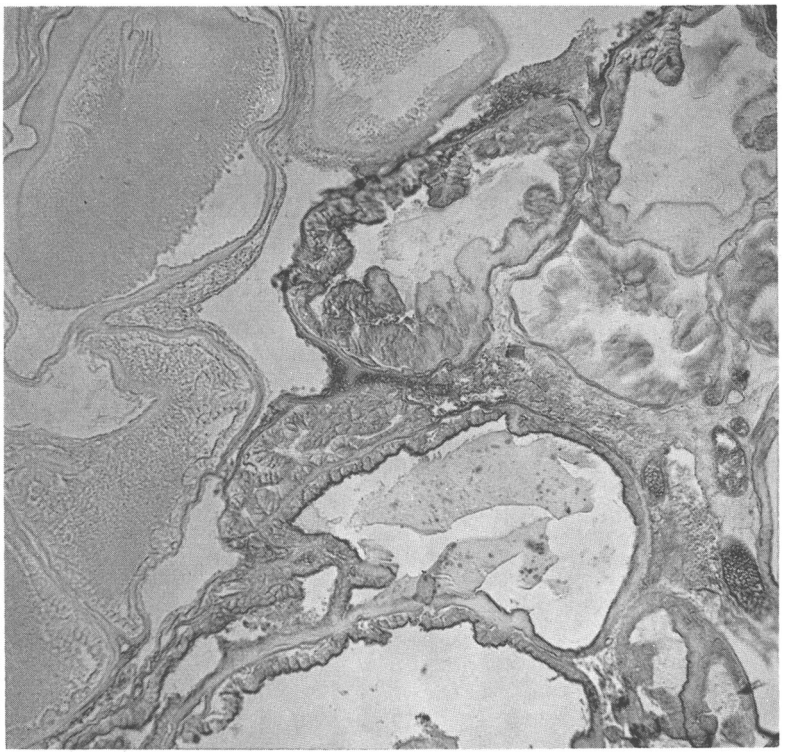

FIG. 4. Zinc stain of dorsolateral prostate of mature rat at junction of dorsal (left) and lateral (right) components of the gland, showing positive zinc stain in the lateral portion (black in photo) and negative reaction in dorsal portion (light gray in photo). Dithizone × 136.

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Received April 3, 1956. P.S.E.B.M., 1956, v92.

Inhibition of Insulin Degradation by Amino Acids and Related Compounds.* (22371)

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Shmidt and Saatchian(1) and Mirsky and Broh-Kahn(2) have shown that insulin is readily inactivated by liver preparations at pH 7.5. Insulin labeled with I^{131} is likewise degraded and the accumulation of radioactive material soluble in 10% trichloracetic acid has been found(3,4) to parallel the biological inactivation of insulin and the formation of non-protein nitrogen from insulin. These ob-

* This research project has been supported in part by grants from the U. S. Public Health Service, the Atomic Energy Commission and the Upjohn Co.

servations suggest that this inactivation of insulin is associated with proteolysis and that liberation of TCA-soluble radioactivity from insulin-I^{131} can be used to measure this process. Although this insulin degradation has been attributed by Mirsky and Broh-Kahn (2) to an enzyme, which they called "insulinase," the enzymatic action is probably not absolutely specific for insulin, since α-corticotropin, casein, glucagon and somatotropin have been found to serve as substrate competitors(5,6). In this manner these compounds decrease the action of the enzyme on insulin. The discovery of a compound that could be given safely, orally, and that would significantly inhibit the insulin-degrading enzyme system might be of great advantage in the treatment of diabetes. Indeed, we have produced evidence in this laboratory to show that this mechanism can account for part of the effectiveness of p-aminobenzenebutylurea (7) and of butyltolylsulfonylurea in the treatment of diabetes. Mirsky(8) has reported on the "insulinase" inhibitory effects of several compounds, including a preparation from the liver, of small molecular weight, which he called "insulinase inhibitor."

In studying a certain type of cheese that a patient claimed benefited her diabetes, we found by *in vitro* studies that insulin-I^{131} degradation was not only inhibited by the cheese but also by some of its bacteria and the culture media used to grow the bacteria. Two of the constituents of the media, trypticase and phytone also exerted significant inhibition. Since trypticase is a tryptic digestion product of casein we investigated a casein hydrolysate preparation (Amigen) consisting chiefly of individual amino acids. This compound was found markedly inhibitory and has also been reported to produce hypoglycemia in normal subjects, following its oral ingestion (9). We then investigated the inhibitory effect of many of the individual amino acids and these results serve as the basis for this report.

