THE RELATIVE SPECIFICITY OF THE INSULINASE ACTIVITY OF RAT LIVER EXTRACTS*

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Insulin is inactivated and destroyed by extracts, homogenates, and slices of liver and other tissues (1-4) as well as by the intact animal (5). This destruction is attributed to the action of an enzyme system, tentatively designated as insulinase, which catalyzes the hydrolysis of insulin (1, 6). The fact that tissues like brain and erythrocytes, which are rich in proteinas and peptidases, are poor in insulinase activity suggested that the latter may be relatively specific (7). In accord was the demonstration that, long after the insulinase activity of an aged liver extract had been lost, the catheptic activity of the extract persisted (7).

More definitive data on the relative specificity of insulinase was obtained from studies on the comparison of the effect of a liver extract on $^{131}I$-labeled insulin with the effect on similarly labeled ribonuclease, pepsin, prolactin, lysozyme, chymotrypsin, and human serum albumin. Whereas there was no significant effect of the extract on the labeled lysozyme, chymotrypsin, and human serum albumin, an appreciable degradation of labeled ribonuclease, pepsin, and, to a lesser degree, prolactin occurred under the conditions which yielded maximal degradation of insulin (6). Studies on the effect of different concentrations of these proteins on the velocity of their degradation by a liver extract indicated that the system responsible for the destruction of insulin is different from that responsible for the destruction of the other proteins. Further evidence of the relative specificity of the system responsible for the destruction of insulin was gained from studies which revealed that the addition of the above proteins to the incubation mixture containing labeled insulin did not influence the degradation of various concentrations of insulin (6).

Although Tomizawa and Williams (3) have obtained similar data and concede that the insulin-inactivating system of the liver has some degree of specificity, they state that "it would be judicious to avoid using the term 'insulinase.'" This caution is derived from their observations that the addition of various proteins to the incubation mixture of a liver extract and labeled insulin results in a diminution in the percentage of the radio-

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activity which appears in the trichloroacetic acid-soluble fraction. Thus, whereas human serum albumin, bovine plasma albumin, α-lactalbumin, and ribonuclease did not influence the percentage of the radioactivity of the trichloroacetic acid-soluble fraction, casein, α-corticotropin, trichloroacetate, glucagon, and growth hormone decreased the rate of insulin degradation.

The hydrolysates of a fairly large number of proteins can inhibit the destruction of insulin in vitro and in vivo (8). Since some of the proteins which are alleged to compete with insulin are rapidly destroyed by homogenates of liver (9–11), it is quite possible that the observations of Tomizawa and Williams are due to an inhibition of insulin destruction by hydrolytic products rather than to substrate competition. Accordingly, it was deemed pertinent to determine whether the aforementioned proteins are degraded by extracts which destroy insulin and, if that be the case, whether the system responsible for the destruction of insulin differs from the system (or systems) responsible for the destruction of the other proteins.

Methods

Extracts of livers from fed, male Carworth rats were prepared as described previously (6). The action of each extract on the degradation of I\textsuperscript{131}-labeled crystalline insulin, glucagon, crystalline ribonuclease, corticotropin, and growth hormone was determined. All the proteins\textsuperscript{1} were labeled by a procedure which is essentially similar to that described by Talmage, Dixon, Bukantz, and Dammin (12). Unless otherwise stated, the incubation mixture consisted of 1 ml. of extract plus 1 ml. of Sörensen’s m/15 phosphate buffer (13) containing 1 mg. of a mixture of I\textsuperscript{131}-labeled and unlabeled protein. As a control, 1 ml. of boiled homogenate plus 1 ml. of buffer containing a similar quantity of the I\textsuperscript{131}-labeled and unlabeled protein was incubated at the same time. At the end of the incubation period of 30 minutes at 37° and pH 7.8, 2 ml. of 10 per cent trichloroacetic acid were added to the incubation mixture. The quantity of protein degraded by the extracts was computed as previously described (6) from the percentage of the total radioactivity which appeared in the trichloroacetic acid filtrate. The difference between the unboiled and boiled extracts represents the quantity of protein degraded by the heat-labile system.

After determining the quantity of each protein that was hydrolyzed, the

\textsuperscript{1} We are indebted to Dr. W. R. Kirtley of Eli Lilly and Company for supplies of crystalline insulin and glucagon, to Dr. C. J. O’Donovan of The Upjohn Company for adrenocorticotropin, and to Dr. Irby Bunding of the Armour Laboratories for growth hormone.
A fresh extract was permitted to age for 44 hours at 5° and its activity on the degradation of the same quantities of protein was determined again. The relative activity of the aged extract was expressed in terms of the activity of the fresh extract. Similar experiments were performed with extracts which were dialyzed against distilled water for 5.5 hours at 5°.

In addition to the above, a comparison was made between the activity of fresh extracts incubated with the labeled proteins dissolved in Sörensen's phosphate buffer and in MacIlvaine's citrate-phosphate buffer (14).

