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Insulinase-Inhibitory Action of Metabolic Derivatives of L-Tryptophan.* (23150)

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A decrease in blood sugar concentration of normal rats is produced by a number of metabolic derivatives of L-tryptophan administered by stomach tube(1). Since these compounds are not effective in the severely diabetic alloxanized rat, the hypoglycemic response produced in the normal animal cannot be due to either a decrease in availability of some hyperglycemic agent or to an acute hepatotoxic action and inhibition of enzyme systems involved in glycogenolysis. Insulin dependency of the hypoglycemic response, however, may result from either an increase in secretion of insulin or a decrease in destruction of endogenous insulin consequent to an inhibition of insulinase. Accordingly, the influence of various metabolic products of L-tryptophan on the activity of insulinase *in vitro* and *in vivo* was determined.

Methods. Extracts of livers from fed, male Carworth rats were prepared as described previously(2). The incubation mixture consisted of 1 ml of extract plus 1 ml of Sörensen's M/15 phosphate buffer(3) containing 20 units of a mixture of I^{131} labeled and unlabeled insulin as well as various concentrations of the metabolic products of L-tryptophan. As a control, the same mixture was incubated without the L-tryptophan derivatives. At the end of 30 minutes of incubation at 37°C and pH 7.8, 2 ml of 10% trichloracetic acid was added to the incubation mixture and the quantity of insulin degraded was computed as previously described(2) from the percentage of total radioactivity which appeared in the trichloracetic acid filtrate. The metabolic products of L-tryptophan† used, were indole-3-acetic acid, anthranilic acid, kynurenic acid, picolinic acid, quinolinic acid, nicotinic acid, nicotinamide, nicotinuric acid, 5-hydroxytryptophan, 5-hydroxytryptamine creatinine sulfate, 5-hydroxyindoleacetic acid, indole and skatole. These compounds were dissolved or suspended in 0.5% sodium bicarbonate and adjusted to pH 7.8. To determine the effect of the various compounds on insulinase activity of the intact animal, groups of 10 fed male mice were given a subcutaneous injection of 0.004 mM of the L-tryptophan derivatives in 0.05 ml/gram body weight. Immediately thereafter the mice were given a subcutaneous injection of 1 ml of 5.5% solution of glucose. One hour later,

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one group of mice was given an intraperitoneal injection of 0.4 unit insulin in 0.02 ml acidified water/gram body weight and another subcutaneous injection of 1 ml of the 5.5% glucose solution. The insulin consisted of a mixture of crystalline zinc insulin and tracer quantities of I^{131} labeled insulin. Groups of 10 mice which were given sodium bicarbonate instead of the L-tryptophan derivatives served as controls. All mice were sacrificed one hour after injection of the insulin. The preparation of the animals and determination of the quantity of insulin destroyed/hour/100 g of mouse was the same as that described previously(4).

Results. The data on the effect of various metabolic products of L-tryptophan on destruction of insulin by fresh extracts of rat liver are summarized in Table I. All but 2 of the compounds exerted some degree of insulinase inhibition. Whereas nicotinic acid produced no effect, nicotinamide produced an increase in rate of destruction of insulin by the fresh extracts.

The action of some of the compounds on destruction of insulin by intact mice is depicted in Fig. 1 which also illustrates the action of equivalent dosages of the same compounds by mouth on blood sugar concentration of rats. Those compounds which produced a decrease in blood sugar concentration of the rat produced also a statistically significant ($P<0.001$) inhibition of the destruction of exogenous insulin by intact mice. Quinolinic acid which had no significant influence on blood sugar concentration of rats and nicotinamide which induced a marked hypoglycemia, produced a significant increase in insulinase activity of mice. The action of nicotinic acid, indole and skatole on destruction of insulin by intact mice was not established since these compounds were toxic in the quantities employed.

TABLE I. Effect of Metabolic Products of L-Tryptophan on Inhibition of Destruction of Insulin by Extracts of Rat Livers.

Compound	Inhibition, mean ± % .1 M	.05 M	.025 M
Kynurenic acid	63.5 ± 1.4	35.5 ± 1.3	39.0 ± 1.6
Indole-3-acetic acid	60.6 ± 1.6	56.9 ± 2.0	26.9 ± 5.2
5-Hydroxyindole acetic acid	57.7 ± 3.2	65.0 ± 3.2	32.5 ± 2.7
L-Tryptophan	56.6 ± 1.3	57.0 ± 1.1	29.2 ± 1.6
5-Hydroxytryptophan	56.4 ± 2.0	57.8 ± 1.3	18.8 ± 1.6
Nicotinuric acid	56.1 ± 1.2	49.7 ± 1.6	15.6 ± 2.3
Anthranilic acid	45.8 ± 1.5	36.5 ± .3	2.4 ± 1.3
5-Hydroxytryptamine	43.8 ± 3.7	13.6 ± 2.4	8.8 ± 1.1
Picolinic acid	42.5 ± 4.3	38.4 ± .6	8.8 ± 2.0
Quinolinic acid	35.0 ± 6.6	38.4 ± .6	19.3 ± .6
Indole	25.6 ± 1.7	23.3 ± .9	13.5 ± 2.9
Skatole	12.5 ± 5.8	16.6 ± 1.2	8.6 ± 4.3
Nicotinic acid	1.3 ± 1.0		5.1 ± 3.1
Nicotinamide			−50.4 ± 1.3

