FORMATION OF TRYPSIN FROM CRYSTALLINE TRYPSINOGEN BY MEANS OF ENTEROKINASE

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The mechanism of formation of trypsin from trypsinogen by means of enterokinase has been a matter of almost continuous controversy since the discovery of enterokinase by Schepowalnikow in Pawlow's laboratory in 1899. Pawlow and Schepowalnikow considered enterokinase to be a typical enzyme. Studies of the kinetics of activation have shown, however, that while the rate of activation of a definite concentration of trypsinogen was proportional to the concentration of enterokinase used, the ultimate amount of trypsin formed was not independent of the concentration of enterokinase, as would be expected were enterokinase a true enzyme. This led to the suggestion that the formation of trypsin from trypsinogen by enterokinase is essentially a stoichiometric combination between trypsinogen and enterokinase to form an active enzyme, "trypsin-kinase" (1).

The isolation (8) of crystalline trypsinogen from fresh beef pancreas, its autocatalytic transformation at pH 7.0–9.0 into active trypsin without the aid of any outside activator, and the isolation of the active trypsin in pure crystalline form offer proof against the assumption that trypsin is a stoichiometric compound of kinase and trypsinogen. It was also found that pure crystalline trypsinogen can be changed into active trypsin at pH 3.0–4.0 by means of a kinase obtained from a mold of the genus *Penicillium* (2). The trypsin formed was crystallized and found to be identical in crystalline form, solubility, and specific activity with the crystalline trypsin obtained by spontaneous autocatalytic activation of trypsinogen at pH 8.0. The action of mold kinase was that of a typical enzyme, the process of activation following the course of a catalytic unimolecular reaction and the ultimate amount of trypsin formed being independent of the concentration of mold kinase used.

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This paper deals with the kinetics of the formation of trypsin from crystalline trypsinogen by means of purified enterokinase obtained from swine duodenum contents. Enterokinase acts best in the range of pH 6.0–9.0 where autocatalytic formation of trypsin from trypsinogen occurs readily. The percentage rate of this reaction, however, is proportional to the concentration of trypsinogen. Hence, by using very dilute trypsinogen solutions the rate of the autocatalytic activation may be made negligible compared with that of the activation brought about by a significant amount of enterokinase (3). The autolysis of the trypsin formed, which generally occurs in the range of pH 7.0–9.0 (4), is also minimized by using dilute trypsinogen and by employing temperatures not higher than 5°C.

A further complication exists at pH 7.0–9.0 since under these conditions trypsinogen in the presence of trypsin is partly changed to an inert protein (5) which can no longer be transformed into trypsin either by enterokinase or mold kinase. This complication is minimized in solutions more acid than pH 6.0 where the rate of transformation of trypsinogen into inert protein is greatly reduced. When activation by enterokinase is allowed to proceed at pH below 6.0 enterokinase acts almost like a typical enzyme. The reaction follows approximately the course of a theoretical unimolecular reaction with a velocity constant proportional to the concentration of enterokinase used and the ultimate amount of trypsin formed is practically independent of the concentration of kinase.

If, on the other hand, the activation is allowed to proceed at pH above 6.0 a great portion of the trypsinogen is transformed into inert protein, the more so the lower the concentration of enterokinase used. As a result, the ultimate amount of trypsin formed is less as the concentration of enterokinase used is decreased in agreement with the findings of earlier workers (6).

The kinetics of the formation of the trypsin from crystalline trypsinogen by means of enterokinase under conditions where part of the trypsinogen is changed into inert protein can be derived mathematically as follows:

Let G_o = initial concentration of trypsinogen
E = concentration of enterokinase
A = concentration of trypsin at any time t

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A_e = final concentration of trypsin
I = concentration of inert protein formed from trypsinogen in any time t
I_e = final concentration of inert protein
$G_o - A - I$ = concentration of trypsinogen at any time t.

Assuming (1) that the rate of formation of trypsin is proportional to the concentration of enterokinase and to the concentration of trypsinogen and, (2) that the rate of formation of inert protein is proportional to the concentration of trypsin and to the concentration of trypsinogen, we have the following equations:

$$\frac{dA}{dt} = K_e E(G_o - A - I) \tag{1}$$

$$\frac{dI}{dt} = K_2 A(G_o - A - I) \tag{2}$$

Hence

$$\frac{dI}{dA} = \frac{K_2 A}{K_e E} \tag{3}$$

and

$$I = bA^2 \tag{4}$$

where $b = \frac{K_2}{2K_e E}$ and K_e and K_2 are the velocity constants of the reactions.

