

EFFECT OF THE FORMATION OF INERT PROTEIN ON THE
KINETICS OF THE AUTOCATALYTIC FORMATION
OF TRYPSIN FROM TRYPSINOGEN

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Vernon as early as 1901 discovered the remarkable fact that both "tryptic and rennet ferments" can be formed from their corresponding zymogens by the action of trypsin itself (1). Vernon's results were fully confirmed by Kunitz and Northrop who found that trypsin brings about the formation of chymotrypsin from crystalline chymotrypsinogen (2) as well as the formation of trypsin from crystalline trypsinogen (3). The process of formation of chymotrypsin by trypsin follows the course of a catalytic unimolecular reaction; the formation of trypsin by trypsin, on the other hand, follows the course of an autocatalytic reaction. The rate of the autocatalytic formation of trypsin is greatly enhanced in the presence of high concentrations of ammonium or magnesium sulfate.

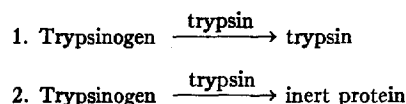
Recently Anson (4) found that trypsin brings about the catalytic formation of carboxypeptidase from its inactive precursor found in beef pancreas.

Trypsin has been found (5) to have an accelerating effect on the clotting of blood. Waldschmidt-Leitz, Stadtler, and Steigenwaldt suggested (6) that trypsin acts like thrombin on fibrinogen. Eagle and Harris found (7) that crystalline trypsin coagulated citrated plasma of the rabbit, guinea pig, horse, and man. They believe that the action of trypsin is due to its catalytic transformation of prothrombin into thrombin. Mellanby and Pratt (8) disagree with the conclusions of Eagle and Harris. According to the English authors trypsin liberates active thrombokinase which acts on prothrombin to form thrombin in the presence of calcium ions. As a source of trypsin

Mellanby and Pratt used pancreatic juice of cats activated by means of enterokinase.

This paper describes a new enzymatic action of crystalline trypsin, namely the transformation of crystalline trypsinogen protein into an inert protein which can no longer be changed into trypsin protein either by the addition of trypsin, enterokinase, or mold kinase (9). The formation of inert protein occurs whenever crystalline trypsinogen containing a trace of trypsin is dissolved in dilute buffer solution of pH 5.0–9.0. This range of pH is also favorable for the autocatalytic transformation of trypsinogen into trypsin. Hence the presence of a trace of trypsin in a solution of crystalline trypsinogen in this range of pH brings about a gradual change of the trypsinogen partly into trypsin and partly into inert protein with the result that the process of formation of trypsin under these conditions diverges from the reaction of the simple autocatalytic type.

The two simultaneous processes may be represented schematically as follows:



The first reaction, as mentioned before, proceeds in accordance with the law of an autocatalytic unimolecular reaction, namely that the rate of formation of trypsin is proportional to the concentration of trypsinogen as well as to the concentration of trypsin in solution. With regard to the second reaction, experimental studies show that the transformation of trypsinogen into inert protein is catalyzed by trypsin and that the process follows the course of a simple catalytic unimolecular reaction, the rate of formation of inert protein being proportional to the concentration of trypsin (the catalyst) as well as to the concentration of trypsinogen (the substrate).¹

The relative rate of formation of the two proteins from trypsinogen depends on the pH of the solution, the rate of formation of inert protein being increased more rapidly with increase of pH than that of formation of trypsin. Hence the proportion of trypsinogen changed

¹ This reaction also complicates the kinetics of the formation of trypsin from trypsinogen by enterokinase (11).

into inert protein is greater in the alkaline range of pH, while in the range of pH 5.0–6.0 the proportion of trypsinogen changed into trypsin is greater.

In acid solution no inert protein is formed and hence trypsinogen may be completely transformed into trypsin by mold kinase at pH 3.0 (9).

Theoretical Study of the Kinetics of Formation of Trypsin and Inert Protein from Trypsinogen by Trypsin

The mathematical derivation of the equations for the kinetics of the simultaneous formation of trypsin and inert protein from trypsinogen is as follows:

- Let G_o = original concentration of trypsinogen
 A_o = original concentration of trypsin
 A = total concentration of trypsin at any time t
 $A - A_o$ = concentration of trypsin formed at any time t
 A_e = final total concentration of trypsin
 $A_e - A_o$ = final concentration of trypsin formed
 I = concentration of inert protein formed in any time t
 $G_o + A_o - A - I$ = concentration of trypsinogen at any time t
 $G_o + A_o - I$ = "available activity" = trypsinogen + trypsin
 I_e = final concentration of inert protein formed.

All concentrations are expressed in milligrams protein nitrogen per milliliter activation mixture.

