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ENDOCRINE and NEUROENDOCRINE MECHANISMS of AGING

Richard C. Adelman
George S. Roth

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Endocrine and Neuroendocrine Mechanisms of Aging

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PREFACE

One of the most important and fastest growing areas of biogerontological research is that of neuroendocrine and endocrine regulation. This fact is not so surprising since such regulation concerns nearly all physiological functions, many of which exhibit decrement with increasing age. In addition, these studies help to bridge the gap in aging research between the molecular and the physiological. Sound bodies of data exist both with respect to the molecular bases of hormone action and the varied mechanisms by which neuroendocrine and endocrine factors regulate normal physiology.

It is the purpose of this volume to present a representative sampling of those neural and hormonal studies which have been the focus of the most intense interest in recent gerontological research. To this end we have been fortunate to enlist the aid of some of the most competent and innovative investigators in the field. More than this, however, an attempt has been made to provide detailed methodological as well as theoretical evaluation of the areas considered. It is our hope that those researchers interested in this area of regulation during aging will be able to utilize the information contained herein as a basis both for critical analysis as well as for the designing and execution of further experiments in this most important area.

Richard C. Adelman
George S. Roth

EDITORS-IN-CHIEF

Dr. Richard C. Adelman is currently Executive Director of the Temple University Institute on Aging, Philadelphia, Penn., as well as Professor of Biochemistry in the Fels Research Institute of the Temple University College of Medicine. An active gerontologist for more than 10 years, he has achieved international prominence as a researcher, educator, and administrator. These accomplishments span a broad spectrum of activities ranging from the traditional disciplinary interests of the research biologist to the advocacy, implementation, and administration of multidisciplinary issues of public policy of concern to elderly people.

Dr. Adelman pursued his pre- and postdoctoral research training under the guidance of two prominent biochemists, each of whom is a member of the National Academy of Sciences: Dr. Sidney Weinhouse as Director of the Fels Research Institute, Temple University, and Dr. Bernard L. Horecker as Chairman of the Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, N.Y. His accomplishments as a researcher can be expressed in at least the following ways. He is the author and/or editor of more than 70 publications, including original research papers in referred journals, review chapters, and books. His research efforts have been supported by grants from the National Institutes of Health for the past 10 consecutive years, at a current annual level of approximately $300,000. He continues to serve as an invited speaker at seminar programs, symposiums, and workshops all over the world. He is the recipient of the IntraScience Research Foundation Medalist Award, an annual research prize awarded by peer evaluation for major advances in newly emerging areas of the life sciences. He is the recipient of an Established Investigatorship of the American Heart Association.

As an educator, Dr. Adelman is also involved in a broad variety of activities. His role in research training consists of responsibility for pre- and postdoctoral students who are assigned specific projects in his laboratory. He teaches an advanced graduate course on the biology of aging, lectures on biomedical aspects of aging to medical students, and is responsible for the biological component of the basic course in aging sponsored by the School of Social Administration. Training activities outside the University include membership in the Faculty of the National Institute on Aging summer course on the biology of aging; programs on the biology of aging for AAA's throughout Pennsylvania and Ohio; and the implementation and teaching of Biology of Aging for the Nonbiologist locally, for the Gerontology Society and other national organizations, as well as for the International Association of Gerontology.

Dr. Adelman has achieved leadership positions across equally broad areas. Responsibilities of this position include the intergration of multidisciplinary programs in research, consultation and education, and health service, as well as advocacy for the University on all matters dealing with aging. He coordinates a city-wide consortium of researchers from Temple University, the Wistar Institute, the Medical College of Pennsylvania, Drexel University, and the Philadelphia Geriatric Center, conducting collaborative research projects, training programs, and symposiums. He was a past President of the Philadelphia Biochemists Club. He serves on the editorial boards of the *Journal of Gerontology, Mechanisms of Ageing and Development, Experimental Aging Research,* and *Gerontological Abstracts*. He was a member of the Biomedical Research Panel of the National Advisory Council of the National Institute on Aging. He chairs a subcommittee of the National Academy of Sciences Committee on Animal Models for Aging Research. As an active Fellow of the Geronological Society, he is a past Chairman of the Biological Sciences section; a past Chairman of the Society Public Policy Committee for which he prepared Congressional testimony and repre-