Substituting bA^2 for I in Equation 1, we get

$$\frac{dA}{dt} = K_e E(G_o - A - bA^2) \tag{5}$$

At the end of the reaction when $\frac{dA}{dt} = 0$ we have

$$G_o = A_e + bA^2_e \tag{6}$$

or

$$\frac{G_o - A_e}{A^2_e} = b = \frac{K_2}{2K_e E} \tag{7}$$

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Substituting $A_e + bA^2_e$ for G_o in Equation 5 we get

$$\frac{dA}{dt} = K_eE[(A_e - A)(1 + bA_e + bA)] \tag{8}$$

which on integration gives

$$\ln \frac{A_e}{A_e - A} + \ln\left(1 + \frac{bA}{1 + bA_e}\right) = mt \tag{9}$$

where

$$m = K_eE + K_2A_e \tag{10}$$

The exponential form of Equation 9 is

$$A = \frac{A_e(e^{mt} - 1)}{e^{mt} + \dfrac{bA_e}{1 + bA_e}} \tag{11}$$

where m is the slope of the straight line obtained when the values for

$$\ln \frac{A_e}{A_e - A} + \ln\left(1 + \frac{bA}{1 + bA_e}\right)$$

are plotted against t, in accordance with Equation 9.

It follows from Equations 7 and 10 that

$$K_eE = \frac{mA_e}{2G_o - A_e} \tag{12}$$

and

$$K_2 = \frac{m - K_eE}{A_e} \tag{13}$$

At pH more acid than 6.0 K_2 becomes negligible and Equations 9 and 11 are then reduced to the approximate forms

$$\ln \frac{G_o}{G_o - A} = K_eEt \tag{9a}$$

and

$$A = G_o(1 - e^{-K_eEt}) \tag{9b}$$

which are the equations of a simple catalytic unimolecular reaction. If an appreciable amount of trypsin, A_o, is present as an impurity in the sample of trypsinogen used then Equation 9 becomes

$$\ln \frac{A_e - A_o}{A_e - A} + \ln \frac{1 + bA_e + bA}{1 + bA_e + bA_o} = mt$$

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where

$$b = \frac{G_o + A_o - A_e}{A^2_e - A^2_o}$$

also Equation 4 becomes

$$I = b(A^2 - A^2_o)$$

The derived equations bring out the following relationship between the trypsin and inert protein formed from trypsinogen in the presence of enterokinase at pH above 6.0: the concentration of inert protein formed at any time during the reaction is proportional to the square of the concentration of trypsin formed and inversely proportional to the concentration of enterokinase used (Equation 4).

It follows then that the higher the concentration of enterokinase used the greater is the percentage of trypsinogen changed into active trypsin.

This relationship, as well as the equation for the kinetics of the enterokinase activation, has been found to check closely with the experimental results.

The transformation of trypsinogen into trypsin in the presence of enterokinase appears thus to be a typical enzyme reaction catalyzed by the enzyme enterokinase. The anomalous results found under certain conditions are due to a secondary reaction by which trypsin changes trypsinogen to an inert protein.

The kinetics of the reaction outlined above applies only to dilute solutions of purified trypsinogen. The activation of concentrated solutions of trypsinogen is complicated by rapid autocatalytic formation of trypsin by the trypsin itself. The activation of crude pancreatic extracts is much more complicated since, as previously noted (7), these extracts contain chymo-trypsinogen in addition to trypsinogen and also a substance which inhibits trypsin (8). In outline the activation of crude trypsinogen by enterokinase proceeds as follows: Addition of kinase transforms the trypsinogen to trypsin which catalyzes the conversion of trypsinogen to form more trypsin and which also catalyzes the conversion of chymo-trypsinogen to chymo-trypsin. If the method of activity determinations used determines both trypsin and chymo-trypsin, as is usually the case, the curves obtained when the activity of the solution is plotted against time are S shaped but

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asymmetrical and resemble those obtained by Vernon (9). These curves generally show a long initial lag period which is partly caused by the interference of the trypsin inhibitor with the catalytic action of the trypsin formed.

Experimental Studies of the Kinetics of the Formation of Trypsin from Crystalline Trypsinogen by Means of Enterokinase. General Procedure

Reaction mixtures were made up of solutions of crystalline trypsinogen and of enterokinase in dilute buffers and allowed to stand at 5°C. The solutions were kept sterile by the addition of 0.1 ml. 1 per cent merthiolate in 1.4 per cent borax solution to 100 ml. of reaction mixture. Samples of 1 ml. were acidified with hydrochloric acid to about pH 2.0 in order to stop the reactions. The concentration of trypsin in the samples was then determined by the hemoglobin method of Anson (10). Samples were also taken in some cases for the determination of the concentration of inert protein formed during the reaction by the method described elsewhere (11) and which consists essentially in adding to the samples a large excess of enterokinase and thus bringing about rapid and complete activation of all the available trypsinogen.