**Results**

The action of eight fresh extracts of rat livers on the degradation of various labeled proteins is illustrated in Fig. 1, where the rates of degradation are expressed as the per cent of the degradation of insulin. It is apparent that extracts which catalyze the destruction of insulin are capable also of catalyzing the degradation of other proteins under identical conditions. Thus, by expressing the rate of insulin destruction by each extract as 100, the mean (± standard error) rates for casein, ribonuclease, growth hormone, corticotropin, and glucagon are 27.0 ± 4.3, 45.9 ± 5.7, 47.9 ± 4.9, 84.4 ± 16.3, and 316.5 ± 39.6, respectively.

The effect of aging of eight rat liver extracts on the degradation of the labeled proteins is depicted in Fig. 2, where the activity of each fresh extract on each protein is expressed as 100 per cent. It is apparent that,
FIG. 2. The effect of aging of liver extracts on the degradation of various I\textsuperscript{131}labeled proteins. The activity of each extract before aging (crosshatched bars) is expressed as 100 per cent and the activity of aging (stippled bars) is expressed as the mean (± standard error) per cent of the former.

FIG. 3. The effect of dialysis of liver extracts on the degradation of various I\textsuperscript{131}labeled proteins. The activity of each extract before dialysis (crosshatched bars) is expressed as 100 per cent and the activity after dialysis (stippled bars) is expressed as the mean (± standard error) per cent of the former.

whereas the activity of the extracts on the degradation of insulin is markedly reduced after 44 hours of aging ($P < 0.001$), there is no statistically significant decrease in the activity of the same aged extracts on the degradation of the other proteins ($P > 0.05$).
The effect of a 5.5 hour period of dialysis of fresh liver extracts on the degradation of the various proteins is depicted in Fig. 3. As with aged extracts, the activity of dialyzed extracts on the degradation of insulin is markedly reduced ($P < 0.001$). The same treatment, however, does not produce a statistically significant effect on the action of the extracts on the degradation of the other proteins.

The observation that citrate enhances the activity of a liver extract on the degradation of insulin\(^2\) led to studies on the effect of the incubation of 

![Fig. 4. Effect of citrate ions on the degradation of various \(\text{I}^{131}\)-labeled proteins by liver extracts. The activity of each extract incubated with phosphate buffer (crosshatched bars) is expressed as 100 per cent, and the activity of the same extracts incubated with MacIlvaine's buffer (stippled bars) is expressed as the mean (+ standard error) per cent of the former.]

\(\text{DISCUSSION}\)

The degradation of \(\text{I}^{131}\)-labeled insulin by liver extracts is associated with a concomitant hydrolysis of the molecule (6) and the destruction of its hypoglycemic action (15, 16). Likewise, the degradation of \(\text{I}^{131}\)-labeled glucagon is associated with hydrolysis of the molecule (16, 17) and the

\(^2\)We are indebted to Dr. Neil C. Davis for this observation.
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loss of its hyperglycemic activity (10, 11, 16, 17). Further, the incubation of adrenocorticotropic hormone with liver homogenates results in the destruction of its biological activity (9). Accordingly, the degradation of labeled growth hormone, corticotropin, and ribonuclease by liver extracts may also reflect the hydrolysis of these proteins and the destruction of their biological activities. However, the relative rates at which the various proteins are hydrolyzed and inactivated by the liver extracts are not necessarily related to the rates at which the labeled fragments are released from the labeled proteins.

That the system (or systems) responsible for the degradation of growth hormone, glucagon, corticotropin, ribonuclease, and casein is different from that responsible for the degradation of insulin is indicated by the demonstration that, while the degradation of insulin is markedly diminished when extracts are aged or dialyzed, the degradation of the other proteins is not changed significantly. Further, whereas the degradation of insulin is increased in the presence of citrate, there is no significant effect on the degradation of the other proteins. In accord is the report of Snedecor and MacGrath (10) on the preparation of a glucagon-inactivating extract of liver, which is relatively impotent in the inactivation of insulin, and Kenny's observation that dialysis does not affect the glucagon-inactivating properties of rat liver extracts (11).

3 hour acid hydrolysates of insulin, glucagon, corticotropin, growth hormone, ribonuclease, and casein contain peptides which can inhibit the destruction of insulin by liver extracts (12). Since these proteins are destroyed by liver extracts, it is quite probable that their hydrolytic products, like those of acid hydrolysates, can act as inhibitors of insulinase. Accordingly, the observation of Tomizawa and Williams (3) that the degradation of insulin is inhibited in the presence of the above proteins may not be due to a competition between insulin and the protein for the enzyme system but to an inhibition of insulinase by some hydrolytic product of the added protein. The competitive nature of the inhibition may be due to a competition between insulin and the hydrolytic product.

The above and previous observations (1, 6, 7) support the hypothesis that the system responsible for the inactivation and destruction of insulin is relatively specific. The determination of absolute specificity, however, must await crystallization of the enzyme and the determination of the precise linkages in the insulin molecule that are ruptured. Until such time, it appears quite appropriate to refer to the system responsible for the destruction of insulin as insulinase.

SUMMARY

Extracts of rat liver, which catalyze the degradation of insulin, catalyze also the degradation of casein, ribonuclease, corticotropin, growth hor-
mone, and glucagon under identical conditions. The relative specificity of the system responsible for the destruction of insulin (insulinase) is indicated by the demonstration that, whereas aging and dialysis decrease and the presence of citrate ions increases the activity of liver extracts on the degradation of insulin, such measures do not influence the degradation of the other proteins.

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