sented the Society on the Leadership Council of the Coalition of National Aging Organizations; and is Secretary-Treasurer of the North American Executive Committee of the International Association of Gerontology. Finally, as the highest testimony of his leadership capabilities, he continues to serve on National Advisory Committees which impact on diverse key issues dealing with the elderly. These include a 4-year appointment as member of the NIH Study Section on Pathobiological Chemistry; the Executive Committee of the Health Resources Administration Project on publication of the recent edition of *Working with Older People—A Guide to Practice;* a recent appointment as reviewer of AOA applications for Career Preparation Programs in Gerontology; and a 4-year appointment on the Veterans Administration Long-Term Care Advisory Council responsible for evaluating their program on Geriatric Research, Education, and Clinical Centers (GRECC).

George S. Roth, Ph.D., is a research chemist with the Gerontology Research Center of the National Institute on Aging in Baltimore, Md., where he has been affiliated since 1972. Dr. Roth received his B.S. in Biology from Villanova University in 1968 and his Ph.D. in Microbiology from Temple University School of Medicine in 1971. He received postdoctoral training in Biochemistry at the Fels Research Institute in Philadelphia, Pa. Dr. Roth has also been associated with the graduate schools of Georgetown University and George Washington University where he has sponsored two Ph.D. students.

He has published more than 70 papers in the area of aging and hormone/neurotransmitter action, and has lectured, organized meetings, and chaired sessions throughout the world on this subject.

Dr. Roth's other activities include fellowship in the Gerontological Society of America, where he has served in numerous capacities, including chairmanship of the 1979 midyear conference on "Functional Status and Aging." He is presently Chairman of the Biological Sciences Section and a Vice President of the Society. He has twice been selected as an exchange scientist by the National Academy of Sciences and in this capacity has established liasons with gerontologists, endocrinologists, and biochemists in several Eastern European countries. Dr. Roth serves as an editor of *Neurobiology of Aging* and is a frequent reviewer for many other journals including *Mechanisms in Aging and Development, Life Sciences, The Journal of Gerontology, Science,* and *Endocrinology*. He also serves as a grant reviewer for several funding agencies including the National Science Foundation. In 1981 Dr. Roth was awarded the Annual Research Award of the American Aging Association.

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TABLE OF CONTENTS

Chapter 1

INSULIN AND GLUCOSE METABOLISM DURING AGING

Gerald M. Reaven and Eve P. Reaven

TABLE OF CONTENTS

I. INTRODUCTION

During the past several decades it has become clear that substantial changes occur in glucose and insulin metabolism during aging. Perhaps the most widely appreciated of these changes is the observation that glucose tolerance deteriorates with age. Indeed, the weight of evidence is so great, and the subject reviewed so frequently,[1–3] that it would not be useful to once again address the question whether or not aging is associated with a deterioration of glucose tolerance. Clearly it is. However, the significance of this statement is clouded by the fact that so many of the published studies of the effect of age on glucose and insulin metabolism do not consider certain methodological and theoretical questions. As a result, the authors of this chapter feel that there still exist two major unanswered questions relevant to the effect of age on glucose and insulin metabolism. The first of these is the most fundamental, and involves a decision as to whether the observed deterioration in glucose tolerance is due to age itself, or is secondary to a number of other factors which occur frequently in older individuals and may adversely modify carbohydrate homeostasis. A second, and related, question concerns the mechanism(s) responsible for the deterioration of glucose tolerance in older individuals. For example, is the observed glucose intolerance a function of an alteration in insulin secretion or of tissue sensitivity to insulin?

In this chapter, the authors will attempt to respond to the questions defined. They will review the available relevant literature, and, at the same time, point out the methodological and theoretical problems which make it difficult to come to definitive answers at this time. As such, the authors have not compiled an inclusive bibliography of experimental data related to the effect of aging on glucose and insulin metabolism. Instead, they have focused attention on what they believe to be the central issues.