Kinetics of Formation of Trypsin by Means of Enterokinase at pH 5.6 and 7.6.—The striking difference in the behavior of enterokinase when allowed to act on crystalline trypsinogen at pH 5.6, as compared with that of pH 7.6, is shown in Fig. 1. At pH 5.6 enterokinase acts almost like a typical enzyme so that the ultimate concentration of trypsin formed in a solution of trypsinogen of a definite concentration varies only slightly with the concentration of enterokinase used, while at pH 7.6 the ultimate concentration of trypsin formed varies markedly with the concentration of enterokinase.

Fig. 2 shows the experimental data for the action of enterokinase on crystalline trypsinogen at pH 5.6 plotted logarithmically (Equation 9*a*).

$$\ln \frac{G_o}{G_o - A} \; vs. \, t$$

In the calculations the value of G_o was taken as 1.5×10^{-3} [T. U.]$^{\text{Hb}}$ per ml. which is the value obtained in the presence of a large excess of enterokinase. In the presence of the concentrations of kinase used in this experiment (Fig. 1, pH 5.6) the activity reaches a maximum value of only 1.3–1.35 $\times 10^{-3}$ [T. U.]$^{\text{Hb}}$ per ml., the difference being

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due to the formation of a small amount (10–20 per cent) of inert protein. In the first part of the reaction this formation of inert

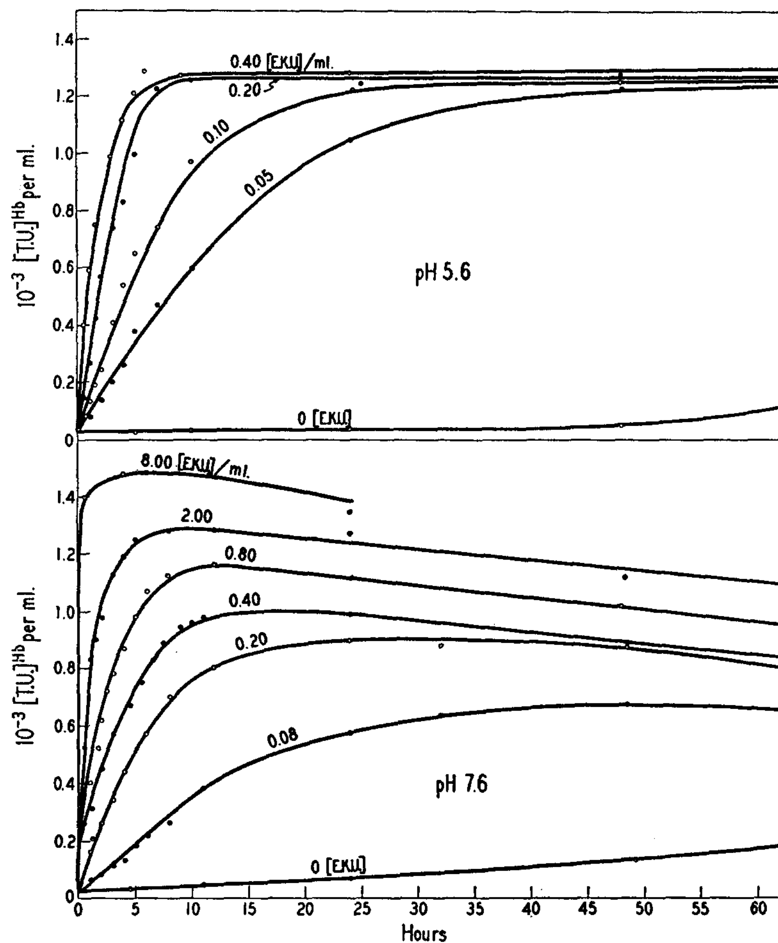

FIG. 1. Formation of trypsin from crystalline trypsinogen by enterokinase at 5°C. Activation mixtures: 5 ml. 0.065 per cent solution of trypsinogen in 0.005 M hydrochloric acid + 10 ml. 0.1 M phosphate buffer + 1.0 ml. enterokinase solution in water + distilled water to 50 ml.

protein is too small to affect the results but in the latter part of the reaction when A approaches the value of G_o the reaction will proceed more slowly than calculated on the simple assumption that no inert

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protein is formed. Thus in Fig. 2, where the results have been plotted by the simple monomolecular equation the theoretical and experimental points agree up to 50–60 per cent activation and the slopes of the lines are proportional to the concentrations of enterokinase used. In the last 30–40 per cent of the reaction the formation of inert protein becomes significant and the experimental points lie below those predicted by the simple equation in which the formation of inert protein was neglected.

