high concentrations can stimulate the growth of neurons sensitive to nerve growth factor. Insulin and nerve growth factor have similar effects in their respective target tissues (16).

Another growth factor which is found in the submaxillary glands of mice is epidermal growth factor (50). This peptide has been isolated and structured. It facilitates anabolic reactions in tissues derived from epidermis.

The relationship of insulin to these other tissue growth hormones promises to be intriguing and may open up new areas of information which will allow us to understand the control of growth in specific tissues.

INSULIN RECEPTORS

Insulin action on adipose tissue, liver, and possibly other cells, appears to result from the interaction of insulin with a specific receptor binding site on the external surface of the cell

membrane (11). The current theories presume that after insulin binds to the receptor, it may alter the conformation of the membrane, thereby allowing changes in hexose, amino acid, and possibly ion transport. Other consequences of the binding of insulin to the receptor may be the release of one or more second messengers that are released and mediate the intracellular effects of insulin. The decrease of cyclic AMP that is the consequence of the action of insulin on some cells could be due either to some inhibition of adenyl cyclase by either a change in the membrane or a messenger which blocks the cyclase, or possibly, the activation of an intracellular phosphodiesterase by some second messenger. Many studies have confirmed the existence of the insulin receptor and have demonstrated that insulin exerts its actions even though it cannot penetrate into the cell. The events subsequent to insulin-receptor interaction are still speculative, with little definitive proof.

DIRECTIONS FOR FUTURE RESEARCH

Future research needs in the area of the therapy of diabetes mellitus with synthetic insulin and insulin analogs must be subdivided into immediate and long-range goals.

A. *Immediate Needs:* The major immediate need is the development of more efficient, more rapid, and cheaper methods for the synthesis of insulin and insulin analogs. Since the A chain is 21 residues and the B chain is 30 residues, the synthesis of the chains presents major problems in obtaining high yields of purified chains. Classical techniques of organic peptide synthesis are very laborious and require extensive purification of each subunit of the chain before it can be coupled with the next subunit. The final yield of chains from the starting material represents only a few percent. The Merrifield solid state synthesis method has been used by several investigators, but the products are heterogenous and very difficult to purify. An immediate need is investment in basic research to develop newer and better techniques for synthesizing peptides. This research will clearly be of benefit in the therapy of many diseases other than diabetes mellitus, since synthetic peptide analogs of many hormones have now been shown to have therapeutic benefits.

Another major problem in the synthesis of insulin and its analogs is the combination of the A and B chains to give high yields of purified synthetic insulin. The techniques worked out by Zahn and co-workers (65), Katsoyannis and Tometsko (27) and the Chinese group [Du et al. (14)] give yields of 2 to 10 percent insulin from combination of the chains (34). This low yield and the contamination with all sorts of erroneous chain combinations make the problem of the synthesis of large quantities of insulin almost insolvable. As a result of knowledge obtained from the three-dimensional structure of insulin, which indicates that Gly A1 and Lys B29 are separated by 8 to 10 angstroms, and previous structure-function studies that showed that the presence of acetyl groups at Gly A1 and Lys B29 did not affect the hypoglycemic activity of insulin, Brandenburg (4) prepared a number of modified insulins in which the amino groups of Gly A1 and Lys B29 were coupled, using a bifunctional reagent. The groups connecting the two residues were dicarboxylic acids ranging from C2 to C13. All of the derivates had significant in vivo hypoglycemia activity (32 to 100 percent) but markedly reduced in vitro activity on fat cells (2 to 14 percent). Of great interest was the observation that $N^{\alpha}A1$, $N^{\varepsilon}B29$ adipoylinsulin could be fully oxidized and then subsequently reduced, to give a 75 percent yield of the starting material (5). These data indicate that the 6 carbon bridge is actiong like the connecting peptide of proinsulin to put the chains in the proper position so reduction gives a very high yield of the proper combination of the A and

B chains. Additional research into this kind of molecular modeling may make it possible to combine A and B chains with an almost quantitative yield.

The development of better methods of chain synthesis and combination would provide for the eventual production of synthetic insulin for treatment of diabetic patients. Eventually, this may become very important as the number of patients with diabetes mellitus is increasing rapidly throughout the world, and we must face the possibility that some day animal sources of insulin may not be sufficient to treat all of the patients who need it. Another benefit of developing better synthetic techniques will be the ability to make large quantities of insulin analogs for potential pharmacologic use, as noted below.

B. *Long-range Needs:* Long range research with insulin analogs has the potential to open new areas of treatment to both diabetic and non-diabetic patients. As noted in the preceeding sections of this monograph, insulin interacts with specific membrane receptors to initiate a wide variety of biological actions. Research to characterize the nature of insulin receptors is essential. Are all insulin receptors the same, or are there different receptors to account for each different type of insulin action? Are the insulin receptors of each insulin-dependent tissue the same, or do they have major differences? Another area which needs extensive investigation is the mechanism(s) by which activation of insulin receptors causes the various biological actions of insulin to occur. Identification of the specific parts of the insulin molecule that interact with insulin receptors needs to be determined. Elucidation of the structure of insulin receptors and the residues of the insulin molecule that interact with them should lead to the synthesis of a variety of simple molecules that could bind to insulin receptors and activate them. This might provide the opportunity for developing parenteral, or oral agents, which have insulin action and could be administered with meals to control the diabetic state. Such a major achievement in therapy would revolutionize the management of patients with diabetes mellitus.

As noted in the sections on insulin actions and the relationship of insulin to other anabolic hormones, the insulin molecule has the potential to effect almost every phase of metabolism. There are clearly many instances in clinical medicine where a therapeutic agent which had some, but not all of these actions, would be very advantageous. For instance, an agent with the anabolic activities of insulin would be useful in the therapy of growth disturbances, in the repair of injury or in post-operative healing. Likewise, a molecule which had insulin-like action, but did not significantly promote lipogenesis might be extremely useful in the treatment of the obese diabetic patient. The synthesis of insulin analogs and careful evaluation of the spectrum of their biological activities has the potential to find such agents. This depends on whether all of the actions of insulin reside in the same amino acid residues of the molecule. Since the mechanism of insulin action appears to be different for certain activities, it is likely that some insulin analogs will show a spectrum of activity that is different from native insulin. None of the modified or synthetic insulins that have been made in the past have received adequate and careful evaluation of the spectrum of their biological activities. Such studies should be a part of long-range research in this area.

Along these same lines of investigation, it would be worthwhile to carefully evaluate the activity of naturally occurring insulins. For example, recent studies have shown that guinea pig insulin has about 10 percent the activity of bovine insulin, when assessed in the mouse convulsion (2.14 I.U.), or rat epidydimal fat, or hemidiaphragm assay, but when assessed by blood glucose

lowering in the guinea pig, it has 1/3 to 1/2 the activity (70).

The relationship of insulin to other anabolic agents, such as somatomedin, nerve growth factor, and epidermal growth factor need to be further clarified. The possibility that insulin analogs might be synthesized, which have increased somatomedin or nerve growth factor activity, should be considered as being highly likely and of great potential therapeutic importance as organ specific growth factors.

POTENTIAL VALUE OF THIS RESEARCH

The potential values of this research are: (1) the assurance of adequate supplies of insulin for patients with diabetes mellitus; (2) the development of new and better analogs for the treatment of diabetes mellitus; (3) the development of new pharmacologic agents for use in diabetes mellitus, and a variety of other diseases.

While these goals seem somewhat remote and theoretical, one needs only to look at the therapeutic agents that research has made available for use today. We would have been equally skeptical of their development 20 or 30 years ago.

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DRUGS ENHANCING INSULIN SECRETION

Harold E. Lebovitz

INTRODUCTION

The main objective in the treatment of patients with diabetes mellitus is to restore their metabolic balance to normal. We assume that goal will be achieved if the diabetic patient's blood glucose is maintained at the same fasting and postprandial levels as that of normal individuals. This will be a valid assumption if the agents used for treatment have the same spectrum of biological activities as insulin, and the defects being treated can be attributed to insulin insufficiency.

If diabetic patients have a total lack of pancreatic insulin (destruction of islet cells or disturbance of insulin synthesis) therapy must consist of the administration of exogenous insulin or insulin-related peptides. If diabetic patients have adequate stores of pancreatic insulin but are unable to secrete it appropriately because of a disturbance in the secretory process, it should be possible to treat those individuals with agents that stimulate the release of insulin. If diabetic patients have adequate or excessive quantities of pancreatic or circulating insulin but its actions are blocked at the cellular level, then therapy must be directed toward administering massive doses of exogenous insulin or modifying the cellular response to the endogenous insulin.

The present chapter explores the development and potential use of agents that increase insulin secretion. We shall first try to define the patients who might benefit from such a therapeutic approach. Next we will review the current state of knowledge about the mechanisms of insulin secretion. We will then examine the possibility that some forms of diabetes mellitus may be due to specific defects in the insulin secretory process. And, finally, we will define the potential approaches that might be taken to develop agents that will stimulate insulin secretion and may therefore be useful in the treatment of some patients with diabetes mellitus.