The authors' inability to arrive at definitive conclusions at this time should not be discouraging. After all, experimentation on this subject has taken place over a period of many years with many different expressed goals. The utility of this presentation will be the degree to which it has successfully redefined the problems and pointed to possible experimental approaches to their solution.

II. AGE AND GLUCOSE METABOLISM

In this section the authors will attempt to review published data concerning the independent effect of age on glucose tolerance. In order to do this, it will be necessary to address two issues. The first problem will be to differentiate between the impact of age on glucose tolerance as distinguished from the effect of environmental factors well known to adversely modify carbohydrate metabolism. Once the initial distinction is made, it will still be necessary to determine if the observed changes in glucose tolerance are part of a normal and inevitable aging process, or are representative of an emerging population of individuals who develop diabetes as they age.

A. Effect of Age on Glucose Tolerance

Most of the available information concerning the effect of age on glucose tolerance is derived from studies of man. In order for such a study to be included in this evaluation, the authors of this chapter felt that certain criteria had to be fulfilled. In the first place, a substantial number of individuals had to be examined and the population studied had to cover a wide span of ages. For example, the demonstration that a group of old individuals (mean age of 70 years) had worse glucose tolerance than a group of young individuals (mean age of 30 years) is interesting, but by itself does not provide much insight as to whether or not age leads to a progressive deterioration of glucose

tolerance. In order to answer this question there must be at least three groups of patients (young, middle, and old age) in the population studied. In addition, the authors felt that all groups (regardless of age) had to consist of healthy volunteers, all of whom were fully ambulatory. Although this latter consideration attempts to take into account the possibility that a decline in physical activity could be responsible for the deterioration of glucose tolerance that occurs with age, it should be pointed out that no published study has seriously analyzed this crucial factor. Finally, the authors felt that consideration had to be given to the effects of both obesity and inadequate carbohydrate intake on the changes in glucose tolerance that occur with age.

The application of these criteria greatly reduces the quantity of available information, but enough data remains to permit some consideration of the effect of age per se on glucose tolerance in man. There is considerably less information concerning the effect of age on carbohydrate metabolism in animals, and the authors' criterion for consideration of this information was less rigorous, i.e., the study had to include a substantial number of animals with one category of animals being at least 9 months of age.

1. Human Studies

a. Oral Glucose Tolerance

There are three studies which have attempted to determine the effect of age on the plasma glucose response to oral glucose in large numbers of healthy volunteers well matched for weight. Unfortunately, no consistent results were seen. The largest population was found in the study of Wingerd and Duffy,[4] who analyzed the results of oral glucose tolerance tests in 2248 females. The subjects were divided into three groups: ages 20 to 30 (711), 40 to 49 (974), and greater than 50 (567). The mean ages of the three groups were 32.5, 44.4, and 53.7 years, and the results indicated that there was a progressive rise in plasma glucose levels over this 20-year time span. The effect was most dramatic 1 hr after the oral glucose challenge, with a mean difference in plasma glucose of 32 mg/dℓ between the youngest and the oldest group. Although there was no formal attempt to stratify by degree of obesity, the relative weight of the three groups was comparable. However, this study has at least two important confounding variables; approximately 25% of the patient population was receiving sex hormone therapy of one kind or another and all of the patients had diabetic relatives. Although Wingerd and Duffy state that statistical analysis permitted the identification of age itself as a factor which led to worsening glucose tolerance, it would seem that this population may have not been an ideal one in which to estimate the effect of age itself on glucose tolerance.

