sult from alimentary lipemia then this biological mechanism is of considerable physiological and probably of pathological significance. One can only speculate concerning the importance of this mechanism in human disease, particularly in atherosclerosis(9), in vascular thrombosis(10), and in multiple sclerosis(8).

Conclusion. Following large fat meals changes occur in the circulation of the cheek pouch of the golden hamster. These changes consist of an increased adhesiveness and aggregation of the red blood cells accompanied by a slowing and even cessation of flow of the blood in the exposed pouch. They appear after the peak of the lipemia has been passed, and develop to their maximum as the lipemia clears. After complete clearing of the lipemia the suspension stability of the blood returns toward normal, and the circulation again assumes a normal appearance.

A 20 minute 16 mm colored cinephotomicrograph with titles has been prepared showing the circulatory changes described in this paper.

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Concerning the Mechanism of Action of Enterokinase.* (20124)

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Lipotropically active fractions from pancreas have been prepared by Dragstedt *et al.* (1) and by Entenman *et al.*(2). Recently Bosshardt *et al.*(3) obtained a fraction possessing lipotropic activity from the residue remaining after extraction of insulin from bovine pancreas. György and coworkers(4, 5) investigated the properties of Bosshardt's preparation and concluded that the lipotropic activity of this fraction depended upon the *in vivo* liberation of a proteolytic enzyme. They established that the lipotropic activity of this fraction was destroyed by boiling and that the fraction itself had no proteolytic activity *in vitro*, but became proteolytic after an inhibitor had been removed. They tentatively identified the inhibitor as pancreatic trypsin inhibitor, the enzyme as trypsin, and the lipotropic fraction as a complex between the enzyme and the inhibitor together with an excess of the free inhibitor. They further showed that the action of the inhibitor could be overcome by a substance present in duodenal juice and presumed to be enterokinase. The latter finding led them to conclude that enterokinase, besides having the well known ability of transforming trypsinogen into trypsin, is capable of overcoming the action of trypsin inhibitor on trypsin.

Since crystalline trypsin, crystalline pan-

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creatic trypsin inhibitor, and crystalline trypsin-trypsin inhibitor complex as well as purified enterokinase were available in this laboratory, it was decided to check whether the postulated new action of enterokinase could be detected in relatively pure systems.

Experimental. Crystalline trypsin, crystalline pancreatic trypsin inhibitor, and crystalline trypsin-trypsin inhibitor complex were prepared according to the methods of Kunitz and Northrop(6). Each of these substances was recrystallized at least 3 times. Stock solutions of appropriate strength were made using the previously established factors for the specific absorption at 280 mμ(7). Purified enterokinase was prepared according to Kunitz (8). We also had a sample of enterokinase prepared by Dr. Kunitz in 1940 and kindly supplied to us on a previous occasion. This sample still showed about 1/7 of its original activity, corresponding to 6 E.K.U. (Kunitz) per mg. Crystalline trypsinogen which was freed from the trypsin inhibitor by purification with trichloroacetic acid was used as substrate in the determination of enterokinase activity (9). The proteolytic activity was determined by the spectrophotometric method of Kunitz (10) using casein as substrate.

Both preparations of enterokinase exhibited a slight but detectable proteolytic activity. When enterokinase was treated with an excess of pancreatic inhibitor, 60% of this activity could be inhibited, suggesting that the preparation of enterokinase was slightly contaminated with trypsin. No definite conclusion could be drawn concerning the residual proteolytic activity of the enterokinase preparation. All further experiments were therefore corrected for the blanks containing enterokinase and casein. With the levels of enterokinase used, the values of these blanks were often identical with, and seldom significantly higher than the values for blanks containing casein only (Table II).

The hypothesis of Haanes and György(5) that enterokinase overcomes the action of trypsin inhibitor could be visualized in one of two ways. Either enterokinase binds the inhibitor, and if so, it must bind it more strongly than trypsin does, or it must partially digest the inhibitor (free or bound). In order to check the first alternative, 2 sets of determinations were performed. First trypsin-trypsin inhibitor complex was exposed to various concentrations of enterokinase at room temperature for a period of 6 hours. All tubes contained 30 γ/ml of trypsin-trypsin inhibitor complex. The amounts of enterokinase in different tubes varied as follows: 0, 10, 20, 50, 100, and 250 γ/ml. One series of tubes contained 0.1 M phosphate buffer, pH 7, and the second series contained 0.1 M acetate buffer, pH 3. The dissociation of the complex at pH 7 was 0 and at pH 3 was 38% $\pm$ 3% at all levels of enterokinase used. The second set of experiments is illustrated in Fig. 1. Addition of enterokinase slightly slowed down the formation of the complex at pH 7 and slightly accelerated the dissociation of the complex at pH 3. The observed differences are on the border line of the accuracy of the method. The results of these 2 sets of experiments rule out the possibility of preferential binding of the trypsin inhibitor by enterokinase.