EVIDENCE THAT ABNORMALITIES OF INSULIN SECRETION ARE IMPORTANT IN THE PATHOGENESIS OF DIABETES MELLITUS

Diabetes mellitus is not a single disease, but rather a group of diseases that are similar in that the resultant metabolic defect is due to insufficient insulin action. Diabetes mellitus in man can be subdivided as shown in Table 1. Spontaneous genetic diabetes mellitus is itself a complex disease which exists in two major and possibly many minor variants. The major variants, ketotic insulin dependent diabetes mellitus and nonketotic maturity-onset diabetes mellitus exhibit many striking differences (64). The ketotic insulin dependent form ordinarily manifests itself prior to 25 years of age. The pancreas contains little or no insulin (97). Plasma insulin levels are low and are unchanged by the administration of glucose or other known stimulators of insulin secretion (74). These patients represent approximately 5 percent of the total diabetic population (63,65). They require exogenous insulin administration for their treatment. Nonketotic maturity-onset diabetes mellitus ordinarily presents after the age of 25 years and is usually associated with obesity, multiparity or some other predisposing environmental factor (63, 65). The insulin content of the pancreas is reduced (mean 50 percent with a range of 20 to 100 percent) compared to matched controls but is still about 1 U/g (mean) (97). Fasting plasma

TABLE 1. Types of Diabetes Mellitus

1. Spontaneous Genetic Diabetes Mellitus
   a. Ketotic Insulin Dependent
   b. Maturity Onset (Nonketotic)
2. Endocrine Induced
   a. Acromegaly
   b. Cushing's Syndrome
   c. Hyperthyroidism
   d. Pheochromocytoma
   e. Carcinoid Syndrome
3. Destruction of Pancreatic Islet Cells
   a. Trauma
   b. Surgery
   c. Inflammation (Pancreatitis)
   d. Tumor Infiltration
   e. Metabolic (Hemochromatosis)
4. Resistance to Insulin Action
   a. Lipoatrophic Diabetes Mellitus
   b. Werner's Syndrome

TABLE 2. Physiologic Factors that Stimulate In Vitro Insulin Secretion

1. Carbohydrates
   a. Glucose
   b. Mannose and other Metabolizable Monosaccharides
2. Hormones
   a. β Adrenergic Receptor Stimulators
   b. Glucagon
   c. ACTH
   d. TSH
   e. Gastro-intestinal Hormones
      1. Secretin
      2. Pancreozymin-Cholecystokinin (?)
      3. Gastrin
      4. Enteroglucagons
      5. VIP (Vasoactive Intestinal Peptide)
      6. GIP (Gastric Inhibiting Peptide)
   f. Cyclic AMP
3. Amino Acids
4. Cholinergic Agents
5. Cations
   a. Increase Flux of Sodium Out of the Cell
   b. Increased Extracellular Potassium
   c. Increased Intracellular Calcium

insulin levels are moderately elevated and the plasma insulin response to oral glucose or meal ingestion is either normal or exaggerated (98). Initially it was thought that this indicated that the primary defect in maturity-onset diabetes mellitus was peripheral resistance to insulin action. More extensive studies, however, indicated that this is not so, for if the blood glucose levels are taken into account and appropriate studies are done to match blood glucose curves in maturity-onset diabetic patients and normal individuals, it is clear that for a given blood glucose curve, maturity-onset diabetic patients secrete less insulin than do normal individuals (43,75,76). In addition, a very striking characteristic of glucose-mediated insulin secretion in maturity-onset diabetic patients is a delay in both the onset of secretion and the time at which peak secretion occurs relative to the time at which the plasma glucose peaks (98). Studies measuring the plasma insulin response to intravenously administered glucose in maturity-onset diabetic patients as compared to age, weight, and sex matched controls confirm the delayed and impaired insulin secretion (88). Maturity-onset diabetic patients comprise about 90 to 95 percent of the diabetic population. Additional support for the concept that impaired insulin secretion is characteristic of maturity-onset diabetes mellitus has come from several studies in which glucose-mediated insulin secretion was shown to be impaired in pre-diabetic patients with normal glucose tolerance (3,10,92).

It seems reasonable to conclude that a major defect in maturity-onset diabetes mellitus is one or more abnormalities in insulin secretion. Thus agents which stimulate the secretion of insulin might be expected to be useful in the treatment of this type of diabetes and an understanding of the exact nature of the defect might lead to development of drugs specific for the particular defect.

The other forms of diabetes mellitus listed in Table 1 are clearly due to lack of pancreatic insulin or peripheral resistance to insulin action. Endocrine induced diabetes mellitus probably representes a combination of impaired insulin release and peripheral antagonism of insulin action. Drugs stimulating insulin secretion might be expected to be useful in these disorders, particularly if, as has been suggested by several investigators, diabetes is induced only in that 15 or 20 percent of the population that may carry some of the genes for spontaneous diabetes mellitus (9, 12,13).

MODEL FOR INSULIN SECRETION

Insulin secretion is a complex process. It has been discussed in some detail in this monograph. In order to understand the development of drugs to enhance insulin secretion it is, however, necessary to review some of the more important aspects of current models of insulin secretion.

Insulin secretion may be thought of as a three-step process which involves (1) recognition of the insulinogenic stimulus by the beta cell, (2) generation of some appropriate intracellular message, and (3) activation of a granule releasing system. Disturbances of insulin secretion could occur through abnormalities in any of these components and drugs that stimulate insulin secretion might do so at any of these sites.

(1) Recognition of the insulinogenic stimulus by the beta cell

Insulin secretion by the beta cell is stimulated by many agents. Table 2 lists some of the more important physiologic factors that directly stimulate insulin secretion in vitro. The most important physiologic agent which stimulates insulin secretion is glucose. Some evidence suggests that the signal for glucose-mediated insulin release is an as yet unknown phosphorylated metabolite of glucose. Sugars that are readily metabolized (i.e., glucose, mannose, and to a lesser extent, fructose) stimulate insulin release(16,17,38). Sugars that are not metabolized (i.e., galactose, 3-O-methyl glucose) do not stimulate insulin release(17,38). D-mannoheptulose, which is an inhibitor of glucose and mannose uptake) inhibits insulin secretion stimulated by carbohydrates, but not by other agents(16,17,18). Matschinsky et al. (66,67) have been unable to show any alterations in beta cell glucose metabolites that could account for glucose-mediated insulin secretion, and they suggest that glucose stimulates insulin release by directly interacting with a plasma membrane glucose receptor. All hormones which stimulate in vitro insulin secretion probably do so by interacting with a specific plasma membrane receptor, activating beta cell adenylate cyclase and generating increased intracellular cyclic AMP (55,84). It is unlikely that ACTH and TSH are significant factors in controlling insulin secretion. Whether alpha cell pancreatic glucagon influences beta cell insulin secretion through intercellular bridges in the islets is unknown. Recent information suggests that the gastrointestinal hormones may be quite important in the physiologic regulation of insulin secretion in response to oral nutrients(2,6,26). The physiologic role of the beta receptor is unclear (53). Butyrylated derivatives of cyclic AMP and drugs that inhibit adenosine 3′5′-monophosphate diesterase (such as theophylline and caffeine) are potent stimulators of insulin secretion (30,55). It is of particular importance, however, to note that cyclic AMP and theophylline can only potentiate glucose-mediated insulin secretion. In the total absence of medium and intracellular glucose, they are unable to stimulate insulin secretion(5,31). Therefore, cyclic AMP and hormones which act through it must be viewed as agents that potentiate glucose-mediated insulin secretion and not as primary stimulators of secretion. Amino acids may stimulate insulin secretion through several different mechanisms (26): (1) direct

interaction with a plasma membrane receptor; (2) transport across the cell membrane; (3) indirectly by releasing adjacent alpha cell glucagon which stimulates beta cell insulin secretion; (4) through metabolites formed intracellularly. Leucine stimulates insulin secretion in the absence of glucose (69) and an unmetabolizable analogue of leucine (BCH), also stimulates insulin secretion (28). These data suggest that leucine itself stimulates insulin secretion. Arginine stimulation of insulin secretion is mediated differently from leucine [diazoxide inhibits leucine-induced, but not arginine-induced insulin secretion; mannoheptulose suppresses arginine but not leucine-induced insulin secretion (26)]. Cholinergic agents potentiate glucose mediated insulin release through a muscarinic action that is blocked by atropine (52,56,89). Cations are not specifically recognized by the beta cell but are involved in either the recognition of insulinogenic stimuli or generation of an intracellular messenger (53,84).

The beta cell therefore recognizes physiologic stimulators of insulin secretion by a variety of mechanisms which include cell membrane receptors, transport across the cell membrane, or the production of a metabolite.

(2) Generation of an appropriate intracellar message

Following the recognition of an insulinogenic stimulus, the beta cell must have one or more mechanisms to transfer this message to the granule releasing system. Evidence for two purportedly interrelated systems exist. The first is an ionic shift in sodium and calcium; the second is the beta cell cyclic AMP system.

Malaisse (54) has characterized agents that stimulate in vitro insulin secretion in relationship to their effects as the concentration of glucose in the medium is increased from none to 750 mg/dl (Fig. 1). Agents which lower the "Km" without affecting the "Vmax" of the secretory process are classified as glucose-stimulating agents. Agents which do not affect the "Km" but increase the "Vmax" of the secretory process are referred to as glucose-potentiating agents. Glucose simulating agents are glucose, other sugars, certain amino acids, such as leucine and different lipid metabolites. Glucose-potentiating agents are adrenergic and polypeptide hormones that act through the adenylate cyclase system.

Dean and Matthews (19,20,21) showed that stimulation of insulin release in the beta cell is accompanied by depolarization of the beta cell membrane. Glucose stimulation of impaled mouse beta cells decreased the membrane potential from -33mV to -16mV. Associated with increasing glucose concentrations were action potentials 1-4mV in amplitude and occurring in bursts with an interval of three to four seconds. Other insulinogenic stimuli produced similar electrical activity. These electrical changes seem to be caused by calcium entering into the beta cell and exchanging with intracellular sodium.