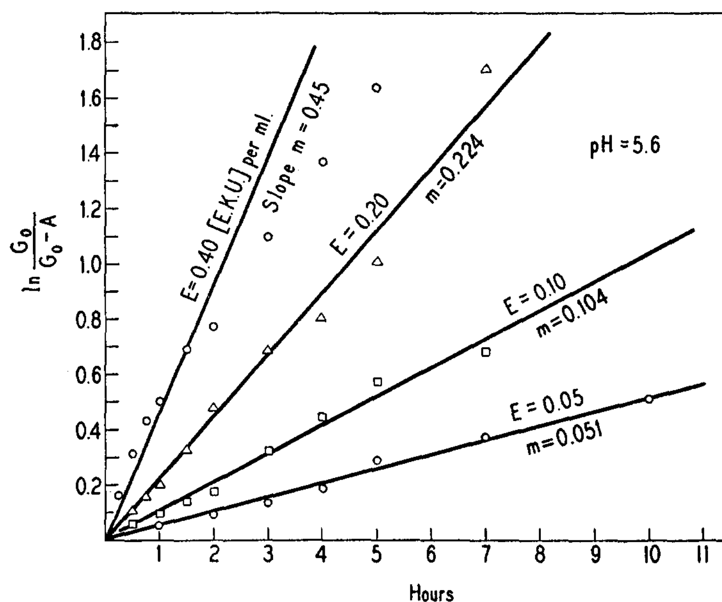

Fig. 2. Logarithmic curves of Fig. 1 pH 5.6 plotted in accordance with the equation of a simple unimolecular reaction.

On the other hand, curve I in Fig. 3 shows that the experimental data for the action of enterokinase at pH 7.6 do not fall in straight lines when plotted logarithmically in accordance with the theoretical equation of a simple unimolecular reaction even in the first part of the reaction. The experimental points, however, do fall in a straight line (curve II, Fig. 3) when plotted according to the more complete Equation 9 which takes care of the complication due to the formation of inert protein. Curve III of Fig. 3 shows the close agreement between

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the observed and theoretical values of A. The last were computed by means of Equation 11 with $m = 0.21$ as given by the slope of curve II. The logarithmic curves (Equation 9) for the whole series of pH 7.6 are shown in Fig. 4. In practically all cases the experi-

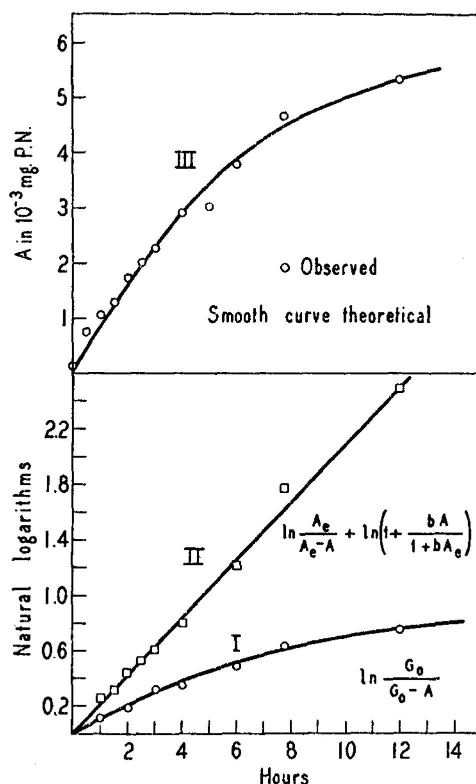

FIG. 3. Comparison between theoretical and observed data on the formation of trypsin from crystalline trypsinogen by enterokinase at pH 7.6 and 5°C. Concentration of trypsinogen 0.01 mg. protein nitrogen per ml. Concentration of enterokinase 0.20 [E.K.U.] per ml.

mental points lie on straight lines. The slopes of the various lines, as well as the calculated values of K_e, K_2, and A_e are given in Table I. The concentrations of G_o and A_e are expressed in mg. protein nitrogen per ml., 1 mg. protein nitrogen being equivalent to 0.15 $[\text{T. U.}]^{\text{Hb}}$.

Formation of Inert Protein.—Fig. 5 shows the time curves for the

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simultaneous formation of inert protein and of trypsin from trypsinogen in the presence of enterokinase at pH 7.6 both determined in-

