RADIOBIOASSAYS

Volume II

Fuad S. Ashkar

Radiobioassays

Volume II

Editor

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FOREWORD

This series of books on Radiotracers in Biology and Medicine is on the one hand an unbelievably expansive enterprise and on the other hand, a most noble one as well. Tools to probe biology have developed at an accelerating rate. Hevesy pioneered the application of radioisotopes to the study of chemical processes, and since that time, radioisotopic methodology has probably contributed as much as any other methodology to the analysis of the fine structure of biologic systems. Radioisotopic methodologies represent powerful tools for the determination of virtually any process of biologic interest. It should not be surprising, therefore, that any effort to encompass all aspects of radiotracer methodology is both desirable in the extreme and doomed to at least some degree of inherent failure. The current series is assuredly a success relative to the breadth of topics which range from in depth treatise of fundamental science or abstract concepts to detailed and specific applications, such as those in medicine or even to the extreme of the methodology for sacrifice of animals as part of a radiotracer distribution study. The list of contributors is as impressive as is the task, so that one can be optimistic that the endeavor is likely to be as successful as efforts of this type can be expected to be. The prospects are further enhanced by the unbounded energy of the coordinating editor. The profligate expansion of application of radioisotopic methods relate to their inherent and exquisite sensitivity, ease of quantitation, specificity, and comparative simplicity, especially with modern instrumentation and reagents, both of which are now readily and universally available. It is now possible to make biological measurements which were otherwise difficult or impossible. These measurements allow us to begin to understand processes in depth in their unaltered state so that radioisotope methodology has proved to be a powerful probe for insight into the function and perturbations of the fine structure of biologic systems. Radioisotopic methodology has provided virtually all of the information now known about the physiology and pathophysiology of several organ systems and has been used abundantly for the development of information on every organ system and kinetic pathway in the plant and animal kingdoms. We all instinctively turn to the thyroid gland and its homeostatic interrelationships as an example, and an early one at that, of the use of radioactive tracers to elaborate normal and abnormal physiology and biochemistry, but this is but one of many suitable examples. Nor is the thyroid unique in the appreciation that a very major and important residua of diagnostic and therapeutic methods of clinical importance result from an even larger number of procedures used earlier for investigative purposes and, in some instances, procedures used earlier for investigative purposes and, in some instances, advocated for clinical use. The very ease and power of radioisotopic methodology tempts one to use these techniques without sufficient knowledge, preparation or care and with the potential for resulting disastrous misinformation. There are notable research and clinical illustrations of this problem, which serve to emphasize the importance of texts such as these to which one can turn for guidance in the proper use of these powerful methods. Radioisotopic methodology has already demonstrated its potential for opening new vistas in science and medicine. This series of texts, extensive though they be, yet must be incomplete in some respects. Multiple authorship always entails the danger of nonuniformity of quality, but the quality of authorship herein assembled makes this likely to be minimal. In any event, this series undoubtedly will serve an important role in the continued application of radioisotopic methodology to the exciting and unending, yet answerable, questions in science and medicine!

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Dr. Colombetti graduated from the Litoral University in his native Argentina with a Doctor in Sciences degree (summa cum laude), and obtained two fellowships for postgraduate studies from the Georgetown University in Washington, D.C., and from the M.I.T. in Cambridge, Mass. He has published more than 150 scientific papers and is the author of several book chapters. He has presented over 300 lectures both at meetings held in the U.S. and abroad. He organized the First International Symposium on Radiopharmacology, held in Innsbruck, Austria, in May 1978. He also organized the Second International Symposium on Radiopharmacology which took place in Chicago in September, 1981, with the active participation of more than 500 scientists, representing over 30 countries. He is a founding member of the International Association of Radiopharmacology, a nonprofit organization, which congregates scientists from many disciplines interested in the biological applications of radiotracers. He was its first President (1979/1981).

Dr. Colombetti is a member of various scientific societies, including the Society of Nuclear Medicine (U.S.) and the Gesellschaft für Nuklearmedizin (Europe), and is an honorary member of the Mexican Society of Nuclear Medicine. He is also a member of the Society of Experimental Medicine and Biology, the Coblenz Society, and the Sigma Xi. He is a member of the editorial boards of the journals *Nuklearmedizin* and *Research in Clinic and Laboratory.*

PREFACE

In the last two decades, radioassay has revolutionized laboratory medicine with its versatility, accuracy, sensitivity, and reproducibility. It has also had a tremendous impact on all scientific areas from biology to medicine including mathematics and physics. Radioassay and its related techniques are still the fastest growing segments in laboratory technology with still-unexplored uses and variations. Its possibilities are endless with new procedures and techniques being developed almost daily.