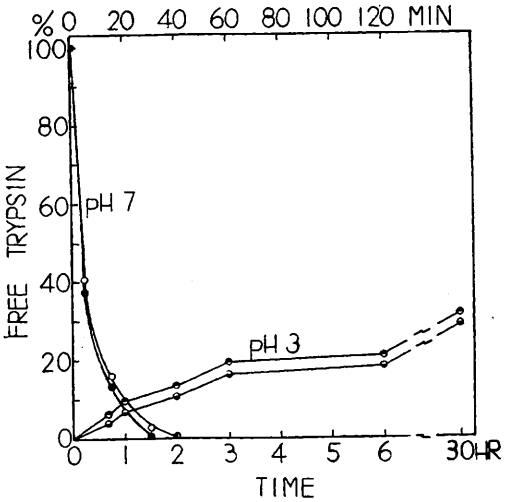

FIG. 1. Effect of time on formation and dissociation of trypsin-trypsin inhibitor complex. Upper abscissa scale in min. refers to tubes 1 and 2. Tube 1 (open circle) contained trypsin 50 γ/ml, inhibitor 25 γ/ml, 1.0 E.K.U./ml of enterokinase, 0.1 M phosphate buffer, pH 7. Tube 2 (solid circle) identical, but no enterokinase. Lower abscissa scale in hr refers to tubes 3 and 4. Tube 3 (circle, solid top) contained 30 γ/ml of trypsin-trypsin inhibitor complex, 0.6 E.K.U./ml of enterokinase, 0.1 M acetate buffer, pH 3.0. Tube 4 (circle, solid bottom) identical, but no enterokinase.

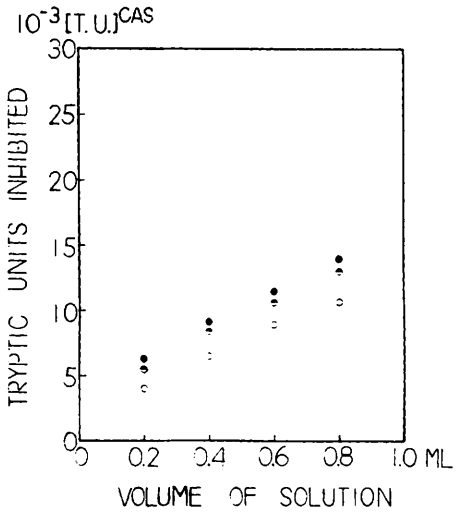

FIG. 2. Recovery of inhibitor. Tube 1 (solid circle) contained 2 γ/ml of inhibitor, 20 γ/ml (0.8 E.K.U./ml) of enterokinase. Tube 2 (open circle) contained 50 γ/ml of trypsinogen, 2 γ/ml of inhibitor. Tube 3 (circle, solid top) contained only 2 γ/ml of inhibitor. 0.1 M phosphate buffer, pH 7, in all tubes. All tubes were allowed to stand 6 hr at room temperature. Aliquots were then assayed against 25×10^{-3}[T.U.]^{cas} of trypsin.

In order to check whether enterokinase inactivated the trypsin inhibitor the experiment illustrated in Fig. 2 was performed. Comparison of values obtained with the samples from tubes 1 and 3 indicate that the inhibitor was quantitatively recovered after 6 hours exposure to the action of enterokinase. A small amount of binding was detected in tube 2, containing trypsin inhibitor and trypsinogen and was due to the partial transformation of trypsinogen into trypsin. Similar experiments in which the mixture of enterokinase and inhibitor were exposed to pH 4.5, 6.0, and 8.0 also showed a complete recovery of the inhibitor. The possibility of the inhibitor being digested by enterokinase was therefore excluded.

The results of these experiments also confirm our previous findings that when sufficient time is allowed, the presence of enterokinase does not interfere with a quantitative formation of trypsin-trypsin inhibitor complex. The above results contradict this part of the hypothesis of Haanes and György(5) which postulates that enterokinase overcomes the action of the inhibitor on trypsin.