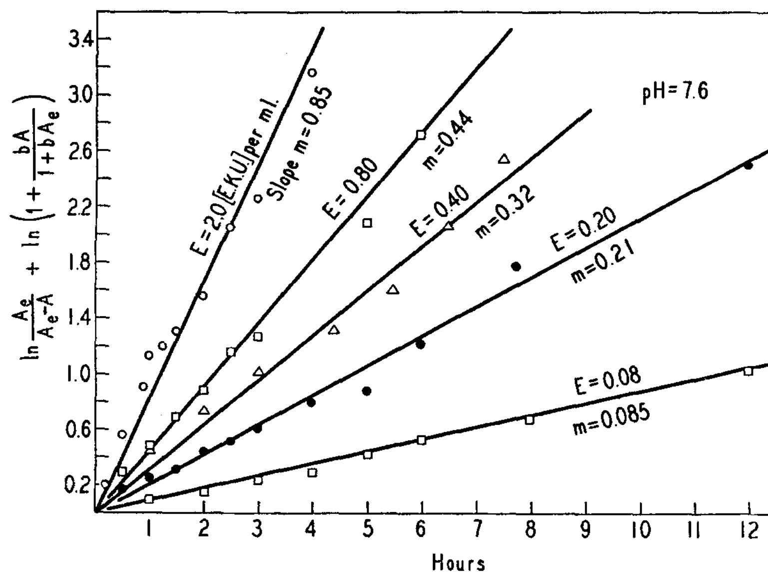

FIG. 4. Logarithmic curves of Fig. 1 pH 7.6 plotted in accordance with Equation 9.

TABLE I

Effect of Concentration of Enterokinase on the Formation of Trypsin at pH 7.6 and 5°C.

Trypsinogen concentration G_o = 0.01 mg. protein nitrogen per ml.

Concentration of enterokinase in [E.K.U.] per ml.	0.08	0.20	0.4	0.8	2.0
A_e in mg. trypsin protein nitrogen per ml.	0.0045	0.0060	0.0066	0.0077	0.0085
Slopes $m = K_eE + K_2A_e$	0.085	0.21	0.32	0.44	0.85
K_e (Equation 12) per [E.K.U.] per hr.	0.31	0.45	0.40	0.35	0.31
K_2 (Equation 13) per mg. trypsin protein nitrogen per hr.	14	20	25	21	27

dependently. The plotted experimental points fall closely on the smooth theoretical curves. The theoretical values of A were obtained by means of Equation 11. The theoretical values of I were calcu-

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lated from the theoretical values of A by means of Equations 4 and 7, namely

$$I = bA^2$$

where

$$b = \frac{G_o - A_e}{A^2_e} = \frac{(10 - 5) \times 10^{-3}}{25 \times 10^{-6}} = 200$$

The relation between the concentration of trypsin and inert protein formed in the reaction mixture at any time is shown graphically in

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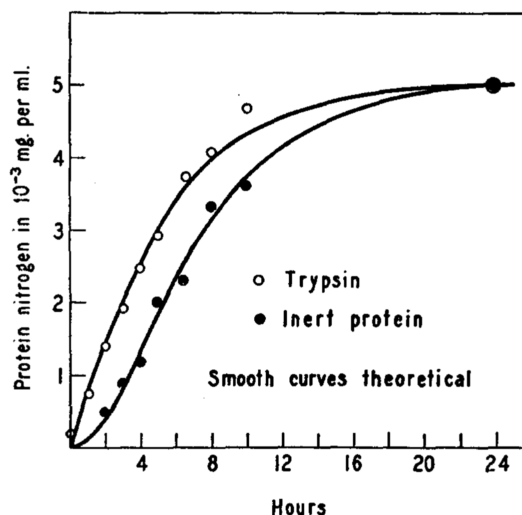

FIG. 5. Comparison between theoretical and observed data on formation of trypsin and inert protein from crystalline trypsinogen pH 7.6 and 5°C. in the presence of enterokinase. Concentration of trypsinogen 0.01 mg. protein nitrogen per ml. Concentration of enterokinase 0.20 [E.K.U.] per ml. in 0.02 M phosphate buffer pH 7.6.

Fig. 6 where the values of I were plotted as a function of A. The smooth parabolic curve is the locus of the theoretical function $I = 200\ A^2$.

It should be observed that in the case of autocatalytic formation of trypsin described elsewhere the relation between I and A is linear (12), while here

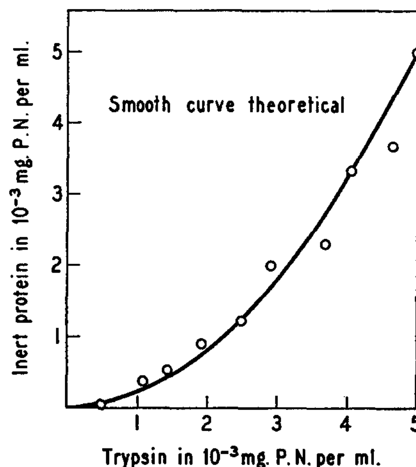

FIG. 6. Relation between trypsin and inert protein formed from crystalline trypsinogen at pH 7.6 and 5°C. in the presence of enterokinase. The smooth curve is the theoretical locus of the equation $I = bA^2$ where $b = \frac{G_o - A_e}{A^2} = 200$.