Radioassay laboratories in the U.S. have grown to in excess of 5000 since the introduction of radioassay procedures into clinical diagnosis. This growth is attributable to the increased use, accuracy, and sensitivity of the technique that has greatly improved the quality and practice of clinical medicine.

The great success of radioassay as an analytical tool has led to wider applications of its principle and the introduction by other useful labels such as enzymes, fluorescence, bacteriophages, and spin-label free radicals. The perfusion of the applications of radioassay and these other related techniques are constantly opening new horizons in the field of medicine and biology.

Fuad S. Ashkar

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Dr. Ashkar is the author and a co-author of numerous contributions to medical literature, editor of three books, and contributor to several others.

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Chapter 1

CLINICAL APPLICATION OF THE RADIOIMMUNOASSAY OF INSULIN

Robert Miller

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I. INSULIN SYNTHESIS AND RELEASE

With the advent of RIA and electron microscopic techniques, a large body of evidence now appears to indicate that the major synthesis of insulin is in the ribosomes and that insulin is then transformed into granules that are manufactured in the endoplasmic reticulum. The endoplasmic reticulum changes in structure and forms a vesicular saccule that aligns along the outer cell surface and exhibits a very cloudy amorphous type of material, the beta granule. These beta granules keep their supply of insulin stored until the appropriate stimulation occurs. At this time there is a margination of the granules in their sacs along the plasma membrane of the beta cells. The walls of the sacs then fuse with the plasma membrane. This subsequently ruptures and the granules are then liberated into the extracellular space, a process called emiocytosis. It was Lacey et al.[1] who suggested that a microtubular microfilamentous system was involved in movement of insulin granules toward the plasma membranes of the beta cell. These organelles are composed of actin-like material. It is believed that microtubules may direct the granules to the cell surface while the microfilamentous web acts as a barrier that controls the access of the granules to the cell membrane.

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. Malaisse[2] demonstrated that the exposure of the islet cell to glucose in the presence of calcium results in an accumulation of calcium and that secretion of insulin is associated with an immediate reduction in calcium efflux. There is a marked increase in calcium extrusion associated with insulin release. It has been postulated that the accumulation of calcium from the extracellular fluid is a necessary prerequisite for insulin release. Beta cell cyclic 3'5'AMP also plays some role in this mechanism and has an effect as a modulating hormone secretion.

With the development of the immunoassay for insulin it has become increasingly apparent that diabetes usually results from a secretory failure in pancreatic beta cells. This will be discussed in more detail later. Briefly, in juvenile diabetics, the failure is severe and complete and is reflected in a total destruction of the pancreatic islet. However, in the adult, the secretory failure is less pronounced. In the glucose tolerance test, there is both a quantitative decrease in total insulin secretion as well as a sluggish response. Cerasi and Luft[3] reported the concept of two separate phases of insulin secretion: an early, rapid burst followed by a later, more prolonged phase. This could indicate that insulin may very well be present in two forms: one in a presynthesized form, which is stored in a small compartment for immediate release and then a second form for slow release that contains newly synthesized insulin that is produced at a longer, slower rate. In the past it was believed that the two chains of insulin were synthesized separately and then joined by means of two disulfide bridges at a postribosomal site.

However, Steiner and co-workers[4] have reported the presence of a large molecule Steiner named pro-insulin. This particular molecule consists of insulin A and B chains linked by an additional peptide to approximately three amino acids. This has been called the C peptide. Its function appears to be that of facilitating the folding of the molecule so that the A and B chains are correctly aligned for the disulfide bonds. Pro-insulin, therefore has a very significant role in maintaining and in establishing the synthesis and structure of insulin.

The enzymes necessary for conversion of pro-insulin into insulin are probably located in the granule membranes and the conversion process takes place in these granules as they move toward the surface to be extruded by the process of emiocytosis. The proteolytic enzymes then leave the pro-insulin at specific sites and the major product of the reaction, therefore, becomes insulin C peptide which is retained with the