Malaisse in a separate series of papers (54,57,58) has presented evidence that glucose-stimulating agents increase the net uptake of calcium by the beta cell. This appears to be due to a reduction in efflux of calcium. Glucose-potentiating agents (5,54) were shown to increase the efflux of Ca^{45} from the cell without altering the uptake of calcium. He has hypothesized that insulin secretion is stimulated by increases in the cytosol calcium concentration. Calcium is thought to be in two intracellular pools: the cytosol and within cell organelles. Glucose-potentiating agents are purported to translocate large quantities of calcium from the organelles to the cytosol, thereby increasing cytosol levels. The increased Ca^{45} efflux is postulated to reflect the movement of some of this increased calcium to the extracellular space. Glucose-stimulating

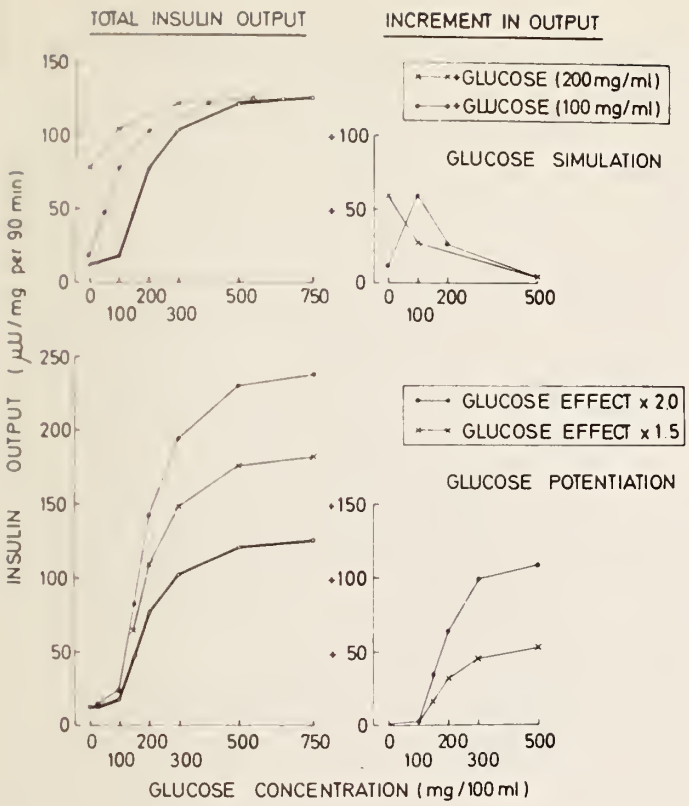

FIGURE 1. Definition of glucose-stimulating (upper panel) and glucose-potentiating (lower panel) insulinotropic agents. The total insulin output (left) and the increment in secretion rate (right) evoked by these agents in pieces of rat pancreatic tissue are shown as a function of the glucose concentration of the incubation medium (indicated along the abscissa). On the left, the rate of insulin output induced by glucose alone is shown by the heavy line. The other curves were derived from the experimental data, as shown in the rectangles, by assuming either that a fixed amount of glucose (1.0 or 2.0 mg/ml) was added to the glucose already present in the incubation medium (glucose stimulation), or that the insulinotropic cation of glucose was multiplied by a constant factor (x1.5 or 2.0; glucose potentiation). From Malaisse, W. J. 1973. Diabetologia 9:167-173. Reprinted with permission from Springer-Verlag.

agents are thought to increase cytosol calcium concentrations by decreasing calcium efflux. The control of insulin secretion by increasing cytosol calcium concentration is a provoking theory for which there is some circumstantial evidence. One must, however, note that such a theory in which a sequestered intracellular calcium pool is hypothesized to interact with an active cytosol pool that is also interacting with the external environment is not presently amenable to testing. Direct measurements of the cytosol calcium concentrations under various states of insulin secretion will be necessary to prove this theory.

The recognition of many agents which increase insulin secretion is accompanied by an increase in intracellular cyclic AMP. Glucagon, theophylline, and glucose have all been shown to increase islet cell cyclic AMP content (15,44,93). Cyclic AMP and theophylline were shown to increase the efflux of Ca^{45} from prelabeled isolated islets and, as noted above, this has been interpreted to mean that intracellular cyclic AMP increases cytosol calcium by translocating calcium from cellular organelles to cytosol. It is not presently known whether the increase in intracellular cyclic AMP mediates other intracellular effects such as phosphorylating proteins or activating gene action.

It is also uncertain whether there are additional intracellular messengers, generated by one or more of the various agents, that stimulate insulin secretion.

3. Activation of granule releasing system

Anatomical studies coupled with insulin secretory studies have suggested the presence of an intracellular transport system for beta granules (45,47). Insulin is synthesized as proinsulin within the endoplasmic reticulum of the beta cell. An energy dependent process transfers it to the Golgi apparatus where distinct beta cell granules are formed and released into the cytoplasm. The granule is surrounded by a smooth membranous sac. Proinsulin is cleaved into insulin and connecting peptide as the granule is formed. Zinc is incorporated into the insulin molecule in the mature beta granule. The beta granules in the cytoplasm attach to microtubules which have a diameter of 200A and a wall comprised of 12 to 14 subunits. The microtubules run perpendicular to the plasma membrane. The beta granules appear to migrate down the microtubules to the plasma membrane. The exact mechanism of movement is unknown. On the inner side of the plasma membrane is a layer of short interconnected fibers which are compact and exclude most of the other components of the cell. This region, called the cell web, is composed of microfilaments (approximately 40 to 70 A°) and appears to be attached to the inner surface of the plasma membrane (59, 73). It creates a zone of exclusion through which the beta granules have to pass on their way to the plasma membrane. When the beta granule reaches the plasma membrane, its membrane fuses with the plasma membrane and both dissolve at the point of contact and discharge the granule's content outside of the cell with formation of microvillous projections at the discharge site. This process is called emiocytosis (47). Recently, the outside of the beta cell plasma membrane has been shown to be coated with a carbohydrate layer composed of glycoproteins and mucopolysaccharides (73). The function of this outer layer is unknown.

Agents which stimulate insulin release appear to do so by causing movement of granules down the microtubular system to the cell surface where emiocytosis takes place(47,48). The evidence that the microtubular system is involved in insulin secretion is: (1) drugs that destroy microtubules such as colchicine, vincristine, and vinblastine inhibit insulin secretion stimulated by all agents (glucose, leucine, sulfonylurea drugs, etc.); (2) chemicals that stabilize microtubules such a deuterium oxide, hexylene glycol (0.15 to 1.0 percent) or ethanol (1.0 percent) cause reversible inhibition of insulin secretion stimulated by glucose, leucine, or sulfonylurea drugs; (3) these antimicrotubular agents inhibit both the initial and late phase of glucose-stimulated insulin release from perifused isolated islets.

The role of the microfilamentous cell web is not as clearly defined. The effect of cytochalasin B on the ultrastructure of the beta cell web and insulin secretion by isolated islets has been studied by Orci et al. (72) and Malaisse et al. (59). Cytochalasin B markedly disrupted the cell web of isolated islets and though it had no effect on basal insulin release, it markedly enhanced glucose-stimulated insulin secretion. This enhancement was reversible and could be inhibited by deuterium oxide. In perifused systems, cytochalasin B enhanced both the early and late phase of glucose-mediated insulin release (49). These studies suggest that the microfilamentous web may serve as a means of deterring insulin secretion. Recent studies with cytochalasin B, however, indicate that this drug inhibits the uptake of glucose, glucosamine and 2-deoxy-D-glucose in HeLa cells and pancreatic islets and also inhibits glycoprotein and mucopolysaccharide synthesis in embryonic cells (68,87). Therefore it is possible that the effects of cytochalasin B are not on the microfilamentous systems but on the cell plasma membrane, or some other component of the cell.

Evidence for emiocytosis as the end process of secretion has been amply documented by both scanning election microscopy and freeze-fracture electron microscopy (46,73).

The granule releasing system is activated by intracellular messages generated by the insulin releasing stimuli. Studies by Grodsky and Bennett (37) and Milner and Hales (70) showed that insulin secretion in vitro could not occur in a calcium free medium regardless of the stimulating agent. Their data and the data discussed above, on Ca^{45} uptake and efflux, have led many investigators to hypothesize that changes in calcium ion trigger the beta cell microtubular system to transport and release beta granules. The mechanism by which calcium ions trigger the microtubular-microfilamentous system is not clear. The mechanism of intracellular cyclic AMP in activating the microtubular-microfilamentous system is also unclear.

As noted above, Malaisse has hypothesized that cyclic AMP increases cytosol calcium ion and that its effects are therefore calcium mediated. Other models of cyclic AMP action have supported this concept. Another possibility, however, is that cyclic AMP may activate a protein kinase that phosphorylates some protein which effects granule transport.

In addition to physiologic agents that stimulate insulin secretion, there are several physiologic factors which are known to inhibit insulin secretion. These are listed in Table 3.

TABLE 3. Physiologic Factors that Inhibit Insulin Secretion

1. α Adrenergic Receptor Stimulators
2. Dopamine
3. Serotonin
4. Amonium Ion

Alpha adrenergic receptor stimulating agents inhibit both in vivo and in vitro insulin secretion (80). They do so through interference with the discharge of beta granules. Turtle and Kipnis (93) indicated that they do so by inhibiting beta cell adenylate cyclase and depressing intracellular cyclic AMP levels. Feldman and Lebovitz (30) have shown that epinephrine and other alpha adrenergic stimulating agents (32) block the in vitro insulin secretory response to dibutyryl cyclic AMP and suggested that epinephrine interferes directly with the granule release process. Brisson and Malaisse (4) have tried to explain epinephrine inhibition on insulin secretion through a decrease in cytosol calcium. Their studies show very small and transient effects of epinephrine in increasing Ca^{45} efflux from the beta cell and they hypothesize that epinephrine provokes a translocation of calcium from the cytosol to some organelles. The data are not very convincing. Dopamine and serotonin are reported to occur in the cytosol of beta cells of some species (8). Studies reported have indicated that both of these monoamines interfere with the final stages of granule secretion. Lebovitz and Feldman (51) have proposed that serotonin and/or dopamine occur in or near the beta granule and tonically inhibit migration down the microtubule. Secretion is thought to be the net balance between stimulatory influences and the tonic inhibition by these monoamines. Serotonin antagonists markedly potentiate glucose and tolbutamide stimulated insulin secretion (33). It is not known whether these monoamines inhibit insulin secretion by altering local calcium concentrations or interfering with microtubular function. The ammonium ion interferes with insulin secretion through altering either the recognition of glucose dependent stimulators or the generation of their intracellular message. It does not interfere with the granule secretory system since it does not block sulfonylurea drug-mediated insulin release (31).

NATURE OF THE INSULIN SECRETORY DEFECT IN MATURITY-ONSET DIABETES MELLITUS

Many studies have been done to try to characterize the nature of the defect in insulin secretion in patients with diabetes mellitus. Difficulties have been encountered in these studies and much of the data are controversial. The problems encountered are related to several factors: (1) age, sex, diet, and degree of obesity influence the insulin secretory response; (2) severity of diabetes and the quantity of insulin remaining in the pancreatic beta cell will affect the magnitude of the insulin secretory response; (3) most clinical studies utilize plasma insulin changes as the index of secretory response; (4) all clinical studies measure the effects of the stimulating agents in the presence of normal or elevated extracellular glucose.

