referable to the combined effects of changes in fluid balance, changes in nitrogen balance, and secondarily to the loss of the sugar in the urine.

Discussion. From the foregoing, it appears that the administration of "pituitary growth hormone" to a diabetic patient results in marked accentuation of the diabetic state. It is possible that the thyrotropin and/or adrenocorticotropin present in the preparation may have played some role in the hyperglycemia and in the increased glycosuria. It seems probable, however, that the role played by these 2 factors was small for the following reasons: 1. The relatively slight change in circulating eosinophils and urinary 17-ketosteroids; 2. Slight increase in nitrogen excretion; 3. Elevation of the respiratory quotient above one, rather than below 0.7. (The latter occurs consistently in diabetics receiving ACTH)(5); 4. Decreased rather than increased potassium excretion; 5. Decreased oxygen utilization.

Summary. Administration of "highly purified pituitary growth hormone" to a diabetic patient, under chemically controlled conditions, resulted in increased hyperglycemia and glycosuria, in minimal increase in blood and urinary ketones, and in minimal change in the nitrogen balance. The data support the concept that the increased hyperglycemia and glycosuria are probably attributable to diminished carbohydrate utilization rather than increased neoglucogenesis. The diminished utilization in turn may be referable to diminished endogenous insulin production as the result of the exhaustion of the beta cells of the islets.

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Identification of Trypsinogen in the Lipotropic Fraction.* (20459)

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Haanes and György(1) analyzed the lipotropic fraction prepared by Bosshardt *et al.* (2) from the residue remaining after the extraction of pancreas for insulin, and identified trypsin and trypsin inhibitor as components of this fraction. Furthermore, they postulated that enterokinase is capable of overcoming the action of trypsin inhibitor on trypsin. Mars, Peanasky, and Laskowski(3) studied a system composed of crystalline trypsin, crys-

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talline pancreatic trypsin inhibitor and highly purified enterokinase, but were unable to confirm the hypothesis of a new activity of enterokinase. In order to explain the discrepancy between their results and those of Haanes and György(1), Mars *et al.*(3) tentatively postulated that, in addition to the components identified by Haanes and György, the lipotropic fraction contained trypsinogen. Through the courtesy of Dr. L. A. Kazal of Sharp and Dohme, Inc., we recently received a small amount of lipotropic fraction and analyzed it for trypsinogen.

Experimental. The lipotropic fraction was treated according to the procedure of Kunitz and Northrop(4) devised for the separation of trypsin and/or trypsinogen from the trypsin inhibitor. 1.250 g of the lipotropic fraction was suspended in 0.0025 N HCl and 0.1 N HCl was added dropwise until the pH of the mixture was 3.0. The insoluble material was centrifuged off and discarded. The yellow liquid, 8 ml, was mixed with 8 ml of 5% trichloroacetic acid (TCA), left for one hour at room temperature, and centrifuged. The supernatant was discarded. The precipitate was washed with 2 ml of 2.5% TCA, was then dissolved in 40 ml of 0.0025 N HCl and left at room temperature for 30 minutes. Solid ammonium sulfate was added (24 g/100 ml) to attain 0.4 saturation. The precipitate which formed was centrifuged off and discarded. To the supernatant, ammonium sulfate (36.1 g/100 ml) was added to attain 0.8 saturation, the resulting suspension was centrifuged and the supernatant was discarded. The precipitate, referred to as 0.8 S.A.S. (saturation with ammonium sulfate) precipitate, was dissolved in 0.0025 N HCl and was assayed, according to the method of Kunitz (5). To some tubes $CaCl_2$ was added, since Gorini(6) and Nord and coworkers(7,8) had shown that calcium exerted a stabilizing effect on trypsin.

A rapid activation of trypsinogen was performed at 37°C, at pH 7.6 (Table I, Exp. 1). All tubes contained 0.8 S.A.S. fraction in a final concentration equivalent to an optical density of 3.24 at 280 μ ($E\,^{1\,cm}_{280} = 3.24$) and 0.1 M borate buffer, pH 7.6. Tubes 1 and 2 contained enterokinase, final concentration

TABLE I. Effect of Enterokinase on Proteolytic Activity of 0.8 S.A.S. Fraction.