The second largest study was that of Nolan et al.,[5] who performed oral glucose tolerance tests (1.75 g/kg body weight) in 707 nonobese and 479 obese females with nondiabetic glucose tolerance test results. The ages ranged from 20 to 60 years and the patients were divided into groups by decade. Fasting blood glucose levels were significantly lower in nonobese females ages 20 to 29, as compared to those aged 30 to 39. However, there was no progressive change in the successive decades. The plasma glucose level 30 min after the glucose challenge was also significantly lower in the 20 to 29 as compared to the 30 to 39 age group, and, as before, there was no further increase with successive decades. There were no significant differences at any other time point, and there were essentially no age-related changes in plasma glucose levels seen in the obese females. At first glance these data seem to indicate that the effect of age on glucose tolerance is minimal and that any deterioration that does take place occurs relatively early in life. However, there are two fundamental drawbacks to this conclusion. In the first place, patients over 60 years were not included, and it is certainly possible that changes in glucose tolerance could occur in those over 60. Second, patients with relatively minor degrees of glucose intolerance (using a series of criteria)

were excluded from analysis. This maneuver results in what is almost a self-fulfilling prophecy; any deterioration of glucose tolerance that occurs with age is designated as the emergence of diabetes. This issue is an extremely important one, and will be discussed in detail in a subsequent section.

A third study of oral glucose tolerance in a large number of patients of all ages was carried out by Boyns et al.[6] These investigators determined the plasma glucose response of 220 healthy volunteers to a 50 g oral glucose load. The ages of the group ranged from 16 to 74, and they were selected by a stratification procedure in a manner that provided equal numbers in each age and sex group and an even distribution of body build in each of the groups. Known diabetics were excluded from the study population. In both sexes the mean blood glucose tended to rise progressively with age. In men there was a significant correlation between age and blood glucose before ($r = 0.24$), 30 ($r = 0.44$), 60 ($r = 0.54$), and 90 ($r = 0.36$) min after the glucose load. The effect of age was less dramatic in women, and significant correlations were only seen 30 ($r = 0.21$), and 60 ($r = 0.31$) min after oral glucose. The maximum quantitative effect of age was observed 60 min after the glucose challenge in both groups: mean blood glucose rose from 86 mg/dℓ in men less than 24 years to 136 mg/dℓ in males greater than 55 years, whereas the increment in females over the same age span was from 92 to 115 mg/dℓ.

Two other studies have attempted to assess the effect of age on oral glucose tolerance in a smaller number of subjects selected in order to minimize the effect of differences in degree of obesity. Kimmerling et al.[7] studied 100 volunteer subjects whose ages ranged from 22 to 69 years. They all had fasting plasma glucose levels less than 110 mg/dℓ, their relative body weights were between 0.75 to 1.20% of ideal body weight, and no correlation was noted between age and relative weight. These authors could find no correlation between age and plasma glucose, either in the fasting state or 120 min after an oral glucose challenge (40 g/m^2). The lack of correlation between age and plasma glucose level 120 min after oral glucose is consistent with the results of Boyns et al.,[6] but the two studies differ in that Boyns et al. could also show a small correlation ($r = 0.24$) between age and fasting glucose level.

Somewhat different results were found in a study by Rehfeld and Stadil[8] of 60 healthy volunteers, matched for height and weight. The group was subdivided into three subgroups of 20 subjects each (young, middle-aged, and old): ages 20 to 32 (mean = 27), 42 to 55 (mean = 48), and 65 to 81 (mean = 74). The mean plasma glucose response of the young and middle-aged groups to 50 g of oral glucose was essentially identical, suggesting that no change in glucose tolerance had occurred as patients aged from 20 to 55 years. However, there was a significant elevation in the mean plasma glucose level of the group of old subjects between 60 and 120 min after the oral glucose load.

There are several other studies in which the effect of age on glucose tolerance has been estimated in populations not matched for degree of obesity, but in which the investigators have attempted to take into account the potential effect of obesity by statistical analysis. Thus, Berger and colleagues[9] found a progressive rise in fasting plasma glucose from 91 mg/dℓ in the 3rd decade to 97 mg/dℓ in the 7th decade in a study of 263 healthy volunteers. There were statistically significant correlations between both age and fasting plasma glucose ($r = 0.25$), and age and percent of ideal body weight ($r = 0.15$). The authors state that when adjustments were made for adiposity the correlation of age with glucose persisted. Similarly, O'Sullivan and colleagues[10] found a "significant rise in blood glucose response of levels with advancing age" in approximately 150 volunteers of various ages. They also found that the degree of obesity increased with age. However, the authors state that "multiple regression analysis indicates that this rise in blood glucose levels with age remains significant." Unfortunately,

they provide no further details as to the degree of correlation between glucose and age that existed either before or after taking degree of obesity into consideration. Thus, the reports of both Berger et al.[9] and O'Sullivan et al.[10] provide support for the notion that glucose tolerance deteriorates with age. On the other hand, it is difficult to ascertain from these data the quantitative nature of the changes that are due to age itself as distinguished from those related to obesity.