In spite of the above difficulties, relevant information is available on the effect of a number of agents on insulin secretion in maturity-onset diabetic patients and prediabetic individuals.

1. Glucose

Many different techniques have been used to study the insulin response to glucose. Oral administration of glucose to patients with mild or moderate maturity-onset diabetes is associated with the following plasma insulin changes: (1) the onset of the rise in plasma insulin is delayed as compared to that occurring in normal individuals, (2) the peak plasma insulin is usually higher than in normals and occurs after the peak plasma glucose is reached, (3) total insulin secretion is frequently greater than that seen in normals (98). Because diabetic patients extract less oral glucose in the liver than normal individuals, their plasma glucoses are much higher than normals. Thus, it is difficult to compare insulin secretion following oral glucose in diabetics to normals since the glycemic stimulus is greater. Perley and Kipnis (75,76) attempted to solve this dilemma by simulating the oral plasma glucose curves with computer programmed glucose infusions. Maturity-onset diabetics had lower plasma insulins than normals with both normal and diabetic simulated glucose tolerance curves. Seltzer et al. (88) attempted to solve this problem by calculating plasma insulin changes relative to plasma glucose changes and expressing it as an index. This calculation also indicated impaired insulin secretion following oral glucose in diabetic patients. The plasma insulin response to oral glucose has been studied in prediabetic patients (in which, of course, glucose tolerance is normal) and are reported to be normal or decreased (27,39,82).

Studies of the plasma insulin response to intravenous glucose have uniformly demonstrated that maturity-onset diabetic patients and prediabetic individuals have impaired insulin secretion in response to glucose (3,88). Most of these studies have used a 25 g glucose bolus as the stimulant. Cerasi and Luft (9,10) have developed an intravenous glucose procedure to measure insulin secretion. They give a priming intravenous injection of glucose followed by a constant infusion for 60 minutes. Plasma glucose and insulin are determined frequently and the insulin response analyzed in relation to the glucose stimulation by an analogue computer. Using this technique they have defined a parameter known as the initial insulin response. They found that this initial response is markedly decreased in patients with maturity-onset diabetes, prediabetic patients, and 15 to 20 percent of normal controls. They suggest that this impaired initial secretory response is a marker of the genetic abnormality of diabetes mellitus.

Defective insulin secretion in response to glucose stimulation appears to be one of the key defects in maturity-onset diabetes mellitus.

2. Amino Acids

Amino acids alone or in mixtures stimulate insulin secretion in man when they are given orally or intravenously. Patients with maturity-onset diabetes mellitus and subclinical diabetes show lower than normal rises in plasma insulin levels during intravenous infusions of amino acids (26,28,35). The plasma insulin response to intravenous amino acids in prediabetic subjects is normal.

3. Beta Receptor Stimulators

Intravenous administration of beta adrenergic agents such as isoproterenol increase the plasma insulin of normal humans (79). Several studies have attempted to implicate a defective pancreatic beta cell beta receptor as the cause of the impaired insulin secretion in diabetes mellitus (11,14). Specifically, infusions of propranolol are reported to significantly inhibit glucose-mediated insulin release. Deckert and colleagues (24), however, showed that isoprenaline infusion stimulated insulin secretion in maturity-onset diabetics just as well as in normals. Robertson and Porte (85) showed that maturity-onset diabetic patients showed the same magnitude of insulin secretion in response to isoproterenol as normals, even though they had markedly impaired insulin secretion in response to intravenous glucose. Isoproterenol, but not glucose-stimulated insulin secretion, could be blocked by propranolol. They concluded that the glucose receptor is distinct from the beta receptor and that the latter is not involved in the insulin secretory defect in diabetes mellitus.

4. Alpha Receptor Stimulators

Intravenous administration of alpha adrenergic agonists in man inhibit insulin secretion (80). Agents that block the alpha adrenergic receptor stimulate insulin secretion in normals and maturity-onset diabetics (77,80). The administration of alpha adrenergic receptor antagonists (phentolamine) increase the insulin secretory response to intravenous glucose in normal subjects (7). Of considerable interest is the observation that even though insulin secretion was greater, the glucose disposal constant was unchanged. Similarly, administration of phentolamine to healthy fasting volunteers caused hyperinsulinemia in response to an intravenous glucose load without amelioration of the glucose intolerance (71). Efendic, Cerasi, and Luft (25) reported that blockage of alpha adrenergic receptors partially restores the initial insulin response (to glucose) in their prediabetic subjects toward normal. They claim that a similar treatment had no effect on early secretion in normal subjects.

5. Secretin and Other GI Hormones

Deckert (22) showed that 75 units of secretin intravenously elicited the same striking rise in plasma insulin in eight maturity-onset diabetics as it did in his five normal volunteers. Six of the eight diabetic patients were also tested by intravenous injection of 25 g of glucose and showed a markedly impaired insulin secretion. Hindberg, Enk, and Persson (41) confirmed the observation that secretin induced insulin secretion is not impaired in maturity-onset diabetics. Vinik, Kalk, and Jackson (96) have indicated that the early insulin response to secretin and impure cholecyotokinin-pancreozymin (probably contaminated with GIP) Gastric Inhibiting Peptide, are normal in diabetic patients.

6. Aminophylline (Adenosine 3′5′-Monophosphate Diesterase Inhibitor

Cerasi and Luft (11) have proposed that the abnormal glucose-mediated insulin response in

some prediabetics is due to a defect in the generation or cellular action of pancreatic beta cell cyclic AMP. This theory is based on studies in which they showed that aminophylline infusions given simultaneously with the glucose infusions normalized or improved the early insulin response to glucose in eight out of nine prediabetics. No such effect was noted in five patients with overt diabetes and only two of eleven nonprediabetic healthy subjects showed an increase in insulin response. Other investigators have shown that intravenous aminophylline causes many hormonal and metabolic changes in normal individuals.

7. Glucagon

Simpson et al. (90) infused glucagon and 25 g of glucose over three minutes into eight maturity-onset diabetics and nine normal volunteers and measured their plasma glucose and insulin responses. Each patient had previously had a similar study done in which only glucose was given. The diabetic patients had no insulin response to glucose. The incremental insulin secretion caused by glucagon was the same in the diabetics as the normals. The incremental insulin secretion increased glucose utilization in the normals, but not the diabetics.

8. *Serotonin Antagonists*

Lebovitz and Feldman (51) have hypothesized that intracellular serotinin and/or dopamine are tonic inhibitors of insulin secretion. In one of their studies (83) they attempted to determine whether the impaired glucose-mediated insulin secretion in maturity-onset diabetics was due to an exaggerated tonic effect of the proposed intracellular serotonin. They performed intravenous glucose studies on normal volunteers and maturity-onset diabetic patients. They treated both groups with a placebo and repeated the intravenous studies. Placebo treatment had no effect on glucose disposal or insulin secretion as compared to the control study. Both groups were then treated with the serotonin antagonist, methysergide maleate, and the intravenous glucose study repeated. Methysergide had no effect on insulin secretion in the volunteers, but increased insulin secretion in the diabetics by 48 percent. In a similar study (1) they have also shown that methysergide potentiates tolbutamide-mediated insulin release in diabetics (39 percent increase).

SULFONYLUREA DRUGS

Several properties of sulfonylurea-stimulated insulin secretion indicate that the mechanism is different from that of glucose and most other stimuli (31,36): (1) sulfonylurea drugs stimulate insulin secretion in vitro in the total absence of glucose; (2) sulfonylurea drug-mediated insulin secretion cannot be blocked in vitro or in vivo by mannoheptulose, 2 deoxy-D-glucose or diazoxide, (3) sulfonylurea drugs stimulate only the first phase of insulin release from perifused or perfused pancreas systems. Sulfonylurea drugs also potentiate glucose and amino acid mediated insulin secretion. Malaisse et al. (61) have suggested that sulfonylurea drugs decrease calcium efflux from beta cells and stimulate insulin secretion by increasing cytosol calcium concentrations. There is some question as to whether sulfonylurea drugs may inhibit beta cell adenosine 3′5′-monophosphate diesterase and act through increased beta cell cyclic AMP (86). This is somewhat unlikely as both butyrylated cyclic AMP derivatives and theophylline have an absolute requirement for glucose in order to stimulate insulin secretion (31,53).

Intravenous administration of 1 g of tolbutamide to maturity-onset diabetic patients ordinarily stimulates a significantly smaller rise in plasma insulin than it does in normal individuals (23,76). Prediabetic subjects have a normal insulin secretory response to intravenous tolbutamide (3).

DIRECTIONS FOR RESEARCH TO DEVELOP DRUGS THAT STIMULATE INSULIN SECRETION

From the information reviewed in this chapter several obvious conclusions can be drawn. The majority of maturity-onset diabetic patients (several million in the U.S.A.) could be successfully treated if drugs were developed which either corrected the specific defect in insulin secretion which occurs in diabetes millitus or augmented nutrient stimulated insulin secretion in general, thereby allowing pharmacologic normalization of the plasma glucose. Such drugs would also be useful to prevent the development of diabetes mellitus in people with the diabetic genetic defect. To develop these drugs it will be necessary to learn more about the mechanisms by which cells secrete granules and specifically how this process is carried out in the beta cell. More research must be done to define the specific defects in insulin secretion which occur in diabetes mellitus. Finally, we must learn how to develop drugs which alter basic processes selectively in the beta cell.

Our knowledge of the mechanisms of insulin secretion are woefully inadequate. How do beta cells recognize the presence of insulinogenic stimuli? Is there a specific glucose receptor? How does glucose interact with the receptor? Do other factors influence the interaction of glucose with the receptor? How is the synthesis of the receptor controlled? If there is not a specific glucose receptor, what is the mechanism by which glucose initiates insulin secretion? Can the beta cell membrane be modified so as to increase the recognition of glucose by the beta cell? Several in vitro studies indicate that this may be possible. Lambert, Henquin, and Orci (50) have shown that preincubation of isolated islets with pronase (2 to 20 micrograms/ml) remarkably enhance subsequent glucose-mediated insulin release. Hellman and co-workers (40) showed that a variety of sulfhydryl reagents which interact at the beta cell membrane increase both basal and glucose stimulated insulin secretion in vitro. They have also presented some data to suggest that the effects of sulfonylurea drugs on insulin secretion may occur through a similar mechanism. Even less is known about the interaction of other insulinogenic stimuli (amino acids, peptide hormones, and catecholamines) with the beta cell plasma membrane.

