The Distribution and Metabolism of Insulin Labeled with Radioactive Iodine (I^{131}) in Normal and Diabetic Subjects

George W. Welsh, 3rd, M.D., Elaine D. Henley, M.D.,**
Robert H. Williams, M.D.,† and Neil J. Elgee, M.D.,‡ Seattle

To investigate possible differences in the distribution and metabolism of insulin in diabetics and normals, insulin labeled with radioactive iodine (I^{131}) was given intravenously to 40 diabetics of varying type and severity, 15 healthy controls, and 11 other nondiabetics with various illnesses. The labeled insulin was given in tracer amounts (50 to 100 microcuries) and test doses varied between 0.5 and 5.0 units of insulin. The dose was diluted in normal physiologic saline solution and injected slowly into fasting subjects over a two-minute period. In most cases therapeutic insulin had been withheld from diabetics for 24 to 96 hours. Plasma samples were drawn at predetermined times, and the amount of protein-bound radioactivity was determined by precipitation with trichloroacetic acid (TCA).

Since labeled insulin is virtually completely precipitated with TCA, the protein-bound radioactivity was assumed to represent undegraded labeled insulin, whereas the TCA-soluble radioactivity was assumed to be comprised of the degradation products of the labeled hormone. Total urinary radioactivity, of which an average of 95 per cent is TCA-soluble, was also measured during the test period. In some subjects surface measurement of radioactivity over various organ areas, such as the thyroid, liver, kidney, and muscle masses, was made.

The TCA-precipitable (protein-bound), presumably undegraded, labeled insulin disappeared rapidly from the plasma of healthy controls and nondiabetics with other illnesses. Samples drawn two minutes after intravenous injection of labeled insulin showed an average of only 47 per cent of the dose remaining in the plasma. This rapid disappearance rate continued for 15 minutes, when only 20 per cent of the dose remained in the plasma, and at 60 minutes only 7.7 per cent remained. The resultant curve was reproducible with small variations in all the controls. Nondiabetics with severe hepatic and renal disease, pancreatitis, carcinomatosis, or thyrotoxicosis, receiving steroid therapy, or under anesthesia, gave essentially the same curves as controls. The degradation products in the TCA-soluble fraction of the plasma increased rapidly to an average maximum of 12 per cent of the administered dose in one hour. Urinary radioactivity, over a one-hour collection period, averaged 10 per cent of the dose.

In contrast, 27 of the 40 diabetics showed a retention of undegraded labeled insulin in the plasma, to markedly varying degree, over the one-hour test period. The average retention amounted to 37 per cent of the dose at one hour. Three of these diabetics retained over 80 per cent of the labeled insulin in their plasma.

Thirteen other diabetics, representing one-third of those studied, exhibited no increased retention, and labeled insulin disappeared from their plasma virtually as rapidly as in controls.

There appeared to be no correlation between the abnormally large retention of labeled hormone in the plasma and the insulin requirements, blood sugar, duration of diabetes, degree of diabetic complications, and clinical condition of the subjects. Four diabetics were not fasted and were given their usual therapeutic insulin three hours prior to testing, and two were given intravenous insulin loads fifteen minutes prior to testing. All these subjects still retained large percentages of undegraded insulin in their plasma.

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From the Department of Medicine, University of Washington School of Medicine, Seattle.

*Trainee, National Institute of Arthritis and Metabolic Diseases and Assistant in Medicine, University of Washington.

†Executive Officer and Professor of Medicine, University of Washington.

‡Resident in Medicine, King County Hospital, Seattle.

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Urinary radioactivity, plasma TCA-soluble radioactivity, and concentration of radioactivity over the thyroid were significantly less in the diabetics, and were roughly inversely proportional to the percentage of dose of undegraded insulin retained in the plasma. No significant differences in concentration of radioactivity were noted over other organs.