Finally, there are two other reports (which indicate that age affects oral glucose tolerance) in which it is even harder to evaluate the impact of obesity. Thus, Nilsson[11] described results which are similar to those of Boyns et al.[6] in that he noted a progressive deterioration of oral glucose tolerance with age in a large group of healthy volunteer subjects. However, Nilsson's data also indicate that the relative amount of body fat increases with age, and he also noted significant correlations between plasma glucose levels during the glucose tolerance test and degree of obesity. He presents data which suggest that these differences in degree of obesity cannot entirely account for the effect of age on glucose tolerance, but it is again difficult to quantitatively assess the relative impact of age itself, as distinct from obesity, on the deterioration of glucose tolerance. Similarly Vecchio and colleagues[12] found a positive correlation ($r = 0.31$) between age and total glycemic response to oral glucose (area under the glucose tolerance curve) in 166 volunteer male prisoners between 20 and 69 years. Although these authors indicate that there was no correlation between absolute body weight and glycemic response, they provide no data as to possible age-related differences in relative degree of obesity in the population studied.

b. Intravenous Glucose Tolerance

In two studies, appropriate design permits an estimate to be made of the effect of age, as distinguished from other metabolic variables, on intravenous glucose tolerance. Franckson et al.[13] estimated the glucose disappearance rate in 325 nonobese and in 150 obese subjects (>25% over their ideal weight). These authors suggest that there is a progressive deterioration in glucose tolerance as both obese and nonobese subjects from 15 to 85 years of age. This result is even more noteworthy in that the study excluded patients with minor impairments of glucose tolerance. On the other hand, inspection of the data suggests that the results of the study may not be so clear. Thus, the tests show that in a small number of very young subjects (less than 15 years) glucose was removed more efficiently than in a small number of very old subjects (above 75 years). On the other hand, it is not so evident that the glucose disposal rate was different in subjects between 25 to 65 years. Furthermore, all subjects were hospitalized patients convalescing from benign illnesses. The possibility that these findings might be a function of state of general health of the population must be considered. Furthermore, the potential impact of a decline in physical activity in the glucose tolerance of the hospitalized elderly is also a significant problem. For all these reasons, the significance of this study is questionable.

A more satisfactory methodological approach to the question of the effect of age on intravenous glucose tolerance is seen in the study by Dyck and Moorhouse.[14] These authors studied 61 volunteers, without known diabetic relatives, all of whom were within 10% of ideal body weight. Although there appeared to be a decrease in the ability of very old subjects to dispose of a glucose load (50 g/1.73 m^2), there was no obvious change up to the age of 50.

c. Tolbutamide Tolerance Tests

Two reports have dealt with the ability of tolbutamide to lower glucose concentrations in aging populations. These reports have described dissimilar results. First, Swerdloff et al.[15] showed that there was a progressive decline in the hypoglycemic effect of in-

travenous tolbutamide with age up to the 6th decade. These studies were carried out in 100 volunteers, aged 21 to 81 years, in whom there was no family history of diabetes, no known diabetes, nor evidence of inadequate carbohydrate intake. No analysis was made of differences in degree of obesity. On the other hand, only six subjects were more than 20% overweight, and the authors state that "within the weight limits of our study group there was no influence of obesity." In contrast, Vecchio et al.[12] determined blood glucose concentration 30 min after the oral administration of tolbutamide, and could find no correlation between the rate of fall of glucose levels and age in 166 male volunteers.