Assuming that the rate of formation of both trypsin and inert protein is proportional to the concentration of trypsin as well as to the concentration of trypsinogen in solution we have:

$$\frac{dA}{dt} = K_1 A (G_o + A_o - A - I) \quad (1)$$

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

hence

$$\frac{dI}{dA} = \frac{K_2}{K_1} \quad (3)$$

and on integration

$$I = \frac{K_2}{K_1} (A - A_o) \quad (4)$$

also

$$I_e = \frac{K_2}{K_1} (A_e - A_o) \quad (4a)$$

Substituting the value for I in equation 1 we get

$$\frac{dA}{dt} = K_1 A (G_o + bA_o - bA) \quad (5)$$

where

$$b = 1 + \frac{K_2}{K_1}$$

When

$$\frac{dA}{dt} = 0 \text{ (at the end of the reaction) } G_o + bA_o = bA_e \quad (6)$$

or

$$A_e - A_o = \frac{G_o}{b}$$

Hence

$$A_e = \frac{K_1}{K_1 + K_2} G_o + A_o \quad (7)$$

Also

$$I_e = \frac{K_2}{K_1 b} G_o \quad \text{or} \quad I_e = \frac{K_2}{K_1 + K_2} G_o \quad (8)$$

Substituting bA_e in equation 5 for $G_o + bA_o$ we get

$$\frac{dA}{dt} = K_1 b A (A_e - A) = (K_1 + K_2) A (A_e - A) \quad (5a)$$

which on integration gives

$$\log \frac{A}{A_e - A} - \log \frac{A_o}{A_e - A_o} = \frac{K_1 + K_2}{2.3} A_e t = \frac{K_1 G_o + (K_1 + K_2) A_o}{2.3} t \quad (9)$$

When A_o is small compared with A_e equation 9 becomes

$$\log \frac{A}{A_e - A} - \log \frac{A_o}{A_e - A_o} = \frac{K_1}{2.3} G_o t \quad (9a)$$

The values of $\log \frac{A}{A_0 - A}$ when plotted against t should lie on a straight line, the slope of which is

$$m = \frac{K_1 + K_2}{2.3} A_0 = \frac{K_1 G_0 + (K_1 + K_2) A_0}{2.3}$$

in accordance with equation 9. The values of K_1 and K_2 are thus easily determinable when G_0 , A_0 , and A_0 are known. Thus

$$K_1 + K_2 = \frac{2.3m}{A_0} \quad (9b)$$

and

$$K_1 = \frac{(K_1 + K_2)(A_0 - A_0)}{G_0} = \frac{2.3m}{G_0} \times \frac{A_0 - A_0}{A_0} \quad (9c)$$

When A_0 is negligible compared with A_0 then

$$K_1 = \frac{2.3m}{G_0} \quad (9d)$$

which also follows from equation 9a directly.

Equation 5a and hence equation 9 are identical with the true equations of an autocatalytic unimolecular reaction (12), namely,

$$\log \frac{A}{A_0 - A} - \log \frac{A_0}{A_0 - A_0} = \frac{K}{2.3} A_0 t$$

except for the presence of two velocity constants and for the specific meaning of the term $(A_0 - A)$ which does not represent the concentration of substrate at any time t as would be the case if there were no complication due to the second simultaneous reaction. (It is to be noticed that in the equation cited A_0 is equal to $G_0 + A_0$ while here

$$A_0 = \frac{K_1}{K_1 + K_2} G_0 + A_0.)$$

The expression for the time rate of formation of inert protein is obtained by substituting in equation 2 values for A in terms of I as obtainable from equation 4, namely, $A = \frac{K_1}{K_2} I + A_0$ and then integrating in terms of I . A simpler solution is to substitute the expression for A in terms of I directly into the integrated equation 9.

We get then the integrated equation for the time rate of formation of inert protein, namely,

$$\log \frac{K_1 I + K_2 A_0}{I_0 - I} - \log \frac{K_2 A_0}{I_0} = \frac{K_1 + K_2}{2.3K_2} (K_1 I_0 + K_2 A_0) t \quad (10)$$

which is similar to equation 9; the plotted curve of I vs. t should resemble an S shaped curve typical for an autocatalytic unimolecular reaction.

The derived equations bring out the following quantitative relationships between the two products formed from trypsinogen by means of trypsin under constant conditions of pH, temperature, and salt concentration.

1. The concentration of inert protein in the reaction mixture at any time t , as well as at the end of the reaction, is proportional to the concentration of trypsin formed.² It follows that the specific activity, *i.e.* the number of tryptic units per milligram protein nitrogen, of the final product is constant and is independent of the original concentration of trypsinogen.

2. The final concentration of trypsin formed as well as the final concentration of inert protein formed is proportional to the original concentration of trypsinogen and is independent of the original concentration of trypsin (equations 7 and 8). The rate of formation of these products is, however, dependent on the original concentration of trypsin in the reaction mixture.

3. The equation for the rate of formation of trypsin still remains autocatalytic in form (equation 9) although it is slightly modified by the correction due to the formation of inert protein.

4. The equation for the rate of formation of inert protein is, like the one for the rate of formation of trypsin, of an autocatalytic form (equation 10).

² All the derived equations would still hold true were inert protein instead of trypsin assumed to be the catalyzing agent in both reactions, since the concentrations of the two products are proportional to each other. The experiments, however, on the effect of varying the initial concentration of trypsin show definitely that the rate of formation of both products is proportional to the concentration of trypsin. It follows, therefore, that trypsin is the catalyst in both reactions.

All these relationships have been found to check closely with the experimental facts.