The intracellular events which occur after the identification of the insulin secretogogue are a mystery. Is the calcium ion the final ultimate intracellular message for the granule discharge system? Techniques need to be developed to measure calcium in the cytosol. Malaisse's theory concerning the effects of hormones and cyclic AMP on intracellular calcium movement needs to be scrutinized and tested. Does cyclic AMP have an intracellular function independent of calcium ion movement? Do intracellular monoamines control insulin secretion through a tonic inhibition of granule discharge? If so, how are the intracellular monoamine levels controlled? What is the mechanism of their inhibitory action? Are there other intracellular messengers that influence insulin secretion?

The function of the granule secreting system also needs to be clarified. What is the function of the microtubular system? How is it controlled? How are the granules moved? Does the cell web impede the release of insulin? If so, what controls the synthesis and function of the web?

In addition to basic research on the control of insulin secretion, it is necessary to carry out applied research to define the specific alterations in insulin secretion in maturity-onset diabetic patients. Glucose, amino acid, and sulfonylurea drug mediated insulin secretion are impaired. Secretin, glucagon, and beta adrenergic agonist mediated insulin secretion seem to be normal. These data suggest that the defect in diabetes mellitus is in stimuli recognition rather

than granule release. What is the defect? Can it be modified? Do intracellular monoamines such as serotonin have anything to do with the diabetic secretory defect? Another very important area to be investigated is why agents such as glucagon, secretin, alpha adrenergic receptor antagonists, and serotonin antagonists increase glucose-mediated insulin secretion in diabetic patients, but do not improve glucose uptake.

Drug development presents even greater problems to be solved. The major difficulty would appear to be to develop agents that affect fundamental cellular processes, but only in the beta cell. Can drugs be made which will affect beta cell recognition of insulinogenic stimuli without affecting other cells? For the present this question is unanswerable, but it needs to be explored. The same question can be asked about drugs that influence the cyclic AMP system. Can pancreatic beta cell cyclic AMP be preferentially increased? Smith (91), in a recent review, has discussed the evidence that drugs may preferentially inhibit the 3′5′adenosine monophosphate diesterase of one tissue. Similar data were presented with reference to activation or inhibition of adenylate cyclase. Chemicals (ionophones) which increase the flow of calcium into cells have recently been made. Their potential effects on insulin secretion are under active investigation. When more knowledge is available about the biochemistry and function of the pancreatic microtubular and microfilamentous systems, it may be possible to develop drugs that will increase granule discharge. It is abundantly clear that there are many exciting avenues opening up for the development of drugs that will stimulate insulin secretion. Their development, however, must be based on increases in our fundamental knowledge about the mechanisms of insulin secretion and the defects in it that are characteristic of the diabetic state.

The only useful drugs currently available that stimulate insulin secretion are the sulfonylureas. It is somewhat surprising that after 20 years, we still do not know how they stimulate insulin secretion, the extent of their usefulness in the treatment of patients with maturity onset diabetes mellitus, the mechanism of their chronic antidiabetic action (pancreatic versus extra-pancreatic), nor the hazards associated with their chronic use (29,34,42,81,94,95). In spite of some suggestions that the newer generations of sulfonylurea drugs are different than the original ones (78), most studies indicate that their clinical usefulness will not be much different, and it seems unprofitable to continue developing additional analogues until answers are obtained to the questions posed above.

POTENTIAL VALUE OF THIS RESEARCH

The potential value of this research is immense. Approximately four million Americans have diabetes mellitus that needs treatment. Many millions more will eventually develop the disease. Oral agents that could stimulate insulin secretin and/or correct the insulin secretory defect in diabetes mellitus would revolutionize the treatment. One could hope to be able to more easily normalize the blood sugar or even perhaps prevent the development of the disease. The effect that this would have in minimizing the complications and sequelae of the disease have been covered in other sections of this monograph.

The basic information on the control of the secretory process would be useful in many other areas of medicine and biomedical research.

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DRUGS ALTERING CARBOHYDRATE AND LIPID METABOLISM

Bernard Robert Landau

INTRODUCTION

With increasing delineation of the biochemical alterations that occur in diabetes mellitus, new pharmacological approaches to its therapy become possible. These approaches differ in the available data supporting their rationale, the biological systems for testing a potential agent, and the "leads" and agents already at hand. In Table 1 many of these leads and agents are grouped according to their presumed mechanisms of action.

While the approaches that follow are referenced in terms of the biochemical process to which the pharmacological agent is or would be directed, alterations in one process invariably result in the alterations in other processes, since these are integrated. Many of the pharmacological agents now under study, while presumed to act on one process, appear to act at several loci, and the primary site of action for most leads or even established agents is still uncertain.

The agents used clinically and other leads now available are inhibitors. The approaches proposed are therefore generally directed toward inhibition rather than stimulation of processes. Inhibitors of biochemical processes are much easier to develop than stimulators, presumably because of the high specificity usually encountered in the activation of biological processes. Progress toward agents which, for example, mimic insulin action on membranes or directly alter an enzyme to increase its activity, will depend upon further definition at the molecular level of these processes and the ingenuity of the chemist using this information in synthesizing compounds that reproduce their function. Further, agents may have to be developed selective in their action toward a given tissue. Despite the knowledge we already have of mammalian membrane structure, transport processes, and hormone action, our knowledge is still woefully inadequate for the design of such agents.

Perhaps more important for any approach toward the prevention, retarding, or reversal of the chronic complications of diabetes, and at least one of these should be the major objective of any approach if it is to provide a major addition to the therapy of diabetes mellitus, is the need for models by which potential agents can be tested for their effectiveness. While measurements in man of basement membrane thickening, conjunctival vessel alterations, etc., may be employed, animal models are needed which allow measurement of responses to agents over shorter durations than in man. Toward this end, lesions similar to those seen in human nephropathy and retinopathy have been reported in animals, but the utility of these preparations is yet to be established.

Most important, the development through basic laboratory procedures of potential agents for the prevention and/or therapy of diabetes mellitus may not require the largest fraction of the investment in manpower and financial resources. As is illustrated by the University Group Diabetes Program, the establishment of efficacy for a drug will almost certainly require a very large investment over many years. Therefore, the finding of "leads" may not prove to be the largest obstacle to success, but rather it may be whether an individual pharmaceutical company or any other

TABLE 1. Therapy of Diabetes Mellitus

Presumed Mechanism of Action	Compound(s)	References
Inhibiting Glucose Absorption	Phenformin	Kruger et al., 1970; Caspary and Creutzfeldt, 1973
Inhibiting Gluconeogenesis	Quinolinic Acid	Veneziale et al., 1967
	Biguanides	Haeckel and Haeckel, 1972; Altschuld and Kruger, 1968
	Pent-4-enoic Acid	Ruderman et al., 1970, Toews et al., 1970
	Metyrapone	Henke and Doe, 1967
Enhancing Insulin Action	Trypotophan	Mirsky et al., 1957
	Indole Acetic Acid	Mirsky and Diengott, 1956
	Biguanides	Davidoff, 1973
Inhibiting Glucagon Release	Diphenylhydantoin	Gerich et al., 1972
Inhibiting Glucagon Action	Des-Histidine Glucagon	Lande et al., 1972
Inhibiting Growth Hormone Release	A Polypeptide	Brazeau et al., 1973
Inhibiting Respiration	Biguanides	Williams et al., 1967; Davidoff, 1968
	Dinitrophenol	Steward and Hanley, 1969
	Salicylates	Steward and Hanley, 1969
	Clofibrate	Hoppel, 1973
Inhibiting Lipolysis	Nicotinic Acid	Carlson, 1969
	Propanolol	Hoppel, 1973
	Dimethylpyrazole	Gerritsen and Dulin, 1965; Hollobaugh et al., 1967
Inhibiting Fatty Acid Oxidation	Pent-4-enoic Acid	Toews et al., 1970; Corredor et al., 1968
	(+)-Decanoylcarnitine	Williamson et al., 1968; Williamson et al., 1969; McGarry and Foster, 1973
	Diphenylene iodonium	Steward and Hanley, 1969
	Dichloroacetic Acid	Stacpoole and Felts, 1971
Inhibiting Aldose Reductase	Glutaric Acid Derivatives	Gabbay, 1973; Morrison and Winegard, 1973; Gabbay and Kinoshito, 1972
Inhibiting Cholesterol Synthesis	Clofibrate	Bergquist, 1970; Harrold et al., 1969
Unknown	Pyridinium Chlorides	Fanshawe et al., 1970; Blickens and Riggi, 1969
	γ-guanidinobutyramide (HL 523)	Butterfield et al., 1969; Schless et al., 1970

similar sized group has the capacity to perform the required clinical testing and, if not, whether other long-term support can be established so that the finding of new pharmacological agents of real benefit can be brought to fruition.

APPROACHES

The approaches that follow (Fig. 1) can be classified in terms of decreasing glucose production or increasing glucose utilization, either one of which will lower blood glucose concentration. In the former group would be the approach of (I) decreasing the absorption of glucose, and (II) decreasing glucose formation (gluconeogenesis) either by inhibiting the catalysts (enzymes) required for formation, or decreasing the availablity of precursors (substrates) needed to form the glucose. In the latter group would be the development of an agent (III) mimicking the acting of insulin, (IV) preventing or retarding the destruction of insulin when present, (V) preventing hormone actions that are counter to insulin's action, (VI) decreasing the yield of energy from glucose metabolism (respiration) so that more glucose would have to be utilized to provide an equivalent quantity of energy, and (VII) preventing the utilization of fat by inhibiting the breakdown of fat (lipolysis) or (VIII) its metabolism (fatty acid oxidation) so that increased quantities of glucose would have to be used as a substitute for the fat and (IX) decreasing blood glucose and/or fatty acid concentration through regulatory controls in the central nervous system. Inhibition of specific pathways of glucose utilization, that is inhibition of (X) the polyol pathway and (XI) glycoprotein formation, and (XII) decreasing cholesterol and triglyceride formation are approaches directed toward preventing the formation of components believed to contribute to the long-term complications seen in the diabetic. A broad screening approach (XIII) would encompass all of the above approaches and others not recognized.

