amino acids, polylysine, polyornithine and polyarginine, accelerate conversion of fibrinogen to fibrin by thrombin; the acidic poly-α-amino acids, polyaspartic, polyglutamic and polycysteic acids, and also heparin, retard this reaction. 2. The acidic polyamino acids neutralize the clot accelerating activity of the basic polyamino acids. 3. The addition of polylysine makes possible the detection of concentrations of fibrinogen too low to be clotted by thrombin alone.

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1. de Vriees, A., Schwager, A., and Katchalski, E., *Biochem. J.*, 1951, v49, 10.

2. Ginsburg, I., de Vries, A., and Katchalski, E., *Science,* 1952, v116, 15.

3. Katchalski, E., Berger, A., and Neumann, Ch., *Nature,* 1954, v173, 978.

4. Stein, Y., de Vries, A., Wislicki, L., and Katchalski, E., *Metabolism,* 1954, v3, 213.

5. Katchalski, E., Bichowski-Slomnitzki, L., and Volcani, B., *Biochem. J.*, 1953, v55, 671.

6. Stahman, M. A., Graf, L. M., Patterson, E. L., Walker, J. C., and Watson, D. W., *J. Biol. Chem.,* 1951, v189, 45.

7. Laki, K., in *Blood Clotting and Allied Problems,* Josiah Macy, Jr. Foundation, New York, N. Y., 1951, v4, 223.

8. Ware, A. G., Guest, M. M., and Seegers, W. H., *Arch. Biochem.,* 1947, v13, 231.

9. Seegers, W. H., McClaughry, R. I., and Andrews, E. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 714.

10. Katchalski, E., Grossfeld, I., and Frankel, M., *J. Am. Chem. Soc.,* 1948, v70, 2094.

11. Katchalski, E., and Spitnik, P., *ibid.,* 1951, v73, 3992.

12. Berger, A., and Katchalski, E., *ibid.,* 1951, v73, 4084.

13. Hanby, E. E., Waley, F. G., and Watson, J., *J. Chem. Soc.,* 1950, vIV, 3239.

14. Katchalski, E., and Berger, A., *Bull. Res. Counc., Israel,* 1951, v2, 314.

15. Astbury, W. T., Dalgliesh, C. E., Darmon, S. E., and Sutherland, G. B. B. M., *Nature,* 1948, v162, 569.

16. Seegers, W. H., Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Science,* 1942, v96, 300.

17. Gerendas, M., *Nature,* 1946, v157, 837.

18. Klein, P. D., and Seegers, W. H., *Blood,* 1950, v5, 742.

19. Biezunski, N., Shafrir, E., de Vries, A., and Katchalski, E., 1954, in preparation.

20. Unpublished observations.

21. Ferguson, W. H., *Am. J. Physiol.,* 1940, v130, 759.

22. Lorand, L., and Middlebrook, W. R., *Bioch. Bioph. Acta,* 1952, v9, 581.

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Inhibition of Degradation of Insulin-I^{131} *in vivo*.* (21287)

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The processes of degradation and of inactivation of insulin by tissues *in vitro* can be inhibited by a number of substances(1,2). Both *in vitro* and *in vivo* the system in which degradation is accomplished does not distinguish between insulin and I^{131}-labeled insulin(1,3). It would seem to be of great importance to determine if the degradation of insulin-I^{131} can be inhibited *in vivo,* because such inhibition might effect potentiation of insulin action and have therapeutic implications. Accordingly, the effects of the following compounds on the degradation of insulin-I^{131} *in vivo* were studied.

(1) *Zinc.* There has been a great deal of study of the chemical and biological interaction between insulin and zinc, and in practice, zinc has been used as a factor in various insulin preparations. It is of interest then, that zinc has been shown to be a potent inhibitor of insulin degradation(1) and inactivation(2) *in vitro,* and accordingly the

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effect of zinc on the *in vivo* degradation of insulin-I^{131} was studied.

(2) *Polyphloretin phosphate.* The enhancement of the ascorbic acid depleting activity of adrenocorticotrophin by the systemic administration of polyphloretin phosphate, (PPP), has been ascribed to anti-enzymatic properties of this substance(4). In view of (a) possible similarities between insulin and adrenocorticotrophin metabolism(5) and (b) the evidence that insulin-I^{131} degradation is proteolytic(1), we desired to find out if PPP affected this degradation.

(3) The effect of *5-isoproplyidene-2,4-dithiohydantoin* (MC 2346) on insulin-I^{131} degradation was investigated because of the report by Houssay *et al.*(6), that this compound reduced the incidence of diabetes in subtotally pancreatectomized rats.