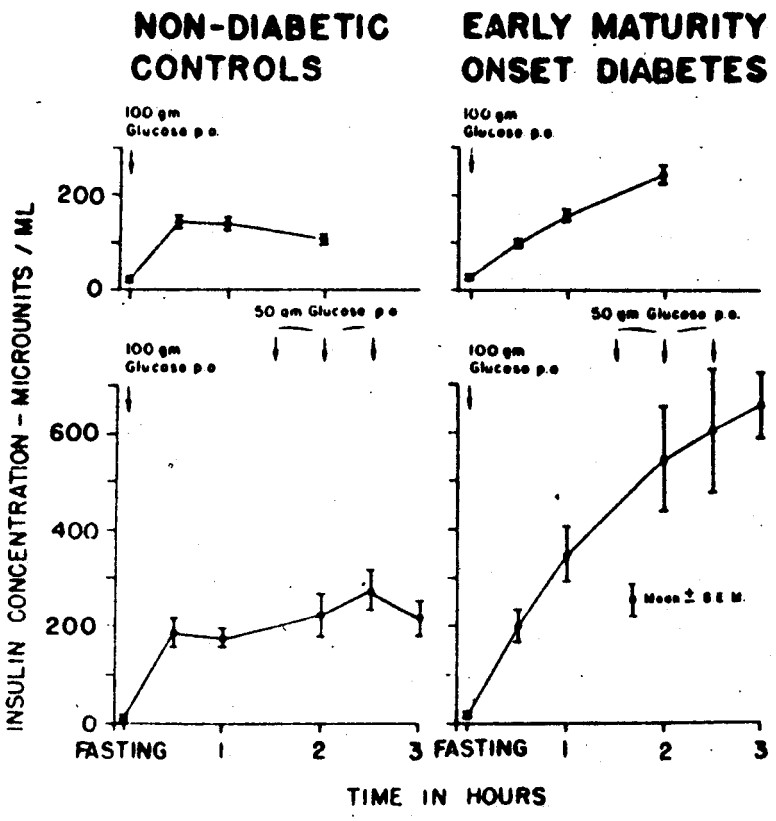

FIGURE 1. Berson and Yalow data on insulin secretion following glucose ingestion in nondiabetic controls and early maturity onset diabetes. (From Berson, A. A. and Yalow, R. S., *J. Diabetes*, 10, 339, 1961. With permission.)

insulin in the granules. The C peptide has now become useful as a marker of beta cell function independent of insulin secretion. This has been particularly useful in diabetics who are on exogenous insulin and in whom attempts are being made to determine whether the source of insulin being measured in the plasma is in fact exogenous or endogenous.

II. DIABETES MELLITUS

With the advent of RIA we have been able to finally establish what has long been conjectured, that the two major forms of diabetes, i.e., juvenile onset and maturity onset diabetes mellitus, are in fact manifestations of degrees of beta cell failure. Following the oral administration of glucose in those with mild diabetes, the plasma insulin concentration rises very sluggishly compared to a normal during approximately the first ½ hr, but within 1 or 2 hr it reaches hypernormal levels. Similar plasma hyperinsulinism is observed in adults with borderline diabetes. However, a hypoinsulin response is noted in individuals with juvenile onset diabetes and those with severe diabetes secondary to primary pancreatic disease. These observations were first brought forth by Yalow and Berson[5] in 1961 (Figure 1).

In Figure 1 it can be noted that following 100 g of glucose by mouth in a nondiabetic control, the insulin levels reach a peak in approximately ½ hr to 1 hr and then sustains at that level, gradually decreasing by the 2 hr mark. However, in the early maturity

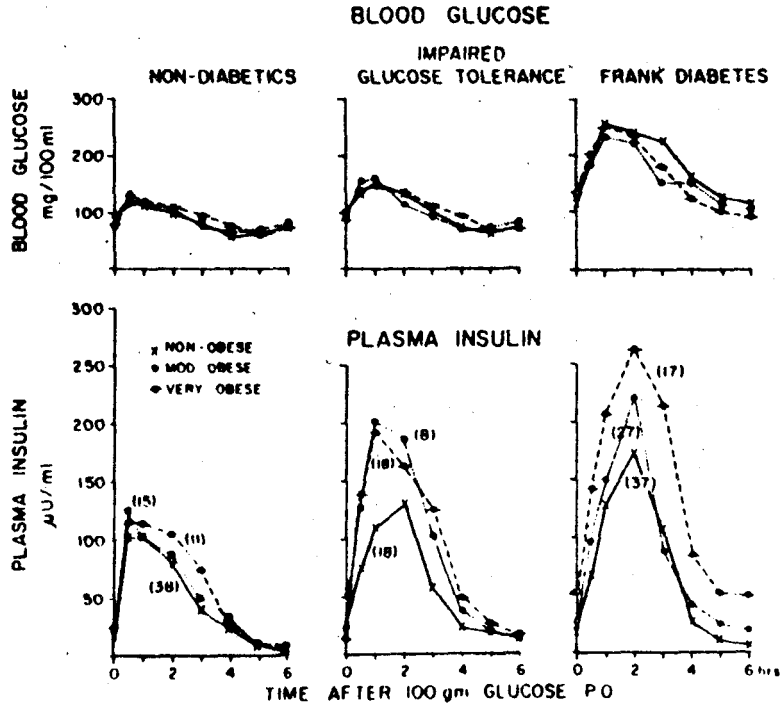

FIGURE 2. Yalow data on blood glucose and plasma insulin in nondiabetics, impaired glucose tolerance, and frank diabetes. (From Yalow, R. S., *Ann. N.Y. Acad. Sci.*, 357, 1965. With permission.)