In vitro experiments were designed to show whether the retention of labeled insulin in the plasma of diabetics was due to factors present in or extrinsic to the plasma itself. Human plasma, labeled insulin, and either rat liver slices or quarter diaphragms were incubated together. These tissues have been shown by other investigators to bind insulin rapidly and firmly, and to degrade labeled insulin, presumably by proteolytic action. Perhaps it could be shown that certain factors in human plasma, particularly diabetics' plasma, would bind labeled insulin in such a manner as to deter its entry into tissues, and degradation might be retarded. Using plasma from controls and from diabetics who retained labeled insulin in their plasma in vivo, it was shown that with diabetic plasma there was markedly less labeled insulin bound to the rat tissues in vitro. The greater the amount of labeled insulin retained in the plasma in vivo, the less the amount was bound to rat tissues in vitro. Similarly, the amount of degradation of labeled insulin in vitro was proportionately less in the presence of diabetic plasma.

It is suggested from these studies that there is a factor in the plasma of many diabetics which tends to bind more insulin in the plasma compartment both in vivo and in vitro. This factor could represent an alteration of the normal binding of insulin by plasma constituents, either quantitatively or qualitatively. The retention of labeled insulin in the plasma compartment in diabetics might reflect only binding of insulin to plasma proteins or other components which are "unsaturated" in the diabetic state, due to insulin deficiency. Preliminary experiments with diabetic plasma incubated in vitro with labeled insulin and rat liver homogenate have shown that the prior addition of nonlabeled insulin to the plasma results in *increased* degradation of labeled insulin by liver homogenate. This prior addition of nonlabeled insulin to diabetic plasma may saturate the carriers of insulin and allow more rapid degradation of labeled insulin added later. However, when nonlabeled insulin is added to normal plasma, in a similar system, there is a *decrease* in the amount of degradation, indicating that insulin is present in excess and competing for the degradation system.

SUMMARY

1. Insulin labeled with radioactive iodine (I^{131}) disappears less rapidly from the plasma of many diabetics, as compared with normal controls and other nondiabetics.
2. There is also less rapid degradation of labeled insulin by these diabetics, as compared with controls.
3. There is no apparent correlation between these observations and the clinical characteristics of the diabetics.
4. In vitro studies suggest that in diabetics the "unsaturation" of plasma components with insulin may be responsible in part for the apparent retention of labeled insulin in the plasma in vivo, and decreased binding and degradation by rat tissues in vitro.

SUMMARIO IN INTERLINGUA

Distribution e Metabolismo de Insulina Etiquettate per Iodo Radio-Active in Subjectos Normal e Diabetic

1. Insulina etiquettate per iodo radio-active (I^{131}) dispare minus rapidemente ab le plasma de multe diabeticos que ab le plasma de subjectos de controlo normal o alteremente nondiabetic.
2. Es etiam a constatar un minus rapide degradation del etiquettate insulina in tal diabeticos que in le subjectos de controlo.
3. Il existe nulle apparente correlation inter iste observationes e le characteristicas clinic del diabeticos.
4. Studios in vitro supporta le these que "non-saturation" del componentes plasmatic con insulina es possibilemente responsabile in parte pro le apparente retention plasmatic de etiquettate insulina in vivo.

DISCUSSION

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): In view of the report by Drs. Milstein and Hausberger, would Dr. Williams and his co-workers consider adding the uptake of insulin by adipose tissue to his many other interesting measurements?

Secondly, have they ever tested insulin in normal subjects who have some allergy to insulin; that is, have they had a nondiabetic with allergy to insulin, and then seen what happened to this?

DR. WILLIAMS: No, we have not made such tests.

FREDERICK M. ALLEN, M.D., (*New York*): In regard to the distribution of insulin, it seems of interest to test large doses as being easiest to follow. I previously published experiments with injecting huge doses in the hind legs of animals (New England J. Med. *219*: 77, 1938; Ann. Int. Med. 12:1263 and 1870, 1939).