Methods. The method used to measure the degradation of insulin-I^{131} was based on the following observations. The radioactivity of insulin-I^{131} is protein-bound‡, but after its injection into a rat, this property is rapidly lost (5). 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, described elsewhere(5), has been adopted to measure insulin degradation and was used in these experiments. A brief outline of the method follows. Sprague-Dawley male rats of similar weight and age were used in all studies. Seventeen hours before an experiment, experimental and control animals had their food and water replaced by 10% dextrose in 0.9% saline (10% D/S). This was removed and the animals fasted for 2 hours before the experiment was begun. Insulin-I^{131} was then injected intravenously in all rats of both groups, and 15 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. Concentration of radioactivity in each fraction was expressed as [T]/[B], a ratio of the concentration of radioactivity in the fraction to the initial overall total body concentration. Values greater than one therefore represented concentrations greater than the initial total body concentration, and values less than one represented lesser concentrations.

Results. Zinc was given to rats in 3 separate experiments. In one experiment, zinc acetate, 0.01 g in 0.2 ml of distilled water, was given to each of 5 rats and sodium acetate, 0.008 g in 0.2 ml distilled water was given to each of 4 control rats. These doses were given subcutaneously twice, once 17 hours before, and finally one hour before the injection of 0.5 unit, 10 μc of insulin-I^{131}. A second experiment was done in 2 parts. Both involved the feeding of zinc carbonate in the diet. A 0.5% Zn diet (Purina Laboratory Chow containing 0.5% zinc as zinc carbonate) was given to 4 rats for 2 weeks and to 5 rats for 4 weeks. Equal numbers of controls of the same weight and age received chow without added zinc for the same intervals. At the termination of the experiments all were given 0.3 unit (10 μc) of insulin-I^{131}. In all of these experiments there was no significant effect of the administration of zinc on the distribution or degradation of insulin-I^{131}.

Polyphloretin phosphate.§ Two experiments were performed using this substance. In one, 8 rats were given 2 mg of PPP per kg intramuscularly in a 1% solution in 0.9% saline 30 minutes before insulin-I^{131} injection and compared with 7 controls who received 0.5% saline only, before the insulin-I^{131}. In the second experiment 40 mg of PPP per kg in a 2% solution in 0.9% saline was given intramuscularly 17 hours and one hour before insulin-I^{131} injection. Again controls of the same age and weight received 0.9% saline only. In both experiments there was no effect of PPP on the distribution or degradation of insulin-I^{131}.

5 - isopropylidene - 2,4 - dithiohydantoin.‖

‡ The insulin was kindly provided by Eli Lilly and Co., and iodinated in 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 then precipitable with trichloracetic acid.

§ The PPP was kindly supplied by Dr. Gustav Martin of the National Drug Co.

‖ The MC 2346 was kindly supplied by Mr. W. A. Lott of E. R. Squibb and Sons.

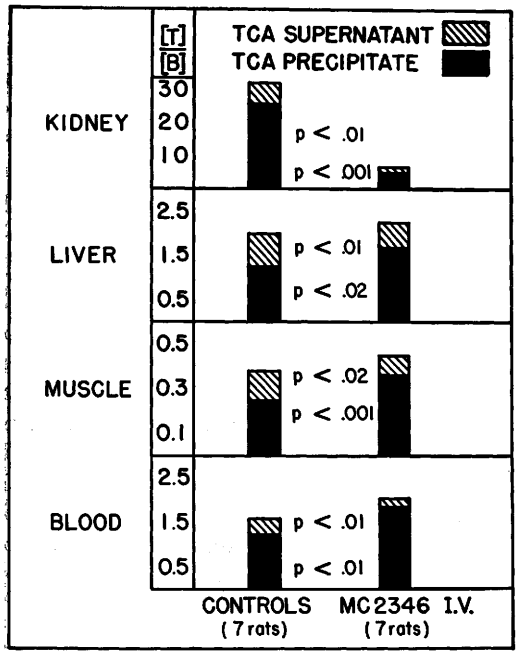

FIG. 1. Effect of 5-isopropylidene-2,4-dithiohydantoin (MC 2346) given intravenously, on concentrations, $\frac{[T]}{[B]}$, of radioactivity in trichloracetic acid (TCA) precipitate and supernatant fractions of tissues 15 min. after intravenous administration of insulin-I^{131} to rats. Figures between columns are p values of differences between respective fractions of treated and control groups.

Three experiments were performed with this substance: 1) Seven rats weighing between 166 and 198 g were each given one ml of a 2% suspension of MC 2346 in 0.9% saline alkalinized to pH 8.9 (alkaline saline) intravenously 2 minutes before the injection of one unit, 10 μc of insulin-I^{131}. Seven controls, weighing between 146 and 192 g, received one ml of the alkaline saline alone and similarly, 2 minutes later, insulin-I^{131}. The results are shown in Fig. 1. Profound changes resulted from the injection of the MC 2346. Supernatant radioactivity was reduced in all tissues. Bound radioactivity was increased in liver, muscle, and blood, and decreased in kidney. We interpret these observations as suggestive that MC 2346 decreased insulin-I^{131} degradation.

2) Six rats weighing between 132 and 174 g were each given one dose of 25 mg of MC 2346 in one ml of alkaline saline by stomach tube 4 hours before insulin-I^{131} injection. Five controls weighing between 155 and 172 g were given alkaline saline only, by stomach tube, and 4 hours later were injected with insulin-I^{131}. The results of this experiment showed no effect of the single oral dose of MC 2346 on the distribution or degradation of insulin-I^{131}.