onset diabetic, the peak effect of the insulin occurs at a much later time and then gradually shows an increase beyond that of a normal individual. Also of significance is that with repetitive challenges of glucose, the insulin level in a normal reaches a plateau with only slightly increased increments at the second and third hour, even following repetitive 50 g-doses of glucose. This is compared to an early maturity onset diabetic in whom one gets a gradual progressive increase in insulin release at the higher levels following repetitive doses of glucose. Investigators have noted abnormally high insulin levels in obese subjects, not only in the fasting state, but also after glucose administration. As is well-known, many diabetics are obese and it has been necessary to evaluate the role of obesity vs. that of diabetes in producing the hyperinsulin response. Yalow found that the peak insulin level occurred in 1 hr in nondiabetic subjects of all weight classes. For all weight classes, the plasma insulin curve was highest in patients with frank diabetes, an average peak was obtained at 2 hr or later (Figure 2). Within a given category, plasma insulin curves were higher in the obese than in the nonobese patients. The differences are not as great in those between diabetics and nondiabetics within a given weight category. Therefore, even though insulin levels are elevated in the obese diabetic the amount of insulin is still inappropriate for the degree of blood glucose elevation.

We are still seeing the fundamental difference which is inadequate amounts of insulin for the blood glucose similar to what we see in a juvenile onset diabetic who is not producing any insulin at all. Those with severe diabetes, whether obese or not, experience a slow-rising plasma insulin level that even after 2 to 3 hr barely reaches the level achieved by a nondiabetic in ½ hr, even though glucose concentrations were 3 to 4 times as great. Those with mild to moderately severe diabetes, obese or not, show the

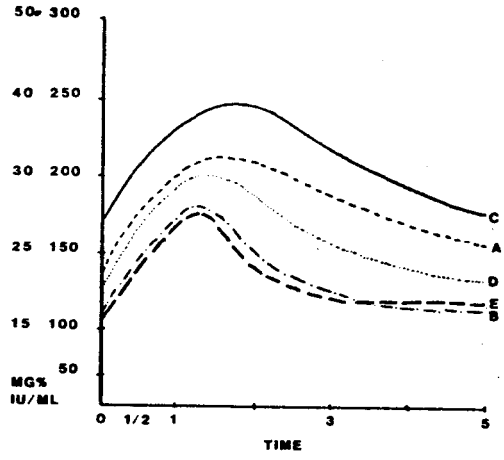

FIGURE 3. Glucose and plasma insulin tolerance curves in (A) obese diabetic insulin level, (B) normal insulin level, (C) obese diabetic glucose, (D) obese normal insulin, and (E) normal glucose level.

highest insulin curves of all the groups with the values in the obese patients being higher than in the nonobese patients. The plasma hyperinsulinism in the adult diabetic is independent of the presence of obesity. Although obesity is associated with hyperinsulinism, it is not associated with impairment of glucose tolerance in the nondiabetic subject, therefore indicating that in nondiabetic obese individuals the ability to produce larger amounts of insulin to match the elevated amounts of blood sugar is appropriate. Therefore they are able to maintain a normal blood glucose level in spite of the obesity as contrasted to the obese diabetic who, although producing more insulin than the obese counterpart, is unable to produce enough insulin to control the elevation in the blood sugar (Figure 3).

III. HYPOGLYCEMIA

Many factors are involved in maintaining glucose homeostasis. Abnormalities in any of these factors can result in hypoglycemia. The major sources of glucose in the blood are by means of food intake, i.e., intestinal tract and the liver production of glucose by means of gluconeogenesis. Most carbohydrate intake is converted to glucose almost immediately following intake of food. Subsequently, elevation of blood glucose occurs. This in turn stimulates the pancreas to release insulin. Some of the stimuli to beta cell release is via the nervous system, some is through other hormones, i.e., the gut hormones, and to an extent, some is via glucagon. Insulin promotes the storage of carbohydrates with the purpose of providing extra supply of these materials to be released on demand because of increasing needs. Most organs require glucose as their major energy source, however, the major consumer of glucose is the brain. Interestingly enough, the brain is one of the few organs that does not require insulin for glucose transport. The kidneys, RBC, bone marrow, and other tissues as well require glucose for energy and their own respective metabolisms.

In the absence of adequate glucose for whatever reason, other sources of energy are provided. These are lipids via fatty acids and protein via breakdown into amino acids. This can be accomplished by several antiinsulin hormones such as somatotropin, glucocorticoids, catecholamines, and glucagon. The inner play of the hormones and free