The effect is extreme depression and with sufficiently high doses (above 1,000 units for the strongly tolerant rat, several thousand units for rabbits, cats or dogs) the animal dies in spite of any glucose administration or any level of hyperglycemia. The hypoglycemia is not greatly different with small or large doses; for example, one rat receiving 20 units and another receiving 500 units may have their hypoglycemic convulsions at about two or two and a half hour intervals, and each recovers with a prompt injection of a fraction of a gram of glucose. But with the small insulin dose the animal acts well and there are only a few repetitions of the convulsions, whereas with the 500 units the depression is marked and the investigator must sit up for about forty-eight hours to combat the recurring hypoglycemia.

Since the depression persists in spite of hyperglycemia, it might hypothetically be attributed to a great excess of insulin throughout the body. But on cutting off the leg that received the insulin, the animal is quite promptly well. In other words, the greater part of the insulin is not diffused through the body but is lying unabsorbed in the leg. The marked toxicity can also be shown with the slowly absorbable protamine insulin.

As regards sugar distribution, years ago I gave a problem to Dr. Walter Palmer for comparison of blood and tissue sugars in diabetic and nondiabetic animals. The results can be found in his paper (J. Biol. Chem. *30*:79, 1917). But I am specially interested today in observations which may throw light on something I published in 1913, namely the diuretic action of sugar. It is true that intravenous glucose can make polyuria by reason of hydremia; but hyperglycemia produced by any other mode of glucose administration in the normal animal fails to reproduce the diabetic polyuria. I speculated that a combined state of sugar in normal blood might explain the difference; but since that was disproved chemically it is now interesting to hear of a combination occurring in the cells. Thus, if free glucose enters into the kidney cells in the diabetic and not in the normal, the difference in diuresis may be explained.

THOMAS H. MCGAVACK, M.D., (*New York*): Have you any clinical evidence about differences between diabetics in which insulin was degraded at different rates?

DR. WILLIAMS, (*closing*): No, we could not find a correlation of the degree of degradation with such factors as severity of diabetes.

The Beneficial Effects of Cooking Food

Cooked foods are more digestible. The cooking of meat loosens the connective tissue so that it is more easily chewed and is of readier access to the gastric juices. Extreme heat, however, long continued, has an opposite effect. Another result of cooking is to render meat more attractive and appetizing, which in turn stimulates the digestive organs. Not only are vegetables made more appetizing by cooking, but, what is still more important, the starch cells under the influence of heat burst and permit the digestive juices to reach the contained starch. Breads and cakes are made light by cooking, for the contained gases expand and the water vaporizes to make the mass still lighter; while this is taking place, the albumins coagulate and "fix" the bread in this light state. The carbohydrate on the surface is partly caramelized, which gives the whole a more attractive appearance and flavor and thus renders it more digestible.

From the book *Nutrition and Diet in Health and Disease* by James S. McLester, M.D., and William J. Darby, M.D., Ph.D. Philadelphia, W. B. Saunders Co., 1952, 6th ed., pp. 141-44.

The Characteristics of a Satisfying Meal

The satiety values of the various foods show that a meal which contains meat gives the greatest degree of satisfaction, and if bread and potatoes are added, particularly the latter, the sense of comfort and well-being is increased. If the bread is buttered, so much the better; and if at the end of the meal something sweet is eaten, the satiety value becomes still greater. Thus is seen the rationale of that type of meal which the race has instinctively chosen: first, a soup (meat extractives); second, meat with potatoes, to which may be added certain other starchy vegetables; then a salad with oil dressing, and finally a dessert. This meal remains longest in the upper portion of the gastrointestinal tract, calls forth the greatest amount of secretory activity, and gives the greatest degree of satisfaction.

From the book *Nutrition and Diet in Health and Disease* by James S. McLester, M.D., and William J. Darby, M.D., Ph.D. Philadelphia, W. B. Saunders Co., 1952, 6th ed., p. 132.