Experimental Studies of the Kinetics of Transformation of Crystalline Trypsinogen into Trypsin and Inert Protein

General Procedure.—Solutions were made of crystalline trypsinogen in dilute buffers and allowed to stand at 5°C.³ In most experiments it was unnecessary to add trypsin since the crystalline trypsinogen used generally contained small but measurable amounts of trypsin which were sufficient to initiate the reactions. Duplicate samples of 1 ml. were taken at various times. One set of the 1 ml. samples was acidified with hydrochloric acid to about pH 2.0 in order to stop the reactions. The concentration of trypsin in these samples was then determined by the hemoglobin method of Anson (10). These measurements provided the data for the rate of formation of trypsin. The 1 ml. samples of the other set were mixed with equal amounts of a concentrated solution of enterokinase (200 [E.K.U.]/ml.) made up in $m/10$ pH 7.6 phosphate buffer and placed at 5°C. This brought about very rapid transformation of all of the available trypsinogen protein into trypsin protein without any formation of inert protein as will be described in a later paper on enterokinase (11). The samples were acidified with hydrochloric acid to pH 2.0 after 2 hours and the concentration of trypsin in these samples was determined. If there were no gradual formation of inert protein in the trypsinogen solution then the concentration of trypsin (available activity) in the enterokinase activated samples taken at various times would remain constant and would be numerically equal to the concentration of trypsin added to or present initially in the trypsinogen solution plus the original concentration of trypsinogen. Actually it was found that in all cases where the pH of the trypsinogen solution was above 4.0 there was a gradual lowering of the concentration of available tryptic activity in the successive samples. This gradual loss in available activity was due to the formation of inert protein since the total concentration of protein in the trypsinogen solution, as measured by the amount of precipitate formed with 2.5 per cent trichloroacetic acid remained practically constant up to the end of the reaction.

The rate of formation of inert protein in the original reaction mixture was calculated as follows:

Let P_0 = initial available activity, and

P_t = available activity at any time t . The concentration of inert protein formed at any time t is then $I_t = P_0 - P_t$

³ It was found necessary throughout these studies to keep all reaction mixtures at a temperature not higher than 5°C. in order to avoid complications due to protein hydrolysis by trypsin.

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.

An experiment illustrating the various measurements is given in Table I. The concentration of trypsin as well as the concentrations of available activity are expressed both in tryptic units $[\text{T.U.}]_{\text{ml.}}^{\text{Hb}}$ and also in milligrams protein nitrogen per milliliter. The last were obtained by dividing the $[\text{T.U.}]_{\text{ml.}}^{\text{Hb}}$ by 0.17 which is the specific activity; *i.e.*, $[\text{T.U.}]_{\text{mg. P. N.}}^{\text{Hb}}$ of pure trypsin.

The experiment shows the following:

1. The final concentration of trypsin formed is only about one-fourth of the initial available activity.
2. The total available activity is diminished rapidly with time until it equals the concentration of trypsin in solution.

TABLE I

Formation of Trypsin and Inert Protein from Trypsinogen at pH 8.0 and 5°C.

Activation mixture: 10 ml. of solution of crystalline trypsinogen in M/200 hydrochloric acid containing 1 mg. protein nitrogen per ml. plus 4 ml. M/2 phosphate pH 8.0 plus water to 100 ml.

Time at 5°C.	Trypsin		Available activity		Inert protein (by difference)	Total protein measured
	$10^{-3} [\text{T.U.}]_{\text{ml.}}^{\text{Hb}}$	$10^{-3} \text{mg. P. N. / ml.}$	$10^{-3} [\text{T.U.}]_{\text{ml.}}^{\text{Hb}}$	$10^{-3} \text{mg. P. N. / ml.}$		
0	0.35	2.0	15.60	90	0	88
3.5	1.22	7.0	11.10	64	26	83
7.0	2.32	13.5	6.08	35	55	88
11.5	3.07	18.0	4.04	23	67	83
23.0	3.70	21.0	3.75	22	68	64
47.0	3.46	20.0	3.50	20	70	49
72.0	3.08	18.0	3.12	18	72	43

3. The total protein concentration is practically unchanged during the time of formation of trypsin; this shows that the rapid loss in available activity is due to the formation of inert protein which can no longer be activated by the excess of enterokinase used.

4. A gradual loss in protein concentration begins at the time when the trypsin concentration of the solution has reached its maximum value. This loss is due to the gradual hydrolysis by the formed trypsin of both the inert protein as well as of the trypsin itself. This gradual destruction of protein by the trypsin is greatly reduced if the pH of the solution is kept below 7.0 and the total concentration of protein below 0.1 mg. protein nitrogen per milliliter.

