Destruction of I^{131} Labeled Insulin by Liver Slices.* (21057)

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The biological activity of insulin is destroyed after incubation with homogenates, extracts and slices of liver(1,2). Two systems appear to be involved in the process, an insulin-inactivating system which has the properties of an enzyme (insulinase)(1) and an inhibitor of this system which appears to be a heat-stable, dialyzable non-protein factor (insulinase-inhibitor)(3). The mode of action or the specificity of insulinase has not been established principally because of the difficulties involved in the biological assay of small amounts of insulin.

With the availability of I^{131} it has become possible to label insulin and to trace the fate of the insulin molecule *in vivo* and *in vitro* (4,5). Since the insulin-inactivating activity of liver slices represents the balance between the actions of insulinase and of insulinase-inhibitor(2) the effect of such slices on the *in vitro* degradation of labeled insulin (I^{131} insulin) was studied.

Methods. Fed male rats of from 150 to 200 g in weight were decapitated and as much blood as possible was drained from the carcass of each animal. The liver was removed and placed in cold phosphate buffer, pH 7.4. Slices of approximately 1 cm^2 in size were prepared with a Stadie-Riggs apparatus(6). After blotting with filter paper, the slices were weighed and the desired amount placed into a flask containing 2 ml of the phosphate buffer. Various concentrations of I^{131} insulin (or I^{131} human serum albumin) in 1 ml buffer were added to the flasks which were shaken in a Dubnoff incubator at a rate of 70 cycles/minute and maintained at 37°C. At the end of the incubation period, an aliquot of the incubation mixture was precipitated with an equal volume of 10% trichloracetic acid. The supernatant and precipitate were separated by centrifugation and after several washings with buffer the radioactivity of the precipitate and of the supernatant plus washings was measured in a well-type gamma counter. Each count was corrected for background and coincidence and the amount of I^{131} insulin degraded was calculated from the percentage of the total counts of the precipitate plus supernatant that was found in the non-protein fraction. In some instances, in addition to the radioactivity measurements, the hypoglycemic activity of an appropriate aliquot of the incubation mixture was determined in rabbits and expressed as the 'pooled percentage of the initial blood sugar level'. An increase in the 'pooled percentage' over that produced by a similar quantity of insulin which has not been incubated serves as an index of insulinase activity(1). Each experiment was performed in triplicate. I^{131} insulin was prepared by subjecting crystalline insulin,† which assayed 25 units per mg to the procedure described by Talmage, Dixon, Bukantz and Dammin(7). From 98 to 99% of the I^{131} insulin was precipitable with 10% trichloracetic acid. I^{131} human serum albumin was prepared in the same manner. The insulinase-inhibitor was prepared from liver by the procedure described in a previous communication(3). Blood sugar was determined by Nelson's method(8).

Results. Incubation of I^{131} insulin with liver slices results in a concomitant reduction in the biological activity of insulin (*i.e.*, an increase in the 'pooled percentage') and a degradation of the insulin molecule (*i.e.*, an increase in the radioactivity of the non-protein fraction of the incubation mixture) (Fig. 1). Accordingly, the insulinase activity of liver slices is associated with a degradation of the insulin molecule.

The relation between the degradation of I^{131} insulin and the length of incubation was

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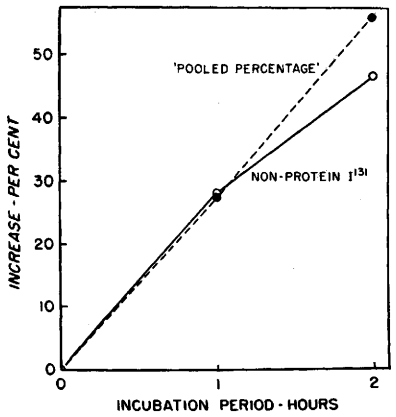

FIG. 1. Inactivation and degradation of I^{131} insulin by liver slices. Each point represents 3 sets of 500 mg liver slices incubated with 3 units of I^{131} insulin at 37°C and pH 7.4 for the designated time.

determined by keeping the quantity of liver slices constant (approximately 500 mg wet weight) and varying the period of incubation. It is apparent from Fig. 2 that the degradation follows zero order kinetics for at least during the first 30 minutes. Thereafter, the reaction follows first order kinetics.

The relation between the quantity of liver and the degradation of insulin was determined by incubating I^{131} insulin with from 1 to 6 slices for 30 minutes. As has been demonstrated previously for insulinase activity(2), the greater the quantity of liver slices, the greater is the degradation of the I^{131} insulin (Fig. 3).

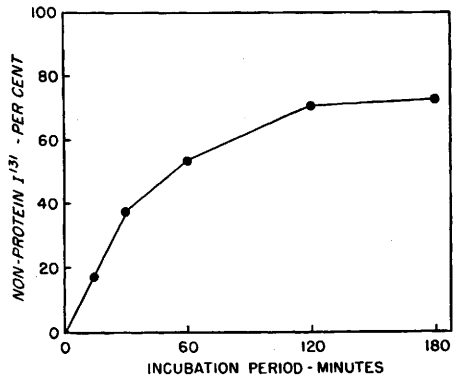

FIG. 2. Relation between length of incubation and degradation of I^{131} insulin. Each point represents mean effect of 3 sets of 500 mg liver slices incubated with 3 units I^{131} insulin at 37°C and pH 7.4 for the designated time.