TABLE II

Effect of pH

Activation mixture: 1 ml. 1 M $KH_2PO_4 + K_2HPO_4$ mixtures, plus 1.0 ml. enterokinase, 5 [E.K.U.] per ml. water, plus 5.0 ml. crystalline trypsinogen, 0.1 mg. protein nitrogen per ml. M/200 hydrochloric acid, plus water to 50 ml. Samples 1.0 ml. plus 1.0 ml. 0.04 M hydrochloric acid for activity measurements.

Trypsinogen concentration G_o = 0.01 mg. protein nitrogen per ml.

Enterokinase concentration E = 0.1 [E.K.U.] per ml.

pH	5.26	5.78	6.12	6.38	6.55	6.72	6.94	7.18	7.45	7.75
A_e in 10^{-3} mg. trypsin protein nitrogen per ml.	8.1	7.3	6.1	5.6	4.6	4.3	3.8	3.4	3.2	2.9
$K_eE + K_2A_e$ per hr. (from logarithmic plot Equation 9)	0.053	0.13	0.20	0.24	0.27	0.25	0.30	0.35	0.36	0.40
K_e per [E.K.U.] per hr. (Equation 12)	0.36	0.75	0.87	0.93	0.79	0.68	0.71	0.72	0.68	0.67
K_2 per mg. trypsin protein nitrogen per hr. (Equation 13)	2	8	19	26	41	43	60	82	93	113

in the presence of enterokinase I is proportional to the *square* of A. Both relations, however, are derived mathematically on the basis of the same assumption that the formation of inert protein is catalyzed by the trypsin formed.

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Effect of pH on the Velocity Constants of Both Reactions

The striking difference in the kinetics of formation of trypsin by means of enterokinase at pH 5.8 and at pH 7.6 was shown in Fig. 1. A summary of a series of experiments on the effect of pH in the region of 5.26–7.75 on the kinetics of formation of trypsin from trypsinogen in the presence of enterokinase at 7°C. is given in Table II and also in Fig. 7.

The results show that there is a gradual decrease in the amount of trypsin formed with the increase of pH. At pH more acid than 5.0

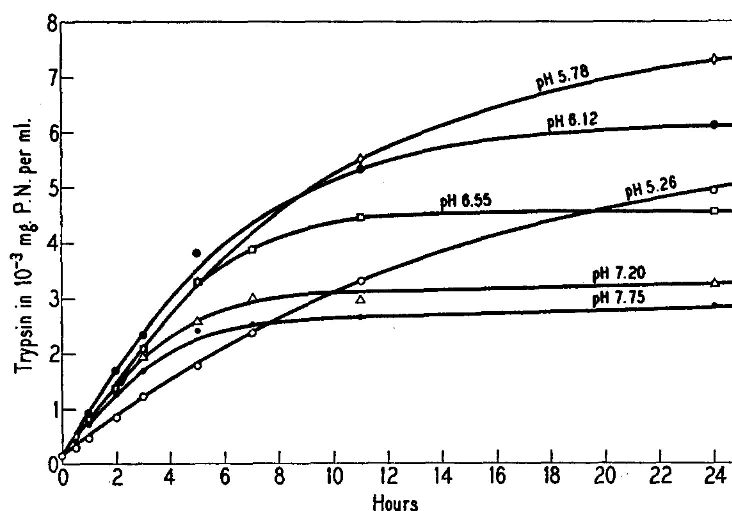

FIG. 7. Formation of trypsin at 7°C. by means of enterokinase at various pH. G_o = 0.01 mg. trypsinogen protein nitrogen per ml. Enterokinase = 0.1 [E.K.U.] per ml.

there is, however, a sudden drop in the amount of trypsin formed due to destruction of enterokinase. The optimum range of pH for the rate of formation of trypsin by means of enterokinase is at about 6.2 as shown on curve K_e, Fig. 8. The values of K_2 for the various pH, as calculated from the slopes of the logarithmically plotted curves, are of the same magnitude as those obtained under the same approximate conditions of temperature, salt, and trypsinogen concentration in the absence of enterokinase during the autocatalytic formation of trypsin (13).

