CLXXXVIII. THE ACTIVATION OF PANCREATIC JUICE BY ENTEROKINASE.

By TRILOK NATH SETH.

Department of Biochemistry, University of Cambridge.

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CHEPOVALNIKOV [1899] was the first to observe that inactive pancreatic juice developed its proteoclastic activity when treated with intestinal juice. Pawlow [1901], as a result of his extensive researches on the subject, came to the conclusion that enterokinase was a specific enzyme, which converted trypsinogen into trypsin. This view of the enzyme nature of enterokinase has been amply confirmed by the work of later investigators, and is now accepted on all hands. However, various hypotheses have been put forward, from time to time, to explain the mechanism of interaction between trypsinogen and enterokinase, though it cannot be said that any one of them has yet been definitely established, and it was in the hope of throwing some more light on this interesting, though complicated, problem that the present work was undertaken.

METHODS.

The general technique adopted during the course of the present work was essentially the same as that used by Mellanby and Woolley [1912, 2], and consisted in incubating at a definite temperature a mixture of known quantities of pancreatic juice and enterokinase, and estimating the proteoclastic activity of the fluid from time to time by the milk coagulation method, using 0.1 cc. of the fluid for each estimation.

Pancreatic juice. Fistula-pancreatic juice obtained from dogs by the method of Bayliss and Starling [1902] was used during the course of the present investigation. About 100–150 cc. of pancreatic juice were obtained from each dog and it was always found to be clear and limpid, with a p_H 8.4–8.8. It was also observed that the alkalinity of the fresh juice was due almost entirely to the presence of HCO_3' , and not CO_3'' , as suggested by previous workers. When collected carefully in sterile tubes, without contamination with intestinal mucosa, and kept at -1° to -2° , the pancreatic juice did not develop any appreciable proteoclastic activity, even after three months.

Enterokinase. The second 13 cm. (approximately) of the small intestine of a dog was removed immediately after death, washed thoroughly, and the

lightly scraped mucous membrane then ground up with washed sand and distilled water (two parts mucous membrane to five parts water). After standing overnight under toluene at 0° , the fluid was filtered. The slightly opalescent filtrate was diluted ten times with water, and this clear and almost colourless fluid was used for the activation of pancreatic juice. When kept under toluene at -1° to -2° , the fluid retained its enterokinasic properties for several months.

Estimation of the proteoclastic activity of pancreatic juice. A method elaborated by Mellanby and Woolley [1912, 2], depending upon the coagulation of milk, was employed for the estimation of the proteoclastic activity of pancreatic juice. The one great merit of this method is its rapidity, which enables one to follow the course of activation through its successive stages by quick estimations at short intervals.

However, the identity of pancreatic rennin and trypsin being still in dispute, the use of the milk coagulation method as an accurate measure of tryptic activity may be questioned. In this connection it may be pointed out that Mellanby and Woolley [1912, 1] put forward the view that all proteoclastic enzymes coagulate milk in the presence of an adequate amount of calcium ions. Again, Vernon [1913], while claