The Fate of Insulin in Altered Metabolic States

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The insulin insufficiency of clinical diabetes mellitus and of experimental diabetes can be influenced quantitatively by many metabolic alterations. Ingle[1] has recently considered these relationships in a review of what he calls "heteropoietic" factors affecting carbohydrate metabolism. We have investigated the effects of some of these factors on the degradation of insulin labeled with radioactive iodine (insulin-I^{131}) in an attempt to elucidate their mechanism of action and to further the study of the metabolism of insulin.

METHODS

The radioactivity of insulin-I^{131} is essentially all protein-bound.† It has been found that after injection into a rat, this property is rapidly lost. A fraction of radioactivity appears that is not protein-bound, as measured by trichloracetic acid (TCA) precipitation, and thereby represents degradation of the insulin-I^{131}.

A standard procedure, fully described elsewhere,[2] has been adopted to measure this degradation and was used in these experiments.

In essence, there were two groups of rats of similar age, weight, and sex in each experiment. One, the experimental group, received a particular metabolic alteration, and the other group served as control. Both received a standard dietary preparation, and then all rats of both groups were given a standard dose of insulin-I^{131} intravenously. Fifteen minutes later the animals were sacrificed, and gastrocnemius muscle, blood, liver, and kidney removed. The radioactivity of these tissues was divided into a protein-bound fraction and a supernatant fraction by means of TCA precipitation. The concentration of radioactivity in these fractions was expressed as a ratio of the tissue concentration (per cent dose/gm.) to the initial total body concentration, to compensate for variations in body weight brought about by some of the metabolic alterations.

EXPERIMENTS AND RESULTS

In figure 1, the results of the experiments are summarized. The concentrations of radioactivity in the tissue fractions are shown as being either increased, decreased,

TCA SUPERNATANT = / TCA PRECIPITATE =	KIDNEY	LIVER	MUSCLE	BLOOD
INSULIN-I^{131} LOAD				↓ / ↑
INSULIN LOAD	↓ / ↓	↓ / ↑	↓ / —	↓ / ↑
HEPATECTOMY	— / —		↓ / ↑	— / ↑
NEPHRECTOMY		—	—	↓ / ↑
HYPOPHYSECTOMY	↓ / ↓	↓ / ↑	↓ / ↑	↓ / ↑
ADRENALECTOMY	— / —	— / —	— / —	— / ↓
GROWTH HORMONE	— / —	— / —	— / —	— / —
HYDROCORTISONE	— / —	— / ↓	— / —	— / —
GROWTH HORMONE and HYDROCORTISONE	— / —	— / ↓	— / —	— / —
THYROIDECTOMY	— / ↑	— / —	— / —	↓ / —
THYROXINE	— / —	— / ↓	— / ↓	— / ↓
TRIIODOTHYRONINE	↓ / ↓	— / ↓	— / —	— / ↓
GLUCOSE	↓ / ↓	— / —	— / —	— / —
FRUCTOSE	↓ / ↓	↓ / ↑	— / ↑	↓ / —
MC 2346	↓ / ↓	↓ / ↑	↓ / ↑	↓ / ↑

FIG. 1. The arrows indicate whether a particular metabolic state caused an increase, a decrease, or no change in the concentrations of radioactivity in the trichloracetic acid (TCA) precipitate and supernatant fractions of tissues measured fifteen minutes after intravenous insulin-I^{131}.

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†The insulin-I^{131} was obtained from the Abbott Laboratories at Oak Ridge. In the preparation of the material, unbound I^{131} was removed by dialysis. Essentially all of the radioactivity remaining was precipitable upon the addition of trichloracetic acid.

or unchanged as compared with the control values for each experiment. In order to be charted as increased or decreased, differences had to have statistical significance of $p < .02$.

Insulin Loads

(A) *Insulin-I^{131}*. The experimental group was given a load of 222 *ug.* and the control group 2 *ug.* of insulin-I^{131}. With the load (222 *ug.*), there was proportionately less degradation as measured by blood levels. There was an increase in the fraction of the blood radioactivity in the TCA precipitate and a decrease in the fraction in the supernatant. This can be interpreted as showing that the degradation of insulin-I^{131} is rate-limited.

(B) *Insulin.* Of great significance is the fact that this same limitation of rate was found to result when both control and experimental groups received the same small (2-*ug.*) dose of insulin-I^{131}, but the experimental group received in addition, 220 *ug.* of nonlabeled insulin.* The nonlabeled insulin interfered with the degradation of the insulin-I^{131}; the TCA precipitable radioactivity was increased and the supernatant radioactivity was decreased, just as was the case with an insulin-I^{131} load. It would appear therefore that the system in which insulin-I^{131} is degraded does not distinguish between labeled and nonlabeled insulin. This gives weight to the supposition that the radioisotope technique gives information pertinent to the study of the metabolism of insulin.