I. GLUCOSE ABSORPTION

Rationale: If glucose absorption is decreased, blood glucose concentration will rise less on carbohydrate ingestion.

Discussion: The diabetic should ingest a normal quantity of carbohydrate, since there is evidence that when a low quantity of carbohydrate is ingested, glucose tolerance, that is the ability to utilize glucose, is impaired. Further, with the inhibition of absorption of any significant quantity of carbohydrate, diarrhea should ensue. This approach then has very little, if any, promise.

Systems: A number of procedures for measuring glucose transport in vitro (intestinal sacs, strips, etc.) and in vivo (intestinal intubations) are well established.

Leads: Phenformin (DBI^{R}) has been shown to inhibit glucose absorption in man (18,56). Since intestinal glucose transport is energy dependent, decreased absorption may be consequent to the effect of phenformin on the respiration (see Approach VI) of the intestinal epithelium. In accord with this, intestinal amino acid transport is also inhibited (18). The effect on intestinal transport is unlikely to contribute significantly to phenformin's overall hypoglycemic effect.

II. GLUCONEOGENESIS

Rationale: Since the diabetic's elevated blood glucose concentration is due to overproduction as well as underutilization of glucose, decrease in glucose formation should lower blood glucose concentration. Glucosuria should then be diminished and, if the chronic complications of diabetes are due to elevated blood glucose concentrations, these should be lessened. Since the

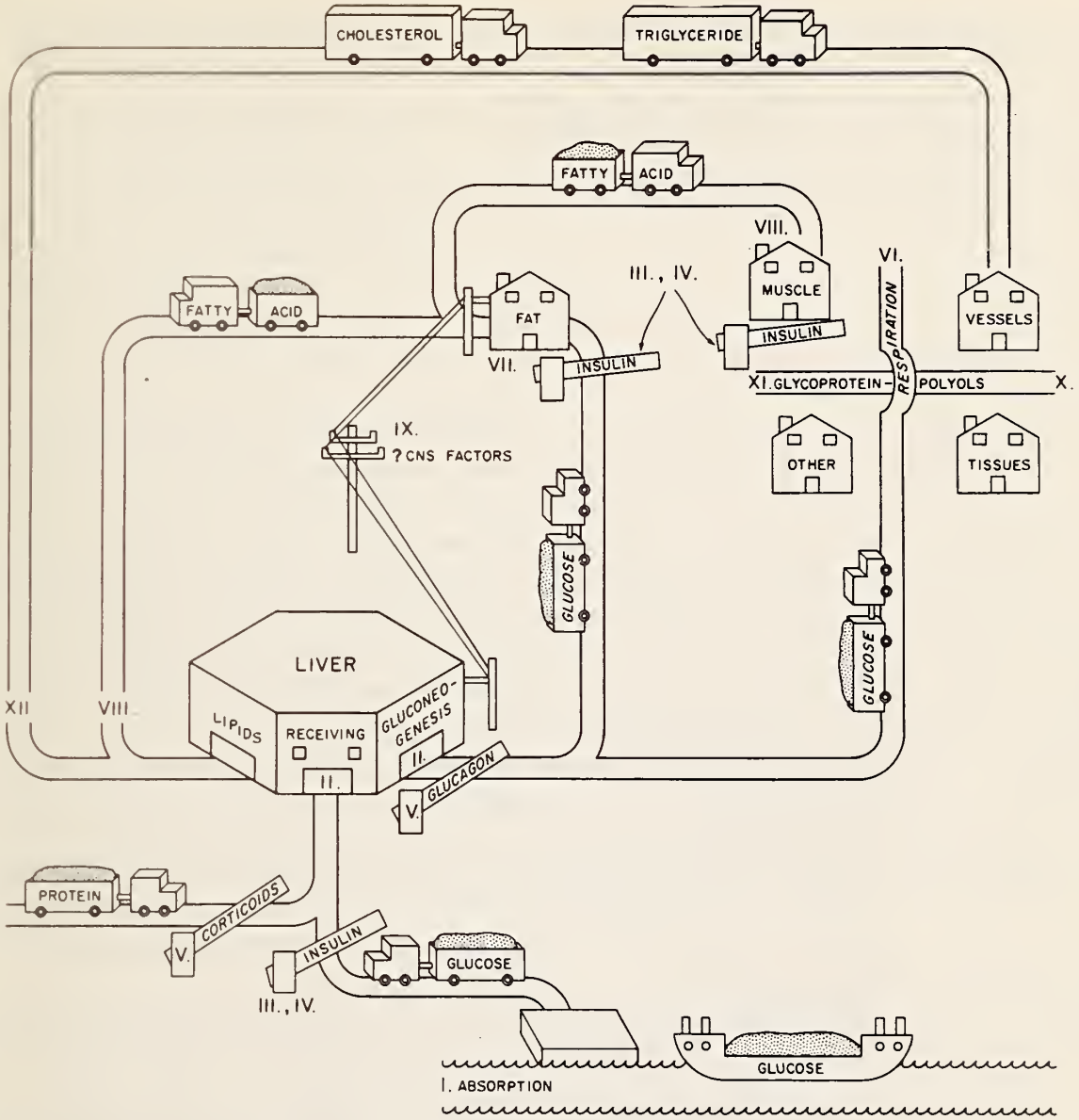

FIGURE 1. Possible sites of action (I - XIII) of drugs altering carbohydrate and lipid metabolism.

liver is the prime site of glucose formation (the kidney and possibly intestine participating under some circumstances), an inhibitor of hepatic glucose formation should be sought.

Discussion: Since the defect in the diabetic is also decreased glucose utilization, this approach in general is not attractive for the diabetic. An elevated blood glucose concentration, at least at concentrations near 300 mg percent or 400 mg percent, favors through mass action glucose utilization at a similar rate to that at 100 mg percent in the presence of insulin (78). While glucose utilization at these elevated concentrations will be directed relatively more in the diabetic than in the normal to insulin-independent tissues, a lowering of blood glucose concentration solely by decreasing production of glucose in the diabetic should result in a still further decrease in glucose utilized by the peripheral tissues, muscle, and fat. Thus, increased mobilization of fatty acids, inhibition by fatty acids of glucose utilization by muscle, and increased ketone formation by liver would be expected. These expectations are perhaps realized in alcoholics who present themselves in ketoacidosis, but with relatively low blood glucose concentration, ethanol presumably having decreased gluconeogenesis. Administration of glucose and fluids is often the only required treatment. Inhibition of gluconeogenesis may prove of some therapeutic value in such acute emergencies as the occasional hyperosmolar coma, where glucose concentrations of 800 mg percent or more are encountered, but insulin and fluids seem to offer for these circumstances a much better therapeutic approach. A decrease in blood glucose concentration could attenutate some of the load on the beta cells of the pancreas and could reduce the diversion of glucose to such pathways as the polyol pathway (see Approach X).

Systems: Key enzymes in the control of gluconeogenesis have been identified, isolated, and purified so that screening for agents that inhibit them is possible. Much is already known of the processes regulating gluconeogenesis so that alterations in the activities of concentrations of natural factors which stimulate or inhibit gluconeogenesis should be possible. Liver (and kidney) slices and perfusion systems have been refined and are available for screening for, and testing of, potential agents, and there are procedures for measuring glucose production in animals and man both directly and using isotopes as tracers.

Leads: Ethanol administration decreases blood glucose concentration presumably by decreasing the availability of substrate for gluconeogenesis. Its ability to decrease glucose production has been shown in liver slices and its ability to lower blood glucose concentration has been shown in man (47). Quinolinic acid inhibits phosphoenolpyruvate carboxykinase, a key enzyme required for gluconeogenesis, and in accord with this it inhibits glucose formation from pyruvate by liver (87).

Phenformin, along with its other possible modes of actions (see Approaches I, IV, and VI), has been shown to inhibit gluconeogenesis in perfused liver of the rat and guinea pig (1,45). Phenformin is the phenylethyl derivative of biguanide and is prescribed in the United States as an oral hypoglycemic agent for the management of the diabetic. Other derivatives of biguanides are prescribed in other countries. They are similar in their actions, although potencies vary.

In man phenformin increases glucose utilization. This appears to be associated with an increase rather than a decrease in glucose production (55,60,61). However, it is postulated that the increase in glucose production is less than would normally be expected in response to the increased utilization, and thus phenformin's inhibition of gluconeogenesis contributes to its hypoglycemic action.

Pent-4-enoic acid also inhibits hepatic gluconeogenesis (73,85), but this appears to be through its primary action of inhibiting fatty acid oxidation (see Approach VIII).

Antagonists of glucocorticoids would be expected to inhibit gluconeogenesis, but the likely response of the pituitary then to increase glucocorticoid production and the dangers of inadvertently producing adrenal insufficiency with an excessive dose, if effective, would probably severly limit such an agent. Metyrapone, which reduces cortisol production by inhibition of adrenal 11-β-hydroxylation, could serve as a lead (49).

III. INSULIN MIMICS

Rationale: Since diabetes mellitus is a disease of relative insulin deficiency, an agent which had all the actions of insulin and could be taken orally would provide effective and convenient therapy.

Discussion: The extent of the contribution such an agent would make depends upon whether insulin deficiency occurs concomitant with the occurrence of diabetic vascular complication or is responsible for the complications. An oral agent that mimics insulin's action would be presented first to the liver, as is the case for insulin released from the pancreas, rather than to peripheral tissues as occurs with exogenous insulin administration. The difference between parenteral and oral administration may be significant in the control of blood glucose concentration and in the prevention of complications. As a minimum such an oral agent would offer a convenience not possible with parenteral administration.

Systems: Preparations containing insulin receptors have now been isolated (37,77). Some information on the structure of the membrane in relation to the receptor are at hand. There are many systems in vivo and in vitro for characterizing the actions of insulin.

Leads: Insulin itself, and various preparations of insulin (36) have been given orally but, while there is some absorption, their use appears impractical because of extensive destruction in, and very limited absorption of, polypeptides by the intestinal tract.