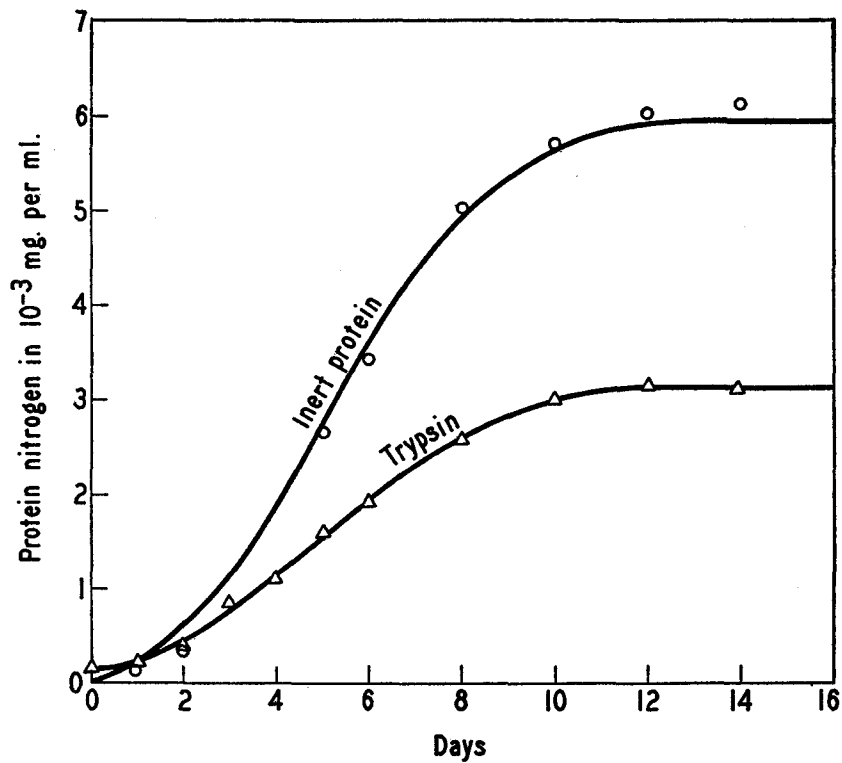


FIG. 1a

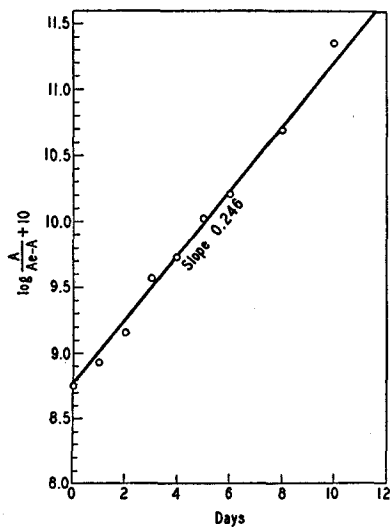


FIG. 1b

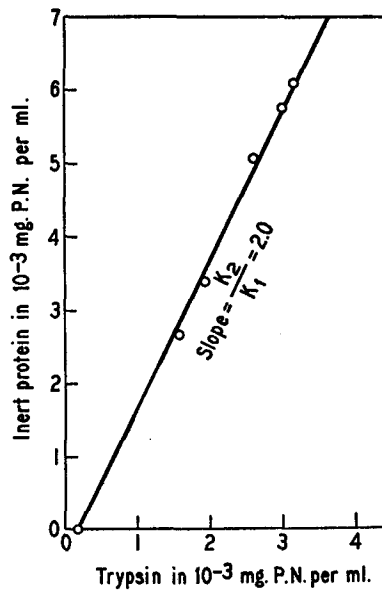


FIG. 1c

FIGS. 1a, 1b, 1c. Formation of trypsin and inert protein at pH 5.8 and 5°C . 5 ml. crystalline trypsinogen, 0.1 mg. protein nitrogen per ml. in 0.005 M HCl, plus 2 ml. 0.5 M K-Na phosphate buffer pH 5.8 plus water to 50 ml. Smooth curves in Fig. 1a drawn through calculated points.

Transformation of Trypsinogen into Trypsin and Inert Protein

Fig. 1 *a* shows graphically the experimental data for the time rate of formation of trypsin and inert protein at pH 5.8. The plotted experimental points for the rate of formation of trypsin as well as for the formation of inert protein fall within the experimental error on smooth theoretical S shaped curves. The theoretical values of A were obtained by means of the exponential form of equation 9*a*, namely,

$$A = A_e \frac{\frac{A_o}{A_e - A_o} e^{\kappa_1 G_o t}}{1 + \frac{A_o}{A_e - A_o} e^{\kappa_1 G_o t}}$$

The theoretical values of I were calculated from the theoretical values of A by means of equation 4, namely,

$$I = \frac{K_2}{K_1} (A - A_o)$$

The values for K_1 and for $\frac{K_2}{K_1}$ were calculated from the slopes of the rectilinear curves in Figs. 1 *b* and 1 *c*.

Fig. 1 *b* shows the plotted points of $\log \frac{A}{A_e - A}$ vs. t . The experimental points fall on a straight line in accordance with equation 9. The proportionality relation between the values of A and I is shown in Fig. 1 *c* in agreement with equation 4.