That the degradation of I^{131} insulin follows first order reaction kinetics is apparent also when the quantity of liver slices are kept constant and the concentration of I^{131} insulin substrate is varied (Fig. 4). The Km of the degradation produced by liver slices from different rats varied markedly. That this variation may be due to the presence of variable quantities of endogenous insulinase-inhibitor in the liver slices is suggested by the effect of exogenous insulinase-inhibitor on the activity of liver slices incubated with varying concentrations of I^{131} insulin (Fig. 5). The Lineweaver-Burk plot of the data in Fig. 5 suggests that the inhibitor is a non-competitive inhibitor(9).

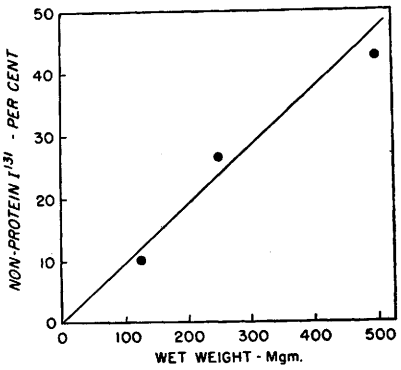

FIG. 3. Relation between quantity of liver and degradation of I^{131} insulin. Each point represents mean effect of 3 sets of liver slices of specified wet weight incubated with 3 units I^{131} insulin for 30 min. at 37°C and pH 7.4.

As a step towards establishing the specificity of the enzyme involved in the degradation of insulin, liver slices were incubated with I^{131} human serum albumin. Negligible amounts of the albumin were degraded during a 3-hour period of incubation while a marked degradation of a similar quantity of I^{131} insulin occurred during the same interval on incubation with similar liver slices.

Comment. The data indicate that relatively intact cells of surviving liver slices are capable not only of inactivating the biological activity of insulin but also of degrading the insulin molecule. If both phenomena are due to a single enzyme system then the rate of degradation of I^{131} insulin can serve as a measure of insulinase activity. The inhibition of both

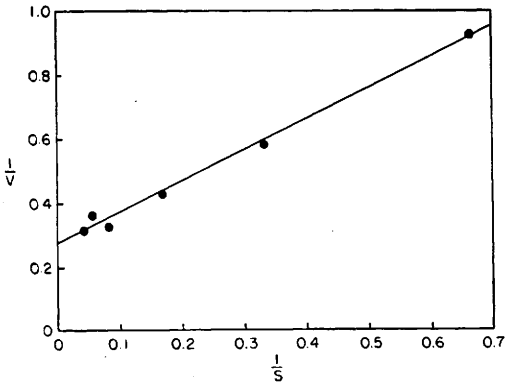

FIG. 4. Effect of concentration of I^{131} insulin on its degradation by liver slices. Each point represents mean effect of 3 sets of 500 mg of liver slices incubated at 37°C and pH 7.4 for 30 min. (V) with different concentrations of I^{131} insulin (S).

insulinase activity and of the degradation of I^{131} insulin by the insulinase-inhibitor supports the possibility that the two phenomena are intimately related and due to an enzyme system which follows first order kinetics. That the enzyme system involved is not some nonspecific proteolytic system is suggested by the inability of liver slices to degrade I^{131} human serum albumin.

The measurement of insulinase activity by the biological technic(1) does not permit the detection of small quantities. With the degradation of I^{131} insulin as an index of insulinase, it is possible to detect relatively minute quantities of the enzyme in blood and other tissues.

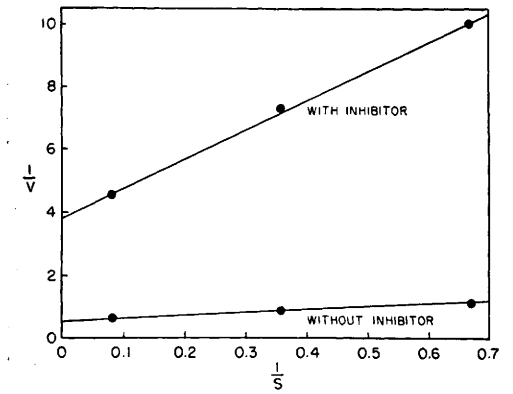

FIG. 5. Effect of insulinase-inhibitor on degradation of I^{131} insulin by liver slices. Each point represents mean effect of 3 sets of 500 mg of liver slices incubated at 37°C and pH 7.4 for 30 min. (V) with different concentrations of I^{131} insulin (S). 0.5 ml of insulinase-inhibitor was added to incubation mixture of one group of liver slices.

There is little doubt that diabetes mellitus in man is due to an insufficiency of insulin relative to the requirements by the tissues. In only a small proportion of instances, however, can the metabolic derangement in man be attributed solely to a diminution in the production of insulin(10). The demonstration that insulin can be destroyed by slices of liver lends support to the hypothesis that the insulin insufficiency of the patient with diabetes mellitus may be due to an increase in the rate of insulin destruction consequent to an increase in insulinase activity or a decrease in the availability of the insulinase-inhibitor(11).

Summary. 1. Insulin labeled with I^{131} was used to determine the effect of liver slices on the degradation of insulin. 2. The insulin-inactivating activity of slices of rat liver (insulinase) is associated with a degradation of I^{131} insulin. 3. The degradation of I^{131} insulin by liver slices follows first order kinetics and is related directly to the quantity of liver slices employed. 4. A non-protein fraction of liver (insulinase-inhibitor) which inhibits the action of insulinase also inhibits the degradation of I^{131} insulin by liver slices.

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