IV. ENHANCING INSULIN'S ACTION

Rationale: An alternative approach to the enhancing of insulin secretion and synthesis in the maturity onset diabetic would be an agent that increases the effect of whatever quantity of insulin is present. One such approach would be through the inhibition of insulin degradation. Since the largest fraction of insulin degradation appears to be in the liver, with the largest portion of the remainder occurring in the kidney and intestine, agents could be sought which inhibit degradation at these sites. Alternatively, agents could be sought which increase the effect of insulin at its site of action.

Discussion: The maximum effect of such an agent is limited by the maximum quantity of insulin being secreted by the pancreas. Thus, such an agent should not be effective in the juvenile diabetic.

Systems: A protease with reasonable specificity for insulin was demonstrated by Mirsky et al. (65). A glutathione transhydrogenase in liver has been shown to cleave the A and B chains of insulin and is believed to participate in the initiation of insulin breakdown, but the enzyme may also catalyze other reactions (52). Recently proteases, claimed to be relatively specific for insulin degradation (12,13), although glucagon is also degraded (27), have been reported to be present in muscle as well as in kidney and liver. Techniques for measuring insulin by

immunoassay and its degradation using iodine tracers are available. The effects of insulin on many isolated tissue preparations, as well as intact animals, have been demonstrated, and agents could be screened in these systems to see if they enhance the effects.

Leads: Tryptophan inhibits insulinase and reduces glucose concentrations in normal but not in the alloxan-diabetic rat (65). Other compounds containing the indole ring also have produced hypoglycemia in animals (64). Biguanides in low concentrations have been reported to augment or amplify insulin action. They have been hypothesized to do this through an action on metal binding sites on membranes (24) (and plasma membrane preparation may be used for initial screening of agents acting in this manner).

V. INHIBITION OF COUNTER REGULATORY HORMONE SECRETION AND ACTION

Rationale: Several hormones are known to counter insulin action, and therefore there would be enhanced insulin action if any of these were inhibited. Evidence has accrued that there may be exaggerated effects of counterregulating hormones in diabetics.

Discussion: Two hormones appear from present information to provide the most suitable targets. Glucagon has been hypothesized to play an essential role in the pathogenesis of diabetes mellitus (86). There is a decreased suppression of glucagon by glucose in the diabetic, relative or absolute hypergluconemia has been identified in every form of endogenous hyperglycemia, and insulin lack when glucon is suppressed does not cause endogenous hyperglycemia nor ketoacidosis (39,41). Inhibition of glucagon's release would then be expected to ameliorate or prevent the disease. A similar statement can be made for growth hormone which has been implicated in the onset of ketosis in the diabetic, has been shown to respond excessively to exercise in the diabetic, and has been proposed to play a role in the development of diabetic retinopathy. Glucocorticoid antagonists are considered in Approach II.

Systems: Glucagon receptor preparations are now being developed which could allow screening for agents in vitro. Immunoassays for growth hormone and glucagon exist.

Leads: Diphenylhydantoin (Dilantin^{R}) inhibits glucagon secretion in vitro, and also inhibits insulin secretion (38). In maturity onset diabetics, diphenylhydantoin can exacerbate hyperglycemia, but in juvenile diabetics, since the beta cell is inoperative, diphenylhydantoin might be beneficial. Derivatives of glucagon are also shown which inhibit its action (57). L-Dopa stimulates growth hormone (9) so that analogs inhibiting this action might be sought.

Most attractive as a lead at present is a polypeptide from ovine hypothalamus that inhibits growth hormone secretion (10) and is called somatostatin (11). It also inhibits the release of insulin and glucagon (54). Because of the roles hypothesized for glucagon and growth hormone in the diabetic syndrome, clinical studies have begun. It was in using somatostatin that evidence was obtained that glucagon is essential for the development of ketoacidosis (41). Its effect on ketoacidosis may be at least in part through direct inhibition of hepatic ketogenesis rather than via glucagon suppression (40). Somatostatin has also been reported to suppress growth hormone levels in acromegalies (93) and insulin hypersecretion in a patient with pancreatic islet cell carcinoma (22). Evidence has been obtained for the presence of somatostatin in human as well as rat brain and pancreas (68,69).

Since somatostatin is a naturally occurring polypeptide, it might be expected to have less toxicity than chemicals foreign to the human body. Toxic effects of somatostatin on platelet aggregation appear to be of concern at concentrations above those being used in the clinical

studies (28). While somatostatin has been said to perhaps be "twice blessed" by inhibiting both growth hormone and glucagon hormone levels, its half life in blood is less than four minutes, so that long-term clinical trials directed in particular toward the therapy microangiopathy await the preparation of a suitable long-acting derivative (15). In addition, analogs of somastatin which are specific for causing inhibition of glucagon release and inhibition of growth hormone release need to be synthesized and tested.

The gestagen, medroxyprogesterone acetate, has been reported through its action upon pituitary function to diminish and confine the progressive course of diabetic retinopathy (16).

RESPIRATION

Rationale: Since the oxidation of glucose to carbon dioxide and water yields about 18 times as much useful energy in the form of ATP as its metabolism to lactate, if respiration (the utilization of oxygen with the production of ATP) is reduced, the body must utilize more glucose to produce similar quantities of energy.

Discussion: The process to be inhibited is critical process for maintenance of body function, and an agent affecting the process would be expected to have dangerous side reactions. For the success of such an approach one might have to be selective in the tissues affected, since certain tissues may be harmed by not being able to increase the utilization of glucose adequately in response to the inhibition.

Systems: Systems in vitro (mitochondria) and in vivo for measuring effects on respiratory function are available.

Leads: Phenformin inhibits respiration in vitro and has been postulated to produce its hypoglycemic effect in vivo by this mechanism (23,82,88). While this effect, as noted above, on theoretical grounds might be considered dangerous, phenformin has proved to be remarkably safe (without including here a consideration of the results of the University Group Diabetes Program Study). Lactic acidosis may be exacerbated by phenformin administration.

Dinitrophenol uncouples respiratory function; that is, oxygen consumption continues in its presence but with a lesser yield of ATP. Salicylates, as aspirin, also uncouple oxidative phosphorylation. Both these compounds lower blood glucose concentrations in animals and in man under selected conditions, but of themselves they do not have any potential as hypoglycemic agents (82). There is a suggestion that salicylates reduce the incidence of diabetic retinopathy (70), but this could be associated with their effect on platelet adhesion (48,67). Clofibrate, a cholesterol and triglyceride lowering agent, recently has also been shown to inhibit respiration (51).

VII. LIPOLYSIS

Rationale: Since ketosis requires mobilization of fatty acids, and since increased fatty acid concentrations may decrease glucose utilization and stimulate glucose production, the inhibition of lipolysis should reverse these.

Discussion: This is a most reasonable therapeutic approach.

Systems: Lipases from adipose tissue and the isolated fat cell, as well as fat pad preparations, can be used for screening. Potential agents, when found, can be tested in intact animals for their ability to inhibit fatty acid release.

Leads: Nicotinic acid has been used, both with only limited success in the diabetic (17). Suppression of lipolysis with nicotinic acid has recently been reported to abolish the nocturnal

rise in plasma triglyceride concentrations that occur with carbohydrate induction, but the practical application of these observations is uncertain (75). Propanolol, a beta adrenergic block, presumably acts through inhibition of epinephrine action on adipose tissue cyclic AMP dependent lipase. Propanolol may be of use in the prevention of ketoacidosis in the brittle diabetic (4). A large number of other β-adrenergic blocking agents have been prepared. 3',5' dimethylpyrazole reduces lipolysis in adipose tissue, but does not decrease fasting blood glucose concentrations, although it does reduce blood glucose concentrations in animals pretreated with glucose (42,50). A number of other compounds inhibit lipolysis, but they have not been reported to decrease glucose concentration (82). Antagonists to the naturally occurring fat mobilizing substance(s) (see section on Weight Reduction), if they are of importance in lipid regulation and are better characterized, may offer an additional lead. The fat mobilizing substances have been reported to be diabetogenic (59).

VIII. FATTY ACID OXIDATION

Rationale: Blocking fatty acid oxidation results in lowered blood glucose concentrations.

Discussion: This approach is supported particularly through the elucidation of the mechanism by which the ingestion of unripened ackee fruit results in hypoglycemia. Hypoglycin in the fruit is converted to an acid which inhibits many enzymatic processes and, in addition, is converted to a carnitine derivative, decreasing available free carnitine. Since carnitine is required for transport of fatty acids into the mitochondria, their oxidation is depressed (21). This results in decreased gluconeogenesis. Hypoglycin inhibits the enzyme catalyzing the oxidation of the amino acid, leucine, and the resulting accumulation of a compound, isosaluric acid, may account for the symptoms following hypoglycin's ingestion (84). The mechanism by which the decreased fatty acid oxidation leads to decreased gluconeogenesis is not certain (21,85).

Systems: The biochemical steps in fatty acid oxidation have been delineated, and these offer systems for screening.

Leads: The toxic effect of hypoglycin precludes its use (19). Pent-4-enoic acid has served as an analog for hypoglycin in studies of its action(21,73,85) (+)-decanoylcarnitine inhibits long-chain acylcarnitine transferase, the enzyme involved in the transport of the fatty acids, and it has been shown to inhibit hepatic ketogenesis in the isolated liver and in ketotic alloxan diabetic rats (63,89,90). When combined with insulin, it produced more of a fall in plasma ketone concentrations than with either agent alone. While the (+)-decanoylcarnitine had no effect on plasma glucose concentrations, it enhanced the hypoglycemic effect of insulin in anesthetized rats. Carnitine itself has been shown to prevent starvation ketosis in children (44). Dichloroacetic acid lowers blood glucose concentration in diabetic animals and the mechanism of its action is evidenced by its inhibition of fatty acid oxidation by an adipose tissue preparation (80).

IX. CENTRAL NERVOUS SYSTEM REGULATION

Rationale: There has been for over a century evidence that the central nervous system contributes to the regulation of blood glucose concentration. Recently, stimulation of the ventromedial hypothalamus has been shown to increase blood glucose and insulin glucagon concentration, but not insulin concentration (5,32). Stimulation of the premammillary but not the ventromedial area increased concentrations of plasma-free fatty acids (5). There is evidence for an insulin-sensitive center in the central nervous system (25,26,83) (see section on Weight Reduction). The stimulation of a receptor in the ventromedial hypothalamus has been postulated to activate

neurons which, via the sympathetic system as well as by direct neural action of the liver, participate in blood glucose regulation (32). Agents which affect this system could result in lower blood glucose concentration, increased insulin secretion, and decreased lipolysis.