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Effect of Concentration of Trypsinogen

With increase in concentration of trypsinogen in solution, the concentration of enterokinase being kept constant, the rate of the catalytic formation of inert protein is greatly increased as compared with the rate of the catalytic formation of trypsin by enterokinase, since the relative rate of formation of the two products is proportional to the

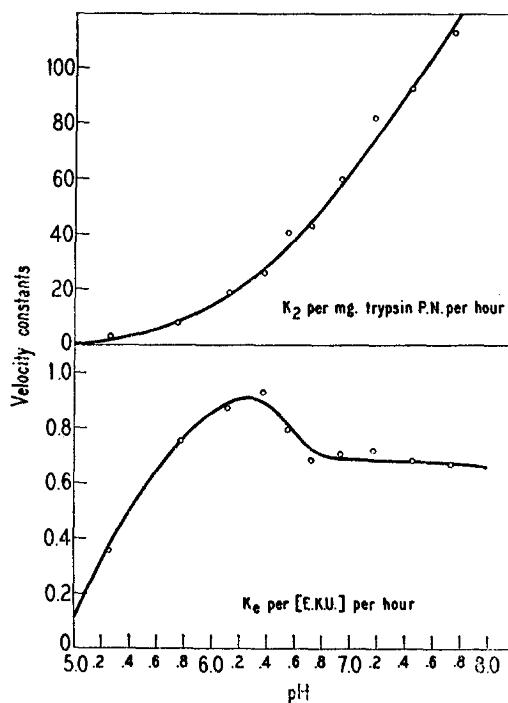

FIG. 8. Effect of pH on the velocity constants

concentration of trypsin formed (Equation 3). The complicating effect of the formation of inert protein on the kinetics of the enterokinase action as the concentration of trypsinogen is increased becomes evident even at pH 5.8. This is shown in Fig. 9 where the percentage of trypsinogen changed into trypsin was plotted against t. The higher the concentration of trypsinogen used the lower was the percentage rate as well as the final per cent of trypsinogen changed into trypsin.

The results of the experiment are summarized in Table III where

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the observed values of inert protein formed are given. In every case the sum of values of A_e and I_e is equal to the corresponding value of G_o.

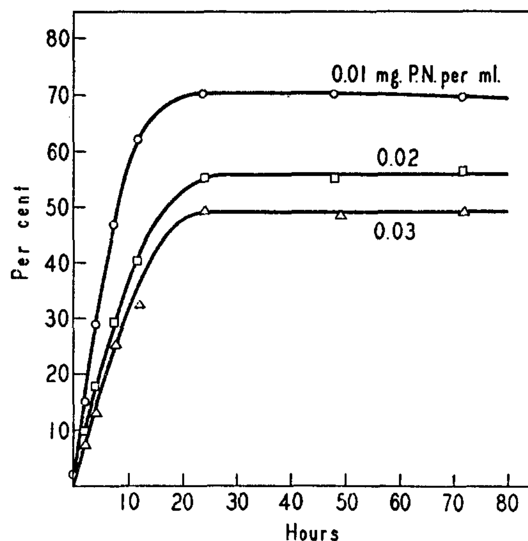

FIG. 9. Effect of concentration of trypsinogen on the formation of trypsin from crystalline trypsinogen by means of enterokinase at pH 5.8 and 6°C. Concentration of enterokinase 0.1 [E.K.U.] ml.

TABLE III

Effect of Concentration of Trypsinogen at pH 5.8 and 6°C.

Concentration of enterokinase 0.08 [E.K.U.] per ml. 0.02 M phosphate buffer pH 5.8 of activation mixture.

Concentration of trypsinogen = G_o, in mg. protein nitrogen per ml. activation mixture	0.01	0.02	0.03
Final concentration of trypsin formed = A_e, in trypsin mg. protein nitrogen per ml.	0.007	0.011	0.015
Final concentration of inert protein formed = I_e (observed) in mg. protein nitrogen per ml.	0.003	0.008	0.015
$\frac{K_2}{K_e} = \frac{2E(G_o - A_e)}{A_e^2}$	10	12	11

The table also shows that the relation between G_o and A_e in each case checks with the theoretical Equation 7 giving a value of $\frac{K_2}{K_e}$

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independent of the original concentration of trypsinogen used. The values of K_e and K_2 as calculated from the slopes of the logarithmic curves appear, however, to decrease with increase in concentration of trypsinogen. A similar effect of increase in concentration of substrate on the magnitude of the velocity constant has been frequently observed in the case of enzymatic reactions (14).

Addition of fresh trypsinogen to the activation mixtures at the end of the reaction always brought about formation of more trypsin, thus proving that the incomplete activation of the original trypsinogen was not due to any insufficiency or possible inactivation of the enterokinase. On the other hand, the addition of excess enterokinase or mold kinase to the activation mixture at the end of the reaction has never brought about an increase in the concentration of trypsin although no significant loss of protein has been noticed. It is evident that the incompleteness of the enterokinase reaction is due to the partial transformation of the trypsinogen into inert protein which cannot be changed into trypsin by any known activator.