Haanes and György(5) identified trypsin-trypsin inhibitor complex and free pancreatic trypsin inhibitor as components of the lipotropic fraction isolated by Bosshardt *et al.*(3). It then occurred to us that one of the plausible explanations of the results of Haanes and György would be to assume that in addition to the above components trypsinogen was also present. Haanes and György split the lipotropic fraction with trichloroacetic acid. They redissolved the precipitate, dialyzed it, and assayed it for proteolytic activity by the methionine method, requiring 24 hours incubation. They found that the precipitate was proteolytically active, and that addition of enterokinase did not increase this activity.

An artificial mixture containing trypsinogen (1 mg), trypsin-trypsin inhibitor complex (4 mg), and free pancreatic trypsin inhibitor (0.5 mg) was treated with trichloroacetic acid. The precipitate was redissolved. A solution of enterokinase was added to one portion, while the other contained no enterokinase but an equivalent amount of water. Aliquots were withdrawn at different time intervals. The results indicate (Table I) that the action of enterokinase can be detected only during the early periods of exposure, using the relatively rapid method of Kunitz(10). The activation of trypsinogen in the absence of

TABLE I. Effect of Enterokinase on Proteolytic Activity of a Mixture of Trypsinogen and Trypsin.

Time	E_{280} 1	2	3	4
0 min.	.208	.210	.286	.283
30	.243	.224	.337	.291
60	.252		.340	.300
90	.256		.351	.303
150		.231		
180	.252		.350	.313
5 hr		.244		
6	.258	.253	.347	.320
20	.044	.041	.352	.340
24	.006	.002	.354	.349

Tube 1 contained trichloroacetic acid precipitate dissolved in enough 0.1 M phosphate buffer, pH 7, to produce a solution having an optical density of 0.350 at 280 mμ, 0.5 E.K.U./ml, room temperature; tube 2 same as tube 1 but without enterokinase. Tube 3 contained trichloroacetic acid precipitate dissolved in enough 0.1 M acetate buffer, pH 5.6, to produce a solution having an optical density of 0.350 at 280 mμ, 0.5 E.K.U./ml, 5°C; tube 4 same as tube 3 but without enterokinase. All figures are corrected for the blanks.

TABLE II. Conversion of Trypsinogen into Trypsin in the Presence of Trypsin Inhibitor.

Tube	E_{280} 0	1 hr	2 hr	4 hr
1	.065	.057	.063	.055
2	.065	.063	.064	.063
3	.092	.270	.390	.460
Casein control	.064	.060	.062	.061

Tube 1 contained trypsinogen 50 γ/ml and inhibitor 2 γ/ml. Tube 2 contained inhibitor 2 γ/ml and enterokinase 20 γ/ml. Tube 3 contained trypsinogen 25 γ/ml, inhibitor 2 γ/ml and enterokinase 10 γ/ml. 0.1 M phosphate buffer in all tubes. 1 ml aliquots were withdrawn at indicated times.

enterokinase was accomplished by trypsin, when sufficient time was allowed. Long exposures to pH 7 resulted in inactivation of trypsin independently of the presence of enterokinase.

Haanes and György(5) recombined the trichloroacetic acid precipitate with the trichloroacetic acid soluble fraction and found no proteolytic activity. Addition of enterokinase brought about a demonstrable proteolytic activity and led the authors to conclude that enterokinase overcomes the action of inhibitor. The experiment shown in Table II indicates that trypsinogen could be activated even in presence of a considerable amount of inhibitor. The activation time is, of course, somewhat increased, which is in agreement with the previous findings of Kunitz(11).

The definite proof that the lipotropic fraction contained trypsinogen could be obtained only by direct analysis of the lipotropic fraction for trypsinogen under rigorous conditions. Unfortunately, the lipotropic fraction was not available to us. The suggested explanation remains therefore tentative. The experiments described in the present paper were made with artificial mixtures of crystalline compounds and they do not rule out a possibility that Haanes and György's preparation(5) contained some additional factor(s) responsible for the dissociation of the complex.

Summary. 1. Using highly purified systems, no evidence was found that enterokinase overcomes the action of pancreatic trypsin inhibitor on trypsin. On the contrary, the inhibitor, after being exposed to the action of enterokinase, was quantitatively recovered, indicating that enterokinase neither formed a strong complex with the inhibitor, nor digested it. 2. It is tentatively suggested that the results which led to the postulation of the new activity of enterokinase could be explained by the assumption that the lipotropic fraction contained trypsinogen in addition to trypsin-trypsin inhibitor complex and free inhibitor.

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