2. Animal Studies

The authors of this chapter could identify only three papers which provided data on animals studied for a time span of 12 months or more. Methodologically, the report of Klimas[16] was the most satisfactory. In this study, oral glucose tolerance tests (750 mg/kg) were performed on 24-hr fasted rats (20 rats each at 1, 3, 6, 10, 14, 18, 26, 30, and 34 months of age). Klimas stated that deterioration of oral glucose tolerance occurred within the first 6 months, but that there was no further change as rats lived to 34 months. The changes that did occur in the first 6 months were quantitatively modest, and upon inspection of the data it was possible to question if there was any real change in glucose tolerance beyond 3 months of age.

Gommers and DeGasparo[17] measured plasma glucose levels and intravenous glucose tolerance in 3-, 12-, and 24-month-old rats. There was no change in fasting glucose levels with age. However, the glucose levels 20 min after the intravenous glucose administration were significantly higher in the 12- and 24-month-old rats as compared to the 3-month-old rats. There appeared to be no significant difference between the 12- and 24-month-old rats. Unfortunately, these authors did not study rats between the ages of 3 to 12 months, and it is difficult to compare these results to those of Klimas who suggested that the changes in glucose tolerance that occur with age take place within the first 6 months of life. Furthermore, in this study rats received 1 g of glucose per kilogram body weight, which means that (on the average) 3-month-old rats received 213 mg of glucose and 12-month-old rats received 333 mg of glucose. It is certainly possible that this disparity in administered glucose could contribute to the differences in plasma glucose level attributed to the effect of age.

The third study which included rats greater than 1 year of age was that of Vranic and Pokrajac,[18] who determined plasma glucose levels in rats aged 20 days, 60 days, and 14 to 18 months. Random glucose levels following ad lib eating were similar in all age groups. At the end of a 24-hr fast the glucose level was higher in rats aged 14 to 18 months than in rats aged 60 days. On the other hand, glucose levels of 20-day-old rats and 14- to 18-month-old rats were comparable when measured at the same time point. Clearly, in order to see if there was age-associated progressive change in glucose tolerance, it would have been necessary to include a group of rats somewhere between 2 and 14 months of age.

Four other studies followed rats to 9 months of age with varying results. Thus, Hoffman et al.[19] found no change in plasma glucose levels (obtained between 9 and 10 A.M.) from rats aged 6 to 34 weeks of age, but did note that the disposal of an intravenous glucose load was impaired in 34-week-old rats as compared to 8- and 16-week-old rats. However, the number of animals was quite small, and the intravenous glucose load was administered on a per kilogram basis. Since the older rats were bigger, they received a greater challenge. As discussed earlier, the impact of differences in glucose load on the change in glucose tolerance is difficult to judge. This variable was controlled by Bracho-Romero and Reaven,[20] who compared the oral glucose tolerance of 3- and

9-month-old rats who had been matched for weight by controlling the dietary intake of the older rats as they aged. Glucose tolerance tests commenced at midday following 4 hr of food restriction. The results of this study showed a slight, but statistically significant, rise in plasma glucose response in older rats. On the other hand, Lavine and colleagues[21] found that the plasma glucose response to the intraperitoneal administration of glucose was constant from 6 to 34 weeks of age. Finally, Berdanier et al.[22] measured 16-hr fasting glucose levels in three different strains of rats at 50, 100, and 300 days of age with intriguing results. None of the three strains had a rise in fasting glucose level as rats aged from 50 to 100 days of age. However, one of the three strains had a significant rise in glucose level from 100 to 300 days of age, another had an intermediate elevation (of doubtful significance), while the third strain underwent trivial and clearly insignificant changes within the same duration of time.

These studies introduce the problem of comparing glucose tolerance in animals of varying sizes. It seems inappropriate to give animals of widely different sizes the same glucose load, but this is no *a priori* reason to assume that the administration of glucose on a per weight basis is any better. Studying animals of equal size by controlling the weight gain of the older ones introduces its own series of additional variables. Of even greater concern is the physical inactivity of small animals allowed to age in captivity, and this is a factor which must be taken into account in future studies.

It should be noted also that many of the described studies were carried out on animals fasted for 16 to 24 hr. This is a substantial fast for small animals, and one should consider the fact that prolonged fasting is accompanied by increased rates of gluconeogenesis which can alter plasma glucose levels. Finally, the possibility that different inbred strains of small animals may respond differently to the effect of age should receive further attention.