	$E\,^{1\,cm}_{280}$		
	0	½ hr	3 hr
Exp. 1, pH 7.6, 37°			
Tube 1, enterokinase, Ca	.520	.610	.505
2, " , no Ca	.370	.405	.185
3, no enterokinase, Ca	.031	.030	.027
4, " " , no Ca	.034	.043	.020
Exp. 2, pH 5.6, 5°	0	5 hr	24 hr
Tube 1, enterokinase, Ca	.118	.170	.280
2, " , no Ca	.044	.146	.298
3, no enterokinase, Ca	.014	.015	.012
4, " " , no Ca	.010	.013	.006

10 E.K.U. (enterokinase units)(9) per ml. Tubes 1 and 3 contained $CaCl_2$, final concentration 0.01 M. At the indicated times 0.1 ml aliquots were withdrawn from each tube, added to a mixture of 0.9 ml of water and one ml of 1% casein solution, and incubated for 20 minutes. Proteolytic activity was determined by spectrophotometric assay of the trichloroacetic-acid-soluble digestion products (5). Control tubes containing an equivalent amount of enterokinase and control tubes containing casein only were incubated simultaneously. The amounts of enterokinase used were too low to exhibit detectable proteolytic activity. The 2 series of controls gave identical results. All figures shown in Table I were corrected for the values of the corresponding controls.

Under the conditions described, the activation occurred very rapidly; the highest peak of proteolytic activity being reached in Tubes 1 and 2 after 30 minutes of incubation. In Tube 1, containing calcium, proteolytic activity decreased slowly during the period of 5 hours. In Tube 2, without calcium, the proteolytic activity decreased sharply, indicating that under these conditions denaturation and self-digestion of trypsin occurred very rapidly(10). Contrary to expectations, only a small part of the potential proteolytic activity was present in the tubes which did not contain enterokinase, indicating that the 0.8 S.A.S. fraction contained predominantly trypsinogen with relatively little trypsin.

An experiment was then performed in which

the 0.8 S.A.S. fraction was subjected to a slow activation at 5°, at pH 5.6 (Table I, Exp. 2). All tubes contained 0.8 S.A.S. fraction, final concentration $E^{1\,cm}_{280} = 0.315$, and 0.1 M acetate buffer, pH 5.6. Tubes 1 and 2 contained enterokinase, final concentration 1 E.K.U. per ml. Tubes 1 and 3 contained $CaCl_2$, final concentration 0.01 M at the indicated time 0.5 ml aliquots were withdrawn and assayed for tryptic activity as before. Control tubes, analogous to those of Exp. 1 were incubated simultaneously. All figures were corrected for the values of the corresponding controls.

Under the conditions described, a steady increase in proteolytic activity during the period of 24 hours was observed in Tubes 1 and 2, containing enterokinase. At 5° and pH 5.6, the rate of denaturation and self-digestion of trypsin was very low(10), and therefore, the protective effect of calcium was not evidenced.

In order to determine whether or not the zymogen was really trypsinogen, it was decided to investigate the action of soy bean trypsin inhibitor on the activated enzyme. Kunitz(5) showed that soy bean trypsin inhibitor reacts stoichiometrically with trypsin, but forms a dissociable complex with chymotrypsin. To 4 ml of solution of 0.8 S.A.S. fraction ($E^{1\,cm}_{280} = 1.2$) were added 4 ml of 0.2 M borate buffer, pH 7.6 and 2 ml of enterokinase solution containing 10 E.K.U. per ml. The mixture was incubated for one hour at 37° C. and five 0.5 ml aliquots were then withdrawn and were mixed with solutions containing 0, 13, 65, 130, and 650 γ of soy bean inhibitor. After 5 minutes casein was added, and the tubes were incubated for 20 minutes. A series of control tubes containing corresponding amounts of inhibitor was incubated simultaneously. For the tube containing no inhibitor, the corrected reading was 0.285. For the tubes containing inhibitor corrected readings varied from 0.050 to 0.040, indicating that about 86% of the proteolytic activity was inhibited, regardless of the amount of inhibitor used. No attempts were made to identify the residual proteolytic activity. Considering the origin and the treatment to which it was subjected, it would appear most likely that chymotrypsin was responsible for the residual proteolytic activity. The results of this experiment agree with the conclusion of Haanes and György(1) that trypsin represented the major proteolytic component of the activated lipotropic fraction.

Summary. The postulation that the lipotropic fraction prepared by Bosshardt *et al.* (2) contained trypsinogen has been confirmed by a direct analysis. In view of this finding, it is no longer necessary to ascribe an additional new activity to enterokinase.

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