Site of Insulin-I^{131} Degradation

When the liver was excluded from the circulation, there was an increase in the protein-bound radioactivity concentration in muscle and blood. Similar results were found in nephrectomized animals. Liver and kidney therefore are sites of insulin-I^{131} degradation. That they are not the only sites of this degradation, however, was shown in an experiment in which both hepatectomy and nephrectomy were done, and some degradation products still appeared in blood and muscle.

Adrenal and Pituitary Influences

Hypophysectomized animals showed decreased supernatant concentrations in all tissues and, conversely, increased protein-bound fractions in all except kidney. Degradation was therefore greatly diminished after hypophysectomy. Adrenalectomy, on the other hand, had very little effect, showing, if anything, some increased degradation as reflected in the blood. Growth hormone was completely without effect, and hydrocortisone reduced only the hepatic radioactivity. The combination of the two hormones had essentially the same effect as that of hydrocortisone alone.

Thyroid Function

After treatment with thyroxine or triiodothyronine, precipitable radioactivity in the tissues was decreased and, presumably, degradation was enhanced. Contrariwise, after thyroidectomy, degradation appeared to be diminished.

Glucose and Fructose Loads

Many experiments were done to assess the effects of these substances on insulin-I^{131} degradation. Large intravenous loads of either substance consistently lowered the renal radioactivity concentration. Other tissue fractions were not altered by glucose, but large doses of intravenous fructose resulted in greater concentrations of nonprecipitable radioactivity in muscle and liver, and lesser concentrations of supernatant radioactivity in liver and blood.

5-isopropylidene-2,4-dithiohydantoin (MC2346)†

This substance has been reported[3] to reduce the incidence of diabetes in rats after partial pancreatectomy. It was therefore given to rats to study its effect on insulin-I^{131} degradation. Pronounced changes resulted after either the intravenous or oral administration of MC2346. Precipitable radioactivity was increased, and supernatant radioactivity decreased, in liver, muscle, and blood, and both fractions of renal radioactivity were reduced. This picture then is essentially the same as that seen after hypophysectomy.

DISCUSSION

The evidence, presented in the insulin load study, is strong that the degradation system does not distinguish between labeled and nonlabeled insulin. Measurement of the rate of degradation of labeled insulin using the isotope method then may help to correlate the interrelation of nonlabeled insulin degradation and insulin sensitivity. Accordingly it is pertinent to consider the effects of metabolic alterations on insulin-I^{131} degradation with particular reference to changes in the biologic activity of insulin known to be brought about by such alterations.

In general, the results indicate that diminished insulin-I^{131} degradation was found in conditions of increased insulin sensitivity—for example, hypophysectomy—and, conversely, increased insulin-I^{131} degradation was found in conditions of relative insulin insensitivity; for example, in thyroxine-treated animals.

*Kindly supplied by Eli Lilly and Company.

†Kindly supplied by E. R. Squibb & Sons.

Therefore, although insulin is known to have an increased biologic action in hypophysectomized animals, yet, as these studies have shown, its degradation is reduced. On the other hand, Houssay[4] has produced insulin insufficiency by treatment with thyroid; our studies have shown that similar treatment (thyroxine or tri-iodothyronine) increases insulin-I^{131} degradation. Biologic activity would therefore, seem to be inversely related to degradation. This pattern was seen to a less pronounced degree in the thyroidectomized animals, who are insulin-sensitive in general and in whom degradation, in blood at least, was reduced.

Such a relationship strongly suggests that insulin degradation does not come about solely as the result of insulin action. It rather suggests that the process of degradation is closely connected with the inactivation of insulin. Many body tissues appear to take part in this degradation,[5] liver and kidney being active sites. That insulin-I^{131} concentrates in kidney has been considered suggestive that degradation represents inactivation, since no biologic function of insulin in kidney is known.[2] It is further of interest that with large glucose loads, renal insulin-I^{131} was decreased, and although insulin was in increased demand and was performing its physiologic action at an accelerated rate to metabolize the glucose, yet insulin-I^{131} degradation was unchanged. This also is consistent with the view that insulin-I^{131} degradation is not simply an accompaniment of insulin action. Fructose appeared to bring about decreased insulin degradation, but it is not known through what mechanism.

Since decreased insulin-I^{131} degradation seemed to be associated with increased physiologic insulin action, it seemed that it might be possible to inhibit insulin-I^{131} degradation as a therapeutic approach to diabetes mellitus. The relative insulin insufficiency of many of these patients might conceivably be relieved and physiologic balance restored, if it were possible to inhibit degradation of endogenous insulin. Many possible substances have been investigated, and of these, MC2346 has shown the most promise. It does inhibit the degradation of insulin-I^{131} and has been shown to reduce the incidence of diabetes after partial pancreatectomy. In the terms of the theories above, then, these two properties may be cause and effect. This substance therefore deserves further investigation.