3) Five rats, weighing between 135 and 171 g were given daily for 5 days the same dose of MC 2346 as described in the previous paragraph, with the last dose being given 4 hours before the injection of the insulin-I^{131}. Five controls, weighing between 152 and 206 g received only alkaline saline solvent in the same dosage schedule. Both groups received one unit, 10 μc, of insulin-I^{131} intravenously. The results are shown in Fig. 2. The changes appear to be similar to those following the administration of MC 2346 intravenously, although perhaps not as marked.

Discussion. The tolerance of the rat for

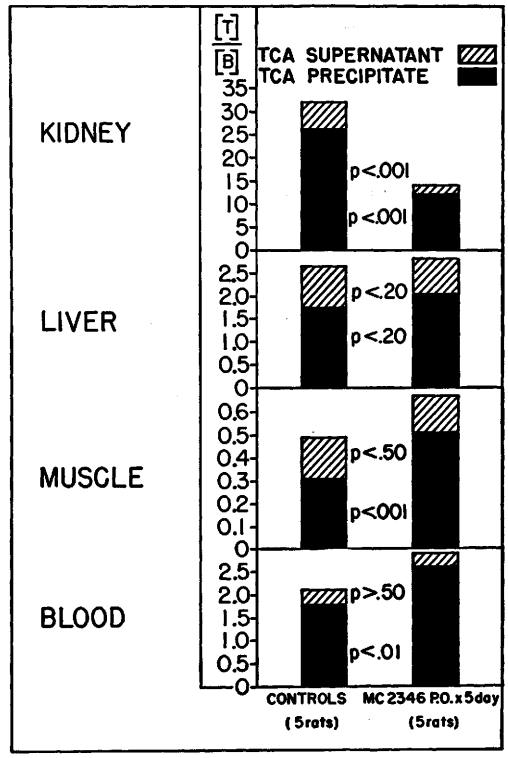

FIG. 2. Effect of prolonged oral administration of MC 2346 on concentrations, $\frac{[T]}{[B]}$, of radioactivity in tissue fractions 15 min. after intravenous insulin-I^{131} in rats.

zinc has been considered to be between 0.5 and 1% of the diet by weight(7). At the lower level, reproduction was found to be impaired, but growth was not. Accordingly this level seemed to be a suitable one for the study of the effect of zinc on insulin-I^{131} metabolism *in vivo*. It was found, however, to be without effect, even after 4 weeks of such a diet. This may mean that different conditions *in vivo* counteract or prevent the inhibitory effect of zinc which has been seen *in vitro,* or perhaps that different dosage programs need to be investigated.

The dose levels of PPP used were ones that have been shown to potentiate adrenocorticotrophin action. The absence of an effect of this dosage on insulin-I^{131} degradation is then of significance and raises the question of the mechanism of action of PPP with respect to adrenocorticotrophin.

The action of MC 2346 on insulin-I^{131} degradation is of great interest, particularly since this substance has been shown to prevent, at least partially, the development of diabetes(6) in the subtotally pancreatectomized rat. The present study has indicated that MC 2346 decreases the degradation of insulin. It seems possible that these 2 functions are related, and, as has been suggested before(5), that decreased insulin degradation results in decreased insulin inactivation. Less insulin can exert the same amount of action as is exerted by a larger quantity which is in the presence of a fully functionary degradation system. The insulin insufficiency resulting from subtotal pancreatic resection may be alleviated by MC 2346 because of a reduction in insulin degradation. Such a concept offers promise of a new therapeutic approach to the insulin insufficiency of diabetes mellitus. Perhaps an inhibitor of insulin degradation could so bolster a relative insulin insufficiency as to make endogenous insulin adequate, and restore physiologic homeostasis.

Summary. Insulin-I^{131} degradation in rats *in vivo* was not inhibited by the administration of zinc in the diet, or by treatment with the substance, polyphloretin phosphate. Marked inhibition was obtained by the intravenous or prolonged oral administration of 5-isopropylidene-2,4-dithiohydantoin and it was suggested that this may explain the protection afforded against diabetes by this substance in the partially depancreatized rat.

1. Tomizawa, H. H., Nutley, M. L., Narahara, H. T., and Williams, R. H., *J. Am. Chem. Soc.,* 1954, v76, 3357.

2. Mirsky, I. A., and Broh-Kahn, R. H., *Arch Biochem.,* 1949, v20, 1.

3. Elgee, N. J., and Williams, R. H., unpublished data.

4. Weidmann, H., *Acta. Endocrinol.,* 1953, v13, 287.

5. Elgee, N. J., Williams, R. H., and Lee, N. D., *J. Clin. Invest.,* 1954, September.

6. Houssay, B. A., Lott, W. A., and Martinez, C, *Compt. Rend. Soc. Biol.,* 1950, v145, 591.

7. Sutton, W. R., Nelson, V. E., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 211.

Received August 5, 1954. P.S.E.B.M., 1954, v87.