Methods. The insulin-degrading enzyme preparation used in all experiments consisted of a dried powder remaining after special treatment of beef liver homogenate with acetone and ether. The portion of 4 mg of this material soluble in 0.5 ml of buffer was placed in a beaker. Either 0.067 M phosphate or, much more frequently, 0.1 M Tris (hydroxymethyl) aminomethane, containing 5×10^{-3} M versene was used as buffer. To the enzyme preparation was added 0.5 ml solution of the test inhibitor and 1 ml of insulin solution containing 0.4 μg of insulin-I^{131} (dialyzed for several hours immediately before using) and 0.1 mg of amorphous insulin. In this system the quantity of enzyme is the limiting factor in the rate of the reaction. The total 2 ml mixture, with the pH adjusted to 7.5, was placed in a Dubnoff Metabolic Shaker at 37.5°C and shaken for exactly 5 minutes. Fifteen seconds before the end of the incubation 1 ml of 2% human plasma in water was added to insure complete precipitation of insulin, and at the end of the incubation period 3 ml of 20% trichloracetic acid was added. After standing for 30 minutes the mixture was centrifuged and the precipitate washed twice with 1 ml of 5% TCA. The 3 supernatant specimens were pooled and the precipitate dissolved in 30% KOH. The radioactivity in each specimen was measured by a well-type gamma counter. Then a calculation was made of the percentage of the total radioactivity found in the supernatant fractions. The following 3 control studies were conducted with each experiment: (a) 1 ml of buffer plus 1 ml of insulin solution, (b) 0.5 ml of buffer plus 0.5 ml of enzyme preparation plus 1 ml of insulin solution, and (c) 0.5 ml of inhibitor plus 0.5 ml of buffer plus 1 ml of insulin solution. All determinations were conducted at least in triplicate.

Results. As shown in Table I most of the amino acids tested did not inhibit the insulin-degrading activity. There was marked inhibition by ergothioneine, 3,4-dihydroxyphenylalanine, cystine, isoleucine, indole-3-acetic acid, and casein hydrolysate (Amigen). The mechanism by which certain amino acids inhibit insulin degradation is not known. However, since there is evidence, recently summarized(10), suggesting that insulin may be degraded by reduction and/or by a proteolytic process the effect of inhibitors on these

TABLE I. Results of Incubation of Insulin-I^{131} plus Insulin-Degrading Enzyme with and without Test Inhibitors.

Inhibitor	Moles/ml $\times 10^{-2}$	Avg % I^{131} in supernatant: No inhibitor*	Inhibitor†	Avg % inhibition
Glutamine	2	6.1 ± .4	6.1 ± .6	NSD
	5	4.5 ± .3	4.6 ± .3	"
Glycine	2.5	6.1 ± .4	6.1 ± .1	"
	5	5.0 ± .1	5.5 ± .3	"
β-Alanine	2.5	6.1 ± .4	5.8 ± .4	"
	5	4.5 ± .3	5.1 ± .2	"
d,l-Asparagine	2.5	6.1 ± .4	6.3 ± .4	"
	5	4.5 ± .3	5.1 ± .3	"
l-Arginine	2.5	6.1 ± .4	6.4 ± .4	"
	5	4.5 ± .3	4.0 ± .3	"
d,l-Serine	2.5	6.1 ± .4	6.4 ± .1	"
	5	5.0 ± .1	5.2 ± .1	"
d,l-Threonine	2.5	6.1 ± .4	6.6 ± .1	"
	5	5.0 ± .1	4.5 ± .1	"
d,l-Leucine	2.5	6.1 ± .4	5.5 ± .1	"
l-Methionine	1	6.9 ± .2	7.6 ± .5	"
	5	4.5 ± .3	4.3 ± .1	"
l-Histidine monohydrochloride	1	6.9 ± .2	7.6 ±1	"
	5	4.5 ± .3	4.4 ± .2	"
Hydroxyproline	2.5	4.2 ± .1	4.0 ± .1	"
	5	4.2 ± .1	4.1 ± .2	"
Tyrosine	.13	4.7 ± .2	4.5 ± .1	"
	.25	4.7 ± .2	4.5 ± .3	"
l-Tryptophane	1	6.9 ± .2	5.1 ± .2	26
	3	6.1 ± .4	1.6 ± .3	73
l-Cystine	.5‡	4.7 ± .2	.1 ± .1	98
l-Isoleucine	2.5	4.2 ± .1	3.3 ± .4	21
	5	4.2 ± .1	2.1 ± .1	50
l-Ergothioneine	.4	5.0 ± .1	1.8 ± .3	64
	2.5	5.0 ± .1	.1 ±0	100
d,l-3,4-Dihydroxyphenylalanine	.5	4.7 ± .2	1.4 ± .1	71
	1.2	5.0 ± .1	1.1 ±0	77
	2.5	4.2 ± .1	.4 ± .2	91
Indole-3-acetic acid	1	4.2 ± .1	1.5 ± .2	64
	2	4.2 ± .1	1.1 ± .1	74
Casein hydrolysate (Amigen)	3 mg/ml	4.7 ± .2	2.6 ± .1	46
	6.3 "	5.0 ± .1	.4 ± .1	92