SUMMARY

1. The process of degradation of insulin labeled with radioactive iodine (insulin-I^{131}) does not distinguish between labeled and nonlabeled hormone.

2. Insulin-I^{131} degradation takes place in many body tissues, notably in liver and kidney.

3. The degradation is reduced after hypophysectomy and probably after thyroidectomy. It is increased by thyroxine or triiodothyronine treatment. Growth hormone, hydrocortisone, and glucose have little effect on the degradation.

4. It is postulated that the degradation of insulin-I^{131} occurs in the process of inactivation of insulin, and if the degradation were to be inhibited the biologic activity of insulin might be enhanced.

5. A substance, MC2346, demonstrated such an inhibitory property, and the therapeutic implications of such a substance are discussed.

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DISCUSSION

GARFIELD G. DUNCAN, M.D., (*Philadelphia*): This important and well conceived study reported upon by Drs. Elgee and Williams is an application of the newest technics in the search to clarify obscure features in the metabolism of insulin.

The ready concentration of radioactivity in the kidney following the intravenous injection of insulin-I^{131} has been interpreted by the authors as indicating that the degradation of insulin in the kidneys is a major and not an incidental process and that the rate of degradation may be altered by influences known to affect the activity of insulin experimentally and clinically. These are new concepts of great potential importance.

The direction in which the studies have been undertaken makes it clear that these investigators fully realized that the tagged element might be a degradation product arriving in the kidney. Strong circumstantial evidences that this is not so have been presented. If this new concept is correct, it may provide another important step in the understanding of metabolic alterations surrounding the problem of diabetes. Comparisons of the behavior of

insulin-I^{131} with other labeled materials give strong support to the author's concept.

Clinically the alteration of the diabetes in patients with intercapillary glomerulosclerosis might represent a delay in the degradation of insulin permitting a more prolonged insulin effect in contrast with the effect in diabetic patients free from this disorder. Personally, I have looked upon the apparent decrease in the severity of the diabetes in these cases as being due largely to nutritional changes related to reduced caloric intake and reduced body weight. Admittedly, this has not been a completely satisfactory explanation of the amelioration of their diabetes.

Assuming that the conclusions arrived at by Drs. Elgee and Williams are correct — and it must be conceded that the evidence is very convincing — their findings might apply to the animal, or to the patient, receiving insulin either subcutaneously or intravenously in which cases the insulin reaches the systemic circulation by other than the portal circulation. It will be interesting to know if this tagged insulin injected into the portal vein, thereby following the same route as insulin in normal animals, follows the same distribution pattern or whether the greater collection of insulin-I^{131} will appear in the liver and less in the kidney. Possibly Dr. Elgee already has this information.

The cause or causes of diabetes are unknown. Damage to islet cell function leading to hyperglycemia and glycosuria probably is a late manifestation of a much more deep-seated process about which we know very little. An analogy of this concept is to be had in hemochromatosis in which diabetes is a relatively late manifestation of a disorder which happens to destroy pancreatic islets.

There are certain clues upon which the later onset of diabetes may be predicted with some certainty. Notable among these are the large babies of mothers long before diabetes becomes manifest. Disturbances in carbohydrate metabolism during pregnancy, the occasional appearance of retinal changes indistinguishable from diabetic retinitis, neuropathies, progressive atherosclerotic changes, changes in lipid metabolism, also may antedate the appearance of the signs upon which we pin the diagnosis of diabetes.

It is in the search for the fundamental fault which initiates these processess that such studies as reported by Dr. Elgee may prove most fruitful. He and Dr. Williams are delving into unexplored territory, and their ingenuity already has been rewarded.

Their further search into the mysteries surrounding the metabolism of insulin may well put a finger on the fundamental fault which instigates the series of abnormalities of which diabetes is but one. It is comforting to those of us who are primarily clinicians that this promising work holds no threat either to the destruction or removal or manipulation of the pituitary gland or of the adrenal gland.

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): I should like to know whether you have tested the rate of destruction after subcutaneous administration.

TOBY LEVITT, M.D., (*London, England*): This most fundamental and important work should help us understand many problems which have been worrying us for quite a long time. One little point which I did not get is, I believe adrenalectomy produced no change in the results, and I wonder why. Secondly, why was it necessary to give these big doses of insulin, and if these big insulin doses were given, could it be possible they affected the other endocrine systems indirectly. I should like to know why. Is it posssible to use other instrumental tools or methods to support these extremely important findings? Was this technic applied to animals or was it in part applied to human beings?

If possible, in their future work, I do hope the authors will be able to correlate the insulin function with the other hormones, especially the thyroid. They should really be complimented for most important work.