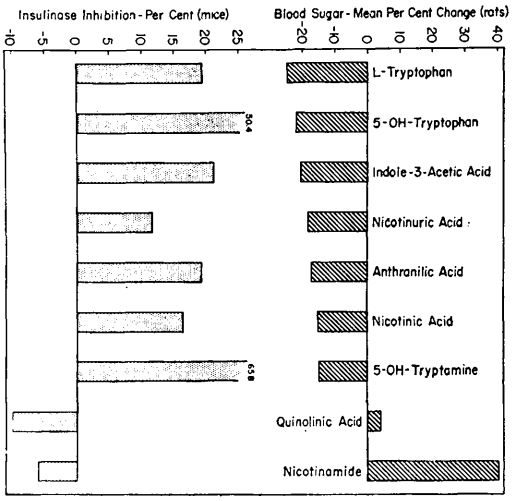

FIG. 1. Effect of metabolic products of L-tryptophan on destruction of insulin by intact mice. The areas represent mean % change in blood sugar conc. of 10 rats during a 5 hr interval after dosage of 4 mM of the compound/kilo body wt. The areas represent % of inhibition of insulinase as computed from difference in rates of insulin destruction by groups of intact mice given sodium bicarbonate and of those given 4 mM of the designated compound/kilo body wt.

It is evident that most of the compounds which exert an insulinase-inhibitory activity *in vitro* exert a similar effect *in vivo*. A discrepancy exists, however, between the action of nicotinic and quinolinic acids *in vitro* and *in vivo*. The positive correlation between effect of the various compounds on blood sugar concentration of rats and effect on rate of destruction of endogenous insulin by mice suggests that the *in vivo* effects are of greater

physiological significance than are the *in vitro* effects.

The data reported herein support the hypothesis that the hypoglycemic action of L-tryptophan and of some of its metabolic derivatives is related to a decrease in the rate of destruction of endogenous insulin. The precise mechanism involved remains unknown.

Conclusions. Metabolic derivatives of L-tryptophan which produce a hypoglycemic response in rats are effective also as inhibitors of the destruction of insulin by fresh extracts of rat livers and by intact mice.

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Action of Ouabain upon Normal and Hypodynamic Myocardium.* (23151)

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The positive inotropic action of cardiac glycosides upon hypodynamic isolated mammalian cardiac muscle has been demonstrated many times. The papillary muscle of the cat has been used most frequently, and the usual experimental procedure has been to stimulate the muscle, suspended in a phosphate medium containing glucose, over a period of hours until it becomes hypodynamic and to then add the drug(1,2). Isolated papillary muscles become hypodynamic within a few hours in phosphate media, in contrast to their stability in bicarbonate media(2). It has been claimed that the typical increase in force of contraction upon addition of non-toxic concentrations of cardiac glycosides does not occur in myocardium which is not hypodynamic, *e.g.*, in bicarbonate medium(2) or in phosphate medium when the drug is added prior to the development of the hypodynamic state(3). We have investigated this matter in guinea pig myocardium.

Methods. Isolated, electrically stimulated strips, prepared from the right ventricular wall of the guinea pig (150-200 g males) heart in a manner similar to that described previously for the rat(4), were used. The basic medium employed had the following composition (m*M*): NaCl 154, KCl 5.6, and $CaCl_2$ 3.2. The bicarbonate medium contained in addition 5 m*M* $NaHCO_3$ and was gassed with 1% CO_2-99% O_2 mixture (final pH 7.4). The phosphate medium contained 1 m*M* sodium phosphate buffer in place of bicarbonate and was gassed with 100% O_2 (final pH 7.4). Both media contained 0.1% glucose. The strips were stimulated at 37°C at a rate of 100/minute at a constant resting tension of 750 mg. The absolute values of the "initial force" were of the same order of magnitude in the two media (approximately 100 mg).

Results. Phosphate medium. Fig. 1 shows the results of typical experiments in which ouabain was added to a hypodynamic preparation (A) and to one in which the force of contraction had not fallen appreciably from the initial value (B). Magnitude and duration of the positive inotropic actions were similar by comparison with the control values.

Bicarbonate medium. Fig. 2 shows the effect of the same concentration of ouabain upon a strip in bicarbonate medium. A positive inotropic action occurred in this typical experiment which persisted above the control value for many hours. The great stability of the muscle in the bicarbonate medium, in contrast to its behavior in phosphate medium, is evident in this figure.

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