* Figures in this column consist of percentage radioactivity in TCA supernatant after incubating insulin-I^{131} with the enzyme preparation, after subtracting the control value which consisted of amount of supernatant radioactivity in beakers containing only insulin-I^{131} and buffer; the latter value usually was less than 1%. Figures shown are avg of 3 determinations; maximal deviation is shown by each.

† Conditions relative to the figures in this column are like those in the preceding paragraph except that test inhibitor was added to control and enzyme-containing beakers.

‡ In order to keep this compound in solution it was necessary to maintain a pH of 8.2; controls for this determination were also at pH 8.2.

NSD = No significant difference.

mechanisms must be considered. It may be hypothesized that cystine protects insulin from degradation by inactivating the enzyme system by oxidation of the sulfhydryl groups. The mechanism by which the other compounds are inhibitory is not known. It is noteworthy that dihydroxyphenylalanine is very active while tyrosine, with a structure differing only in the absence of a hydroxyl group, is inactive.

In comparable investigations, to be reported in detail subsequently, we have found inhibition of insulin degradation by the following compounds: thiamine, thioctic acid, indole, skatole, phenylacetic acid, phenylpropionic acid and indole-3-propionic acid.

Studies are being conducted in mice and man to determine to what extent the effective inhibitors augment the hypoglycemic action of insulin.

Summary. In an *in vitro* system using insulin-I^{131} and a special liver preparation which degrades insulin, it has been shown that certain amino acids and related compounds inhibit insulin-I^{131} degradation, as measured by the accumulation of trichloracetic acid soluble radioactivity. Marked inhibition was demonstrated using cystine, ergothioneine, 3, 4-dihydroxyphenylalanine, isoleucine, tryptophane, indole-3-acetic acid, and casein hydrolysate (Amigen). In the concentrations tested no significant inhibition was exerted by glutamine, glycine, β-alanine, asparagine, arginine, serine, threonine, leucine, methionine, histidine, hydroxyproline or tyrosine.

The authors are very grateful to Drs. Henry H. Tomizawa, Yadviga D. Halsey and Hiromichi T. Narahara for their helpful suggestions.

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Received April 3, 1956. P.S.E.B.M., 1956, v92.

Anti-Inflammatory Activities of Several 9 α-Halo Derivatives of Adrenal Steroids.* (22372)

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The synthesis of a series of 9 α-halo derivatives of adrenal steroids has been reported by Fried and co-workers(1-3). These compounds have been shown to possess unusually high adrenocortical activity in the rat(3-5), in the dog(6,7), and in man(7,8). Several of these compounds are part of a series prepared to study the possible influence of variations in side chain upon adrenocortical action. In the present paper the anti-inflammatory activities of these compounds are reported, using as the anti-inflammatory index the inhibition of formation of granulation tissue around a cotton pellet.

The use of cotton as an irritant in anti-inflammatory studies has been previously reported(9,10). Inhibition of the inflammatory reaction to cotton by either systemic or local administration of cortisone has been demonstrated by Meier, Schuler and Desaulles(9).

Materials and methods. A 5 to 7 mg cotton pellet is inserted high into the upper dorsal area of a male rat (weight range 120-150 g) at the time of bilateral adrenalectomy. The pellet is prepared from non-sterile unbleached cotton, which is rolled into a ball with the fingers(11). The pellet is inserted with long thin forceps, subcutaneously,

* Preliminary report presented at Fed. Am. Soc. for Exp. Biol., San Francisco, April 1955, (*Fed. Proc.*, 1955, v14, 281).