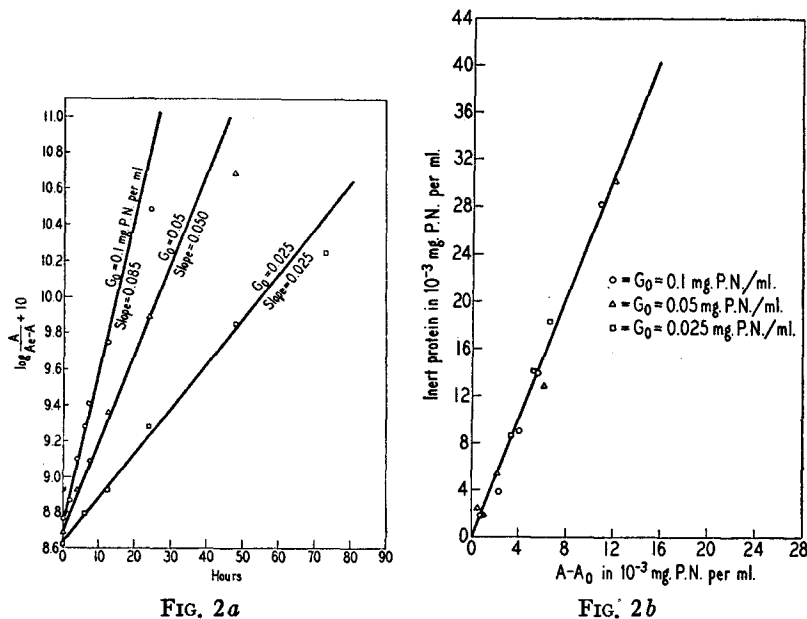
Effect of Varying the Concentration of Trypsinogen on the Kinetics of Formation of Trypsin and Inert Protein

Equation 9 *a* predicts that for various values of initial concentration of trypsinogen the slope of the plotted rectilinear curve of $\log \frac{A}{A_e - A}$ vs. t should be proportional to G_o , provided the value of A_o is small compared with A_e .

Fig. 2 *a* shows the plotted values of $\log \frac{A}{A_e - A}$ vs. t of a series of concentrations of trypsinogen at pH 5.8. The experimental points

lie in all cases on straight lines, the slopes of which are approximately proportional to the initial concentrations of trypsinogen used.

Fig. 2 *b* shows that the linear relation between I and A is independent of the initial concentration of trypsinogen, in agreement with equation 4.



FIGS. 2 *a* and 2 *b*. Effect of concentration of trypsinogen.
10 ml. 0.1 M K-Na phosphate buffer pH 5.8 plus 5.0, 2.5, or 1.25 ml. crystalline trypsinogen, 1 mg. protein nitrogen per ml., plus 0.2 M NaOH to adjust pH to 5.8 plus water to 50 ml.

Effect of Varying the Initial Concentration of Active Trypsin in Solution

The effect of varying the concentration of active trypsin added to a solution of trypsinogen of pH 5.0 is shown in Fig. 3 *a* where the concentration of trypsin formed ($A - A_0$) was plotted against t . It is evident from the curves that the initial rate of formation of trypsin is proportional to the initial concentration of active trypsin in the activation mixture. The final concentration of trypsin formed and hence also the final concentration of inert protein formed is, how-