ARNOLD LAZAROW, M.D., (*Minneapolis*): Although the molecular weight of insulin in concentrated solution is in the neighborhood of 48,000, physical chemical studies have shown that in very dilute solution the insulin molecule dissociates into smaller units which have a molecular weight of 12,000 or possibly 6,000 units. Do you have evidence to show that the dissociated units of insulin are precipitated by trichloroacetic acid? Failure to precipitate the smaller insulin unit would certainly complicate the interpretation of your results.

FRANK L. ENGEL, M.D., (*Durham, N. C.*): I wonder whether you made any studies on the effect of diet on degradation of the insulin? Some years ago, Mirsky reported that the insulinase activity of the liver varied with the diet. Can you make any correlation between Mirsky's observation on liver insulinase and insulin degradation in your system?

NEIL J. ELGEE, M.D., (*Seattle*): All of our studies were done on rats in this particular investigative work. We have done some work with patients — for example, we have found diminished degradation in cirrhotics and in some patients with kidney disease. However, such

studies are still in the preliminary phase since we have been concerned primarily in the past year with insulin-I^{131} metabolism in the rat. Although we visualize that other tools are coming to be of value in this work, at the present time the study of degradation appears to be the most important.

We only used an insulin "load" in one of the experiments reported today. In the others each animal received less than one unit of insulin. The insulin "load" experiment purported to show the specificity of the labeled insulin — that it was not degraded in a different fashion from nonlabeled insulin — and in this experiment large doses were necessary. The animals were sacrificed 15 minutes after the dose. They did not appear ill, perhaps because of the high insulin resistance of the rat.

We have injected insulin-I^{131} into rats in the portal vein and compared distribution and degradation with that found after injection into the vena cava. After injection in the portal vein total radioactivity in liver was increased, and after vena cava injection renal total radioactivity was increased. However, the over-all degradation rate, that is, the relative concentration of tissue radioactivity in supernatant and precipitate fractions was no different. We were very interested in this experiment since work done by Weisberg and co-workers some years ago in dogs showed insulin to be less hypoglycemic when injected into the portal vein. They interpreted this to mean that the liver inactivated the material. We would not be at variance with their conclusions. We just did not demonstrate a similar effect using labeled insulin in rats. I hope to do the experiment in larger animals.

We have not studied the subcutaneous administration of insulin-I^{131}, Dr. Lukens. It could easily be done.

Adrenalectomy did not have any effect, it is true, on the degradation. I can simply say that as far as we could tell, degradation was not related to alterations in carbohydrate metabolism brought about by adrenalectomy.

As to the question of the TCA precipitability of diluted insulin, I think we have sufficient evidence that insulin does not get to a molecular size such that it is not precipitated by TCA, provided protein carrier is present. If carrier protein is not present precipitation may not be complete, but we have greatly diluted insulin-I^{131}, added a little plasma protein as carrier, and been able to precipitate the radioactivity completely.

As to the effects of diet, we have studied that, and the results conflict somewhat with Mirsky's work. He showed that the "insulinase" activity of liver from fasted rats was decreased if extracts or homogenate were used, but tended to be increased if slices were used. We fasted rats for three days, and we found the degradation of the labeled insulin to be somewhat reduced, a finding which fits in perhaps with observations in one of Mirsky's articles and not with the other.

ROBERT H. WILLIAMS, M.D., (*Seattle*): When we add equivalent small amounts of labeled insulin to boiled liver in one beaker and to surviving liver slices in another and permit the mixture to incubate for 15 minutes, we obtain approximately 100 per cent of radioactivity in the TCA precipitate of the boiled liver preparation, but considerably less in the precipitate of the surviving liver preparation. Therefore, the degree of dilution of the insulin would not account for the quantity not precipitated in the instance of the surviving liver slices, but it would be attributable to degradation of the insulin.

SUMMARIO IN INTERLINGUA

Degradation de Insulina in Alterate Statos Metabolic

1. Le systema in que insulina etiquettate a iodo radioactive (I^{131}) es degradate non distingue inter insulina etiquettate e non-etiquettate.

2. Le degradation de insulina a I^{131} occurre in multe texitos del corpore, specialmente in hepate e ren.

3. Le degradation es reducite post hypophysectomia e probabilemente post thyroidectomia. Illo es augmentate per le administration de thyroxina o triiodothyronina. Hormon de crescentia, hydrocortisona, e glucosa influe pauco super le degradation.

4. Nos postula que le degradation de insulina a I^{131} occurre in le processo de inactivation de insulina e que le inhibition del degradation es possibilemente un methodo pro augmentar le activitate biologic de insulina.

5. Un substantia que demonstrava le potentia de un tal inhibition es MC2346. Le implicationes therapeutic de iste facto es discutite.