B. Age, Glucose Intolerance, and Diabetes Mellitus

The results reviewed so far provide some evidence for the notion that the deterioration of glucose tolerance that occurs with age is not entirely due to other variables such as obesity, chronic disease, etc. On the other hand, these data do not necessarily indicate that the observed changes represent a general phenomenon which is a simple function of age. Instead, it is possible that at least some portion of the decline in glucose tolerance seen with aging is due to the fact that an increased number of patients develop diabetes as a function of age.[23] Indeed, there is evidence that this may well occur. Both the Pima Indians of Arizona and the Nauruans of Micronesia have an extremely high incidence of diabetes, and in these populations it is possible to document bimodality of the plasma glucose response to oral glucose.[24,25] Demonstration of bimodality permits objective criteria to be used in the separation of patients at every age into two components: a lower component, presumed to consist of individuals with normal glucose tolerance, and an upper one, which includes patients with diabetes. In both of these populations, the proportion of individuals in the second component increased markedly with age. Furthermore, the magnitude of the deterioration in glucose tolerance that occurs with age was greatly attenuated when those individuals within the second component were removed from the analysis. Thus, in these populations there appear to be two factors which contribute to the glucose intolerance of aging. The first represents a generalized phenomenon which affects the majority of individuals as they age. The second factor is the development of a specific disease, diabetes, in many subjects, and it appears to be this latter event which primarily accounts for the observed deterioration of glucose tolerance that occurs in the whole of the two populations.

Bimodality of glucose tolerance has not been demonstrated in any Caucasian population studied to date, and this makes it much harder to assess the role that an age-

related increase in the development of diabetes might play in the glucose intolerance of aging. On the other hand, there are some data which strongly suggest that this phenomenon may also contribute to the deterioration of glucose tolerance that has been noted in Caucasian populations. Kaufmann et al.[26] determined blood glucose values in 16,699 volunteers as part of a joint detection program for tuberculosis and diabetes. Blood was drawn on subjects who claimed to have been fasted for at least 90 min. Ages ranged from 21 to over 80, and the subjects were divided into 13 groups on the basis of age. The blood glucose values within each age group were broken down by percentile into 1, 5, 25, 50, 75, 95, and 99%. (The one percentile is that blood glucose level below which 1% and above which 99% of the values lie, whereas the 99 percentile is the converse.) These authors noted a distinct rise in glucose level of the 99 and 95 percentile with increasing age in both sexes. The increase in the 75 percentile was quite moderate, and there was little, if any, change with age in the other percentiles. These results indicate that there is not a uniform increase in blood glucose level with age throughout the population, and suggests that the observed change in glucose level noted with age may not be a simple function of age itself. As such, these results strongly resemble those described in the Pimas[24] and the Nauruans.[25] On the basis of these observations, it seems quite likely that at least part of the change in glucose tolerance noted with age in Caucasian populations is due to the development of a disease, diabetes mellitus, that occurs more frequently in older individuals.

III. AGE AND INSULIN

If glucose tolerance deteriorates with age, it is apparent that age must also have an effect on insulin secretion and/or action. In this section an attempt will be made to review the available evidence in this regard, while at the same time emphasizing the methodological issues that must be faced in an effort to define the effect of age on these two aspects of insulin metabolism.

A. Age and Insulin Secretion

The major effort to evaluate the effect of age on insulin secretion has consisted of measurements of plasma insulin levels, either fasting or in response to oral or intravenous glucose. Many such studies have been carried out, and it would not be useful to review these in detail. The results of the majority of these studies have been collated in recent reviews[1–3] and there is a surprising degree of unanimity in the published data. A small minority of these studies suggest that age might be associated with an absolute decrease in the concentration of plasma insulin. In contrast, the majority of available information indicates that insulin levels either remain constant or actually increase as individuals age, and this is true in the fasting state or in response to either oral or intravenous glucose. The simplest interpretation of these data is that insulin secretion is unimpaired with advancing age, and a decrease in insulin secretory response cannot account for the age-related deterioration of glucose tolerance. However, this interpretation has been challenged and the following argument has been proposed.[1] Older subjects have higher blood glucose levels, and it is the blood glucose concentration which is the major stimulus for insulin release. By implication, an insulin response which is unchanged with age may actually reflect a beta cell defect. In order to respond to this issue it is necessary to separate explicitly the question of the role of insulin secretion in the glucose intolerance of aging as distinguished from the effect of age on the beta cell response to glucose. As to the first question, the available evidence seems reasonably straightforward. The vast majority of the published data indicates that age is not associated with an absolute deficiency of insulin, and older individuals appear to have