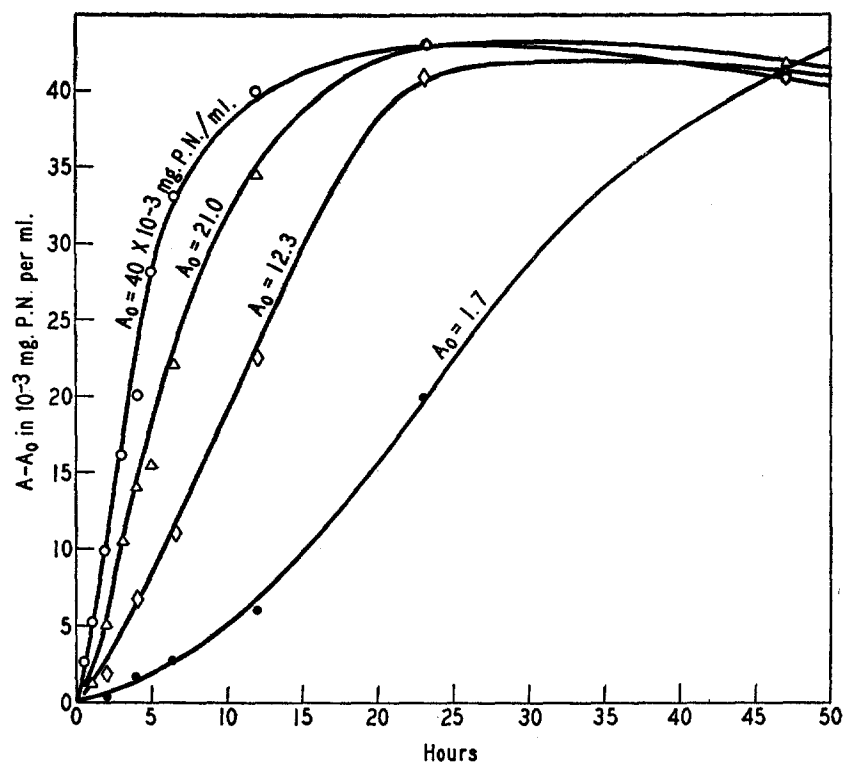


FIG. 3a

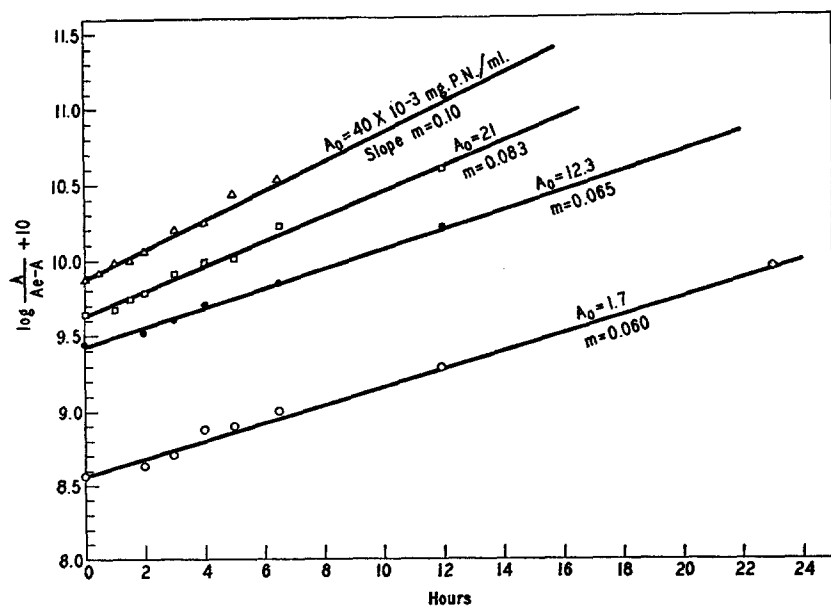


FIG. 3b

FIGS. 3a and 3b. Effect of varying initial concentration of trypsin. 5 ml. crystalline trypsinogen, 1 mg. protein nitrogen per ml. in $m/200$ HCl, plus 0, 5, 10, or 20 ml. crystalline trypsin, 0.1 mg. protein nitrogen per ml. in 0.05 m K-K phosphate buffer pH 5.0, plus 20, 15, 10, or 0 ml. 0.05 m K-K phosphate buffer pH 5.0 plus water to 50 ml.

TABLE II
Effect of Varying the Initial Concentration of Trypsin
Summary of Figs. 3a and 3b

A_o in mg. P.N.....	0.0017	0.0123	0.021	0.040
G_o " " "	0.10	0.10	0.10	0.10
$G_o + A_o$ " " "	0.1017	0.1123	0.1210	0.140
A_e " " "	0.043	0.053	0.064	0.083
$A_e - A_o$ " " "	0.041	0.041	0.043	0.043
Slope $m = \frac{K_1 + K_2}{2.3} A_e$ (Fig. 3b).....	0.060	0.065	0.083	0.100
Hence $K_1 + K_2 = \frac{2.3 m}{A_e}$ (Equation 9b).....	3.23	2.76	3.00	2.76
$K_1 = \frac{2.3 m}{A_e} \times \frac{(A_e - A_o)}{G_o}$ (Equation 9c).....	1.32	1.13	1.29	1.19
K_2 (by difference).....	1.91	1.63	1.71	1.57
Initial slopes m_1 (Fig. 3a).....	0.00025	0.0017	0.0030	0.0052
$\frac{m_1}{A_o}$	0.015	0.014	0.014	0.013

TABLE III
Effect of pH
Summary of Figs. 4a, 4c, 4d

pH.....	4.88	5.92	6.9	7.66	8.5
G_o in mg. P.N.....	0.01	0.01	0.01	0.01	0.01
A_o " " "	0.00012	0.00012	0.00012	0.00012	0.00012
A_e " " "	0.0063	0.00175	0.00063	0.00043	0.00033
Slope $m = \frac{K_1 + K_2}{2.3} A_e$ (Fig. 4c).....	0.13	0.35	0.65	0.75	0.90
$K_1 + K_2 = \frac{2.3 m}{A_e}$ (Equation 9b).....	48	460	2380	4000	6300
K_1 (per mg. P.N./day) = $\frac{2.3 m}{A_e} \times \frac{(A_e - A_o)}{G_o}$ (Equation 9c).....	30	75	120	125	130
K_2 (per mg. P.N./day) by difference.....	18	385	2260	3875	6170
$\frac{K_2}{K_1}$ calculated.....	0.6	5.1	19	31	48
$\frac{K_2}{K_1}$ observed (Fig. 4d).....	1.2	4.7	19	34	43

ever, independent of the initial concentration of trypsin and is identical in all cases, which checks with equations 7 or 8. The plotted curves of $\log \frac{A}{A_e - A}$ vs. t (Fig. 3b) are straight lines the slopes of which are

proportional to the values of A_e in all cases which is in accordance with equation 9. Table II summarizes the results of this experiment.