Discussion: While the recent data indicate the central nervous system may have a major role in the regulation of glucose metabolism, the system is still too vague for the designation of specific sites within it that can be approached chemically.

Systems: Models available require either lesions in or stimuli to the central nervous system or infusions via the circulation of substances into the central nervous system, and all in the whole animal. These appear tedious to use in any screening program involving significant numbers of substances.

Leads: The evidence for the role of the sympathetic system (32,43) appears to offer at present the most likely leads, examining agents which alter autonomic nervous system activities.

X. POLYOL PATHWAY

Rationale: If sorbitol accumulation is responsible for at least some of the complications of diabetes mellitus, then preventing its formation should prevent these complications (35). Since enhanced formation is consequent to an elevation in blood glucose concentration, this would be accomplished by returning blood glucose concentrations to normal. Alternatively, the conversion of glucose to sorbitol could be inhibited.

Discussion: Whether sorbitol does play a role in the development of complications in human diabetes mellitus, and if so to what extent, is uncertain. There is also the claim that the polyol pathway is required for the stimulation of insulin release from the beta cell. Indeed, sorbitol has been reported to stimulate insulin release and an inhibitor of aldose reductase, the enzyme catalyzing the conversion of glucose to sorbitol, has been reported to inhibit glucose stimulation of insulin release (34). If so, then an inhibitor of sorbitol formation could decrease insulin secretion in the maturity onset diabetic and consequently increase blood glucose concentration and obviate or reverse the inhibitor's potential benefits. In the juvenile diabetic, where beta cells are no longer functional, this would not be a concern. While aldose reductase from different tissues from the same species appears to be immunologically similar, an inhibitor that is specific for tissues other than islet tissue could be sought.

Systems: Aldose reductase from several tissues has been identified, isolated, and purified. Agents inhibiting it can be tested for their ability to prevent sorbitol accumulation in vitro in lens, kidney papilla, aorta, nerve, placenta, etc., or in vivo using nerve conduction perhaps as a measure of response.

Leads: Glutaric acid derivatives have been reported to inhibit aldose reductase and in vivo to prevent cataracts and improve nerve conduction observed with galactose administration are presumably due to dulcitol formation from galactose via the polyol pathway (33,66). Industrial sources are making aldose reductase inhibitors available (33,53).

XI. GLYCOPROTEIN SYNTHESIS

Rationale: With the beginnings of characterization of the glycoproteins composing the thickened basement membrane of the glomeruli of the kidney in the diabetic, and with evidence of increased enzymatic activity consistent with an excess of carbohydrate units in the glycoproteins (6,79) an approach through inhibition of synthesis of the glycoproteins becomes feasible. Related

to this approach is the suggestion made some years ago of an increased activity of the glucuronic acid pathway in the diabetic, a pathway by which some of the carbohydrate moieties of glycoproteins are synthesized (91).

Discussion: As yet the relationship of the studies made on kidney basement membranes from other tissues is unknown, and details of the regulation of the enzymatic process are not available. An increase in the activity of the glucuronic acid pathway has not been established, although inhibition of its activity could conceivably be effective.

Systems: Methods for measuring basement membrane composition, enzymatic activities, estimating the glucuronic acid pathway, and the concentrations of its intermediates in blood have been reported. Animals with vascular lesions similar to those seen in the diabetic human are being reported (30,72,92).

Potential Leads: None.

XII. TRIGLYCERIDE AND CHOLESTEROL SYNTHESIS

Rationale and Discussion: Since the accelerated atherosclerosis manifest in the diabetic is associated with elevated concentrations of triglycerides and cholesterol, the same agents now being evaluated for the prevention or retarding of atherosclerosis in the normal individual, through their effects on triglyceride and cholesterol metabolism, should have applicability to the diabetic individuals.

Systems: The same models employed in atherosclerosis studies are available, as well as diabetic animals where elevated cholesterol and triglyceride exist.

Leads: Clofibrate (Atromid^{R}) and its derivatives appear to be most promising (7). Clofibrate may alter the progression of diabetic retinopathy (46).

XIII. MULTIPLE BIOCHEMICAL PROCESSES

Rationale: In addition to synthesizing and screening for agents with their design directed toward specific biochemical processes, broad screens are possible for hypoglycemic agents or agents preventing the onset of diabetes in animals known to be prediabetic or agents preventing or reversing the chronic complications of diabetes in diabetic animals.

Discussion: To seek agents which do not act through insulin synthesis or secretion, animals lacking islets must serve as models.

Systems: Alloxan-induced and streptozotocin-induced diabetic rats are the most readily available diabetic animals. Pancreatectomized or partially pancreatectomized animals can also be used. Spontaneously diabetic animals may also be used (81).

Leads: In general, most of the new chemical structures having hypoglycemic properties reported by the pharmaceutical industry appear to have been detected by broad screens. The compounds have been reported to decrease blood glucose concentration in either alloxan-induced or streptozotocin-induced diabetic rats, but in only a few instances has the biochemical mechanism of their action been localized. Where they are effective in animals lacking a pancreas, they are compared in terms of their prototype, the biguanides, now in clinical use, rather than the other prototype, the sulfonylureas. Pyridinium compounds are examples of such potential hypoglycemic agents (8,31).

A large number of drugs with established actions other than hypoglycemia have on occasion produced hypoglycemia in man (76). A compound with hypoglycemic properties has been reported to lower urea concentrations in the diabetic (14,74) and allows increased protein intake without

increased urea concentrations. Elevated urea levels occur consequent to kidney damage, but such an agent would appear to offer little during the critical period prior to the onset of complications, and alternate therapy is available for management of the uremia. There is no evidence the compound improves kidney function.

WEIGHT REDUCTION

Rationale and Discussion: Obesity will be considered in another monograph. There is no reason to believe that obesity in the diabetic is any different than in the nondiabetic, except that obesity can result in, or enhance the manifestations of, diabetes. Weight reduction's benefits include not only improved glucose tolerance but also decreased cardiovascular stresses.

Various pharmacological approaches can be considered for achieving weight reduction. Prevention of intestinal absorption of food is possible, but the production of the malabsorption syndrome is an extreme which, while used in desperate situations, is unlikely to have general applicability. The uncontrolled diabetic can lose weight from the disease itself, and it is possible to induce diabetes, as well as increase its severity (reversibily as with 2-deoxyglucose, diphenylhydantoin, etc.). Following a selected period of weight loss, diabetic control could then be instituted. However, the weight loss would be through glucose loss in the urine, and large quantities must be excreted to achieve weight loss with attendant discomfort, risk of urinary tract infection, loss of electrolytes, and possibly ketosis. Ketosis with its resulting anorexia could contribute significantly to the weight loss, but this would only be with all its attendant risks. Further the weight loss would be of lean body mass as well as of adipose tissue.

Overall, an agent controlling appetite remains the most reasonable pharmacological approach to weight reduction. However, there is no evidence that the obese diabetic has any more of a recognition of hunger and satiety than the nondiabetic obese individual, nor that psychological problems are any less. There is the suggestion that obesity is produced through increased gluconeogenesis, and this is a stimulus to increased insulin secretion (3). This is postulated to result in the increased lipid deposition, producing a vicious cycle leading to weight gain. However, hormonal and biochemical changes thus far observed in obesity have been shown to be secondary to exogenous obesity.

Appetite control in the diabetic may perhaps be different from normal. While appetite control is undoubtedly dependent on multiple stimuli to the central nervous system, the diabetic is polyphagic despite his elevated blood concentration which, in keeping with the glucostat theory of Mayer (62) would be expected to produce satiety.

There is evidence that the satiety center, at least in the rat, is responsive to insulin (25, 26,83). In the insulin-lacking juvenile diabetic there would be no insulin to direct adequate glucose into the satiety center. The insulin resistance demonstrated in several tissues of obese diabetics may also exist for their satiety center. The anorexia of ketosis could then reflect perhaps in part the providing of ketone bodies as nutrients to the satiety center. This would be in accord with the recent demonstration that ketone bodies as well as glucose can be metabolized by the brain. Much more must be done to elucidate the mechanism of appetite control, but from the evidence at hand the seeking of agents which increase glucose utilization by the satiety center seems attractive.

A number of fat-mobilizing substances have been reported present in the hypothalmic-pituitary axis and in urine (59,71). A naturally occurring substance which mobilized lipid, that is, produced

lipolysis, would be expected to result in weight reduction in an individual, if the mobilized fatty acids then provided the caloric needs of the individual, and as a result his caloric intake decreased. It is reasonable to expect that with increased blood free fatty acid concentrations (and perhaps increased ketone concentrations) appetite would decrease.

Systems: A satisfactory model to screen for appetite inhibitors is yet to be reported. The blood brain barrier, the time-consuming nature of electrode implants in the brain, etc., have thus far restricted its development. The models available include animals trained to eat their meals in short periods and to whom compounds are administered to see their effect on this ingestion. This is gross, and a host of toxic effects of the compounds are encountered. Gold thioglucose treated rats have their satiety center destroyed and therefore do not provide a model for study of agents acting on the center. Fat-mobilizing substances can be identified by their ability to enhance fatty acid (or glycerol) release from adipose tissue in vitro and to increase fatty acid concentrations in vivo.

Leads: Biguanides have been claimed to decrease appetite in the diabetic in the absence of the anorexia they produce. The weight loss they produce is small (2,20), and for them, as for the amphetamines and their derivatives, the duration of effect is short. The existence of the fat-mobilizing substance(s) have proved difficult to confirm and characterize. However, polypeptides from the pituitary and hypothalamus with fat-mobilizing properties have been isolated and the establishing of their structure may be near at hand. Being polypeptides, they almost certainly will be ineffective orally and their synthesis in any quantity seems unlikely. If, however, they can be shown to produce weight loss, perhaps first in experimentally obese animals, and their molecular site of action established, reasonable attempts to obtain an agent mimicking their action can be undertaken.

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