higher glucose levels at any given insulin levels than do younger subjects. Given this information, it seems very difficult to attribute the glucose intolerance of aging to a beta cell defect. The only caveat to this conclusion is the possibility that older individuals secrete a molecular species of insulin which is detected by the immunoassay in a normal manner, but which has less biological activity. For example, if older subjects secrete a greater proportion of proinsulin they could have higher levels of total immunoreactive insulin, but actually have a decline in the quantity of circulating insulin. This possibility has been examined by Duckworth and Kitabchi,[27] and their results suggest that this may be the case. They determined the total immunoreactive insulin and proinsulin-like material in the plasma of 68 nonobese subjects, divided into six groups by decade (15 to 24, 25 to 34, etc.). Subjects were given a 100-g oral glucose load, and plasma concentrations of total immunoreactive insulin and proinsulin were determined 60, 90, and 120 min later. The summed concentration of proinsulin-like material was similar in the 15- to 24-, 25- to 34-, and 35- to 44-year age groups. However, a significant elevation was noted in the 45- to 54-year age group, and the same level of increase persisted in the 55- to 64- and 65- to 74-year age groups. However, the relationship of these observations to the glucose intolerance of aging is questionable. In the first place, the number of patients was relatively small (i.e., 22 in the three age groups above 45 years). Second, the proportion of proinsulin-like material to total insulin was small, the differences seen with age were moderate, and the summed total insulin response after subtracting the value for proinsulin-like material was not decreased in the older subjects. Thus, these patients seemed to have adequate amounts of biologically active insulin even though increased levels of proinsulin were also present. Third, the assay used for proinsulin-like material was an indirect one, and this feature raises further questions as to the significance of the small changes noted. However, this is an important issue conceptually, and it is essential that further consideration be given to the possibility that older individuals may secrete a form of insulin which is biologically different than the insulin of younger individuals.

The question as to the effect of age on the insulin secretory response is more complicated. As Andres and Tobin point out,[1] in order to answer this question it is necessary to determine the ability of an identical stimulus to elicit insulin release from different aged subjects. Unfortunately, this is easier said than done. Andres and Tobin[1] suggest that this can be accomplished by use of the ''hyperglycemic glucose-clamp'' technique, in which the plasma glucose concentration is fixed at a predetermined steady level by rapid analysis of arterial glucose concentration and by appropriate adjustments of a continuous but variable intravenous glucose infusion rate. With this approach, they have maintained steady-state plasma glucose levels at 140, 180, 220, and 300 mg/dℓ in subjects of various ages, and they state that insulin secretion is diminished at the three lower glucose levels in older as compared to younger individuals.[1] Since they argue that the stimulus to insulin secretion is the same in all subjects, beta cell sensitivity must decrease with age. But is the stimulus to insulin secretion the same? Older subjects are relatively glucose intolerant, therefore they receive less glucose intravenously than do young subjects. Thus, young individuals get a bigger glucose load, and it has been shown[28,29] that larger glucose loads lead to increased insulin independently of measured changes in plasma glucose level. Thus, it could equally well be argued that the diminished insulin secretion in older subjects during the hyperglycemic glucose-clamp is simply a function of the fact that they receive less glucose. If both glucose load and glucose level are relevant variables in determining beta cell response, the insulin secretory response of old and young subjects can only be determined when both factors are held constant. Unfortunately this experimental situation can only be attained if the glucose tolerance of the individuals is essentially identical. The authors of this chapter do not