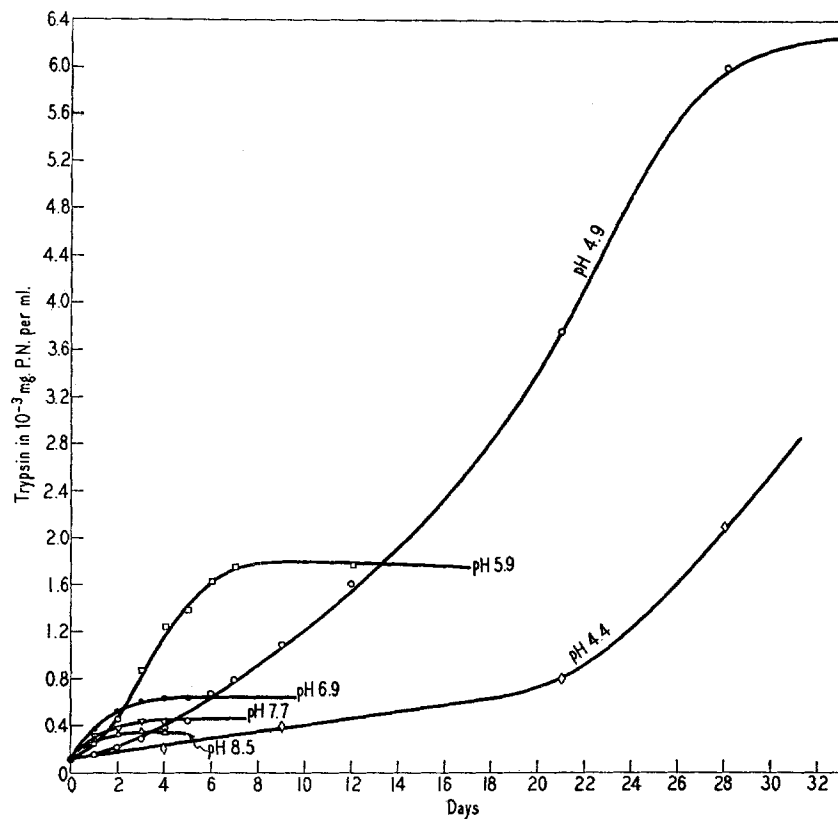


FIG. 4a

Figs. 4a, 4b, 4c, 4d, 4e. Effect of pH on kinetics of formation of trypsin and inert protein at 5°C.

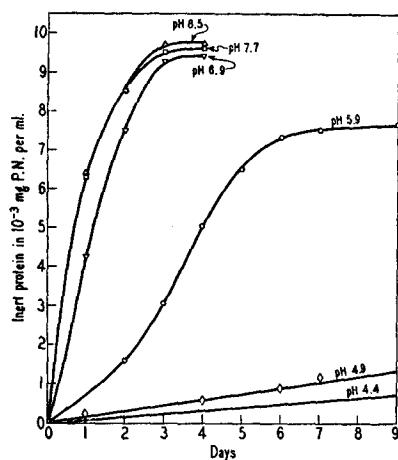
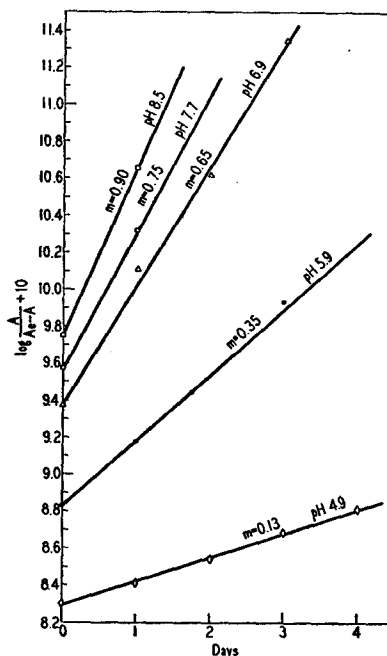
1 ml. M/1 K-K phosphate buffers of various pH plus 5 ml. crystalline trypsinogen, 0.1 mg. protein nitrogen per ml. in M/2,000 HCl plus water to 50 ml.

Potassium salts only were used in the buffer mixtures instead of the usual $\text{KH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$ mixtures in order to eliminate complications due to differences in the relative concentrations of the two cations.

Effect of pH on the Kinetics of Formation of Trypsin and Inert Protein

Figs. 4a and 4b show the curves for the rate of formation of trypsin and inert protein at various pH. The initial rate of formation of

both trypsin and of inert protein increases with increase in pH, but the ultimate amount of trypsin formed decreases with increase in pH because of the larger amounts of inert protein formed in the alkaline solutions. The plotted curves of $\log \frac{A}{A_e - A}$ as well as the plotted curves of A vs. t are straight lines in all cases as shown in Figs. 4 *c* and 4 *d*. The values of the velocity constants K_1 and K_2 for the

FIG. 4*b*FIG. 4*c*

reactions at various pH were obtained from the slopes of the rectilinear curves and are tabulated in Table III. Fig. 4 *e* where the curves for the values of $\log K_1$ and $\log K_2$ vs. pH are given shows the striking difference in the effect of pH on the rate of the two reactions, the rate of formation of inert protein being almost inversely proportional to the hydrogen ion concentration in the range of pH 4.0–7.0. At pH above 5.1 the rate of formation of inert protein is greater than that of formation of trypsin. At pH 5.1 the two velocity constants are