Methods

1. *Preparation of Crystalline Trypsinogen.*—The trypsinogen was prepared by the method of Kunitz and Northrop (8). The crystals were purified and made inhibitor free by means of trichloracetic acid as described on page 993 of the same reference, except for an extra step in the process which was omitted through a typographical error. The corrected procedure for purification by means of trichloracetic acid is as follows: 10 gm. filter cake of trypsinogen crystals is dissolved in 200 ml. N/400 hydrochloric acid and 200 ml. 5 per cent trichloracetic acid added. The solution is left at 20°C. for 1 hour and then filtered with suction and washed several times with small amounts of 2.5 per cent trichloracetic acid and finally with water. The semi-dry precipitate is dissolved in 25 times its weight of N/50 hydrochloric acid, allowed to stand about 30 minutes. Ammonium sulfate is added to 0.4 saturation. The precipitate is filtered off and rejected. The filtrate is brought to 0.7 saturation with solid ammonium sulfate and filtered with suction. The filter cake is dissolved in 3–5 times its weight of N/200 hydrochloric acid and dialyzed for 24 hours at 5–6°C. against running N/200 hydrochloric acid.

2. *Preparation of Enterokinase.*—A stock of enterokinase in water and containing about 1,000 kinase units per ml. was prepared from intestinal contents by the method described elsewhere (15).

3. *Estimation of Enterokinase.*—The quantity of enterokinase in any solution is expressed in terms of the velocity with which it transforms crystalline trypsino-

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gen into trypsin under standard conditions. One enterokinase unit, 1 [E.K.U.] is the amount of kinase that brings about the activation of 0.065 mg. crystalline trypsinogen (0.01 mg. protein nitrogen) in 0.02 M Sørensen's phosphate buffer pH 5.8 at the rate of 100 per cent per hour at 5°C. Under these conditions the activation by enterokinase follows approximately the course of a simple unimolecular reaction so that the plotted values of $\ln \frac{G_o}{G_o - A}$ *vs. t* fall in straight lines (Fig. 2) the slopes of which are proportional to the concentration of enterokinase used. The concentration of enterokinase in each case can be taken as equal to the slope of the lines by assigning the value of unity to the proportionality constant.

The standard method of estimating kinase involves the determination of concentration of trypsin in a series of samples taken at various intervals of time from the activation mixture in order to obtain several points for the logarithmic curve. For practical purposes the following simplified procedure was adopted: *Activation mixture:* 3 ml. 0.02 M phosphate buffer pH 7.6 plus 1.0 ml. enterokinase in 0.02 M phosphate pH 7.6 plus 1 ml. standard crystalline trypsinogen solution (0.1 mg. protein nitrogen per ml.) in N/200 hydrochloric acid. The activation mixture is placed for 30 minutes in a water bath at 25°C. 1.0 ml. of the mixture is then added to 5.0 ml. Anson's urea-hemoglobin solution; its trypsin content $[T.U.]^{Hb}$ is determined as described by Anson (10). The concentration of enterokinase in [E.K.U.] per ml. activation mixture corresponding to the $[T.U.]^{Hb}$ measured is then read off a standard curve. The standard curve is obtained by plotting the data of $[T.U.]^{Hb}$ *vs.* [E.K.U.] for a series of activation mixtures containing various dilutions of a stock of enterokinase of known [E.K.U.] content, as determined by the standard method. 1 [E.K.U.] is equivalent to about 100 mg. of acetone dried pigs' duodenal mucosa.

4. *Estimation of Trypsin.*—Method of Anson (10).

5. *Estimation of Inert Protein.*—Described in preceding paper (11).

The writer was assisted by Margaret R. McDonald.

SUMMARY

Crystalline trypsinogen is most readily and completely transformed into trypsin by means of enterokinase in the range of pH 5.2–6.0 at 5°C. and at a concentration of trypsinogen of not more than 0.1 mg. per ml. The action of enterokinase under these conditions is that of a typical enzyme. The process follows closely the course of a catalytic unimolecular reaction, the rate of formation of trypsin being proportional to the concentration of enterokinase added and the ultimate amount of trypsin formed being independent of the concentration of enterokinase.

The catalytic action of enterokinase on crystalline trypsinogen in

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dilute solution at pH more alkaline than 6.0 and in concentrated solution at pH even slightly below 6.0 is complicated by the partial transformation of the trypsinogen into inert protein which can no longer be changed into trypsin even by a large excess of enterokinase. This secondary reaction is catalyzed by the trypsin formed and the rate of the reaction is proportional to the concentration of trypsin as well as to the concentration of trypsinogen in solution. Hence under these conditions only a small part of the trypsinogen is changed by enterokinase into trypsin while a considerable part of the trypsinogen is transformed into inert protein, the more so the lower the concentration of enterokinase used.

The kinetics of the formation of trypsin by means of enterokinase when accompanied by the formation of inert protein can be explained quantitatively on the theoretical assumption that both reactions are of the simple catalytic unimolecular type, the catalyst being enterokinase in the first reaction and trypsin in the second reaction.

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