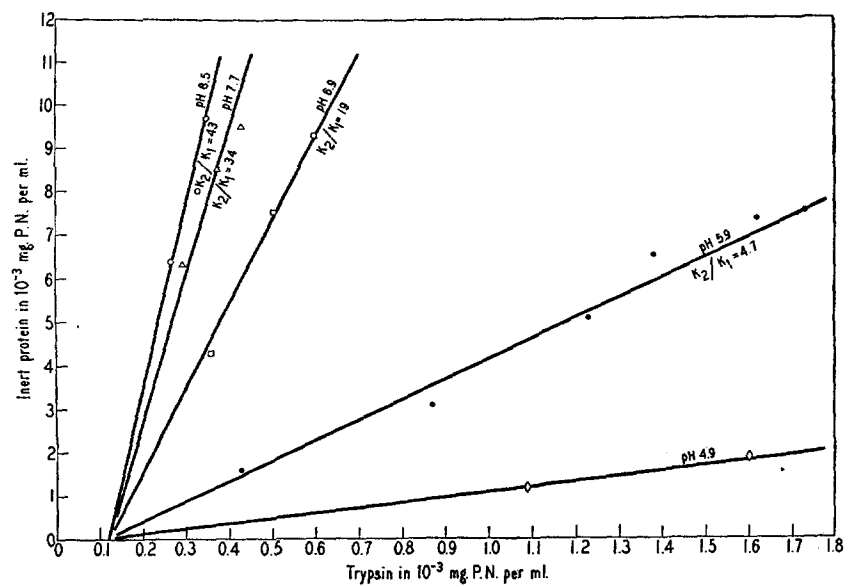


FIG. 4d

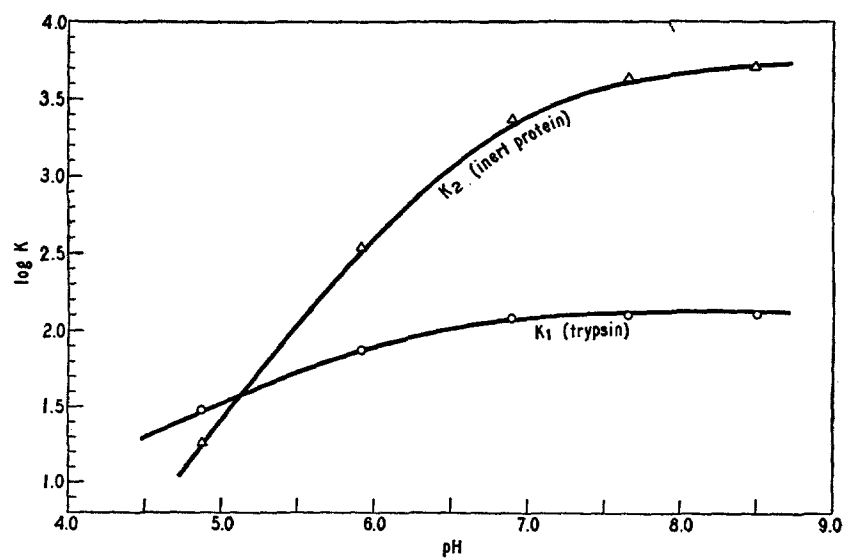


FIG. 4e

equal while at lower pH the rate of formation of inert protein becomes negligible.

The striking difference in the ultimate amount of trypsin formed at pH above 7.0 as compared with that formed at pH below 6.0 may be the explanation of the disagreement between Vernon, on one side, and Bayliss and Starling on the other side in regard to the question whether trypsin itself is able to convert trypsinogen into trypsin. Vernon worked with inactive pancreatic extracts and had no difficulty in activating it by means of trypsin while Bayliss and Starling employed pure pancreatic juice and found that trypsin not only did not activate the pancreatic juice but just the opposite occurred, namely, that it "destroyed the pro-ferment without apparently at any time converting it into ferment" (13). Bayliss and Starling have evidently obtained results comparable to the one represented in Fig. 4 *a* by the curve of pH 6.9 or pH 7.7 while Vernon's results correspond to the curve of pH 4.9. A difference of one or two units between the pH of the pancreatic extract used by Vernon and that of the pancreatic juice used by Bayliss and Starling would amply explain their disagreement.

The writer was assisted by Margaret R. McDonald.

SUMMARY

A solution of crystalline trypsinogen in dilute buffer containing a trace of active trypsin when allowed to stand at pH 5.0–9.0 and 5°C. is gradually transformed partly into trypsin protein and partly into an inert protein which can no longer be changed into trypsin either by enterokinase or mold kinase.

During the process of formation of trypsin and inert protein the ratio of the concentrations of the two products in any reaction mixture remains constant and is independent of the original concentration of trypsinogen protein. This ratio varies, however, with the pH of the solution, the proportion of trypsin formed being greater in the acid range of pH.

The experimental curves for the rate of formation of trypsin, as well as for the rate of formation of inert protein are symmetrical S shaped curves closely resembling those of simple autocatalytic reactions.

The kinetics of formation of trypsin and inert protein can be explained quantitatively on the theoretical assumptions that both reactions are of the simple unimolecular type, that in each case the reaction is catalyzed by trypsin, and that the rate of formation of each of the products is proportional to the concentration of trypsin as well as to the concentration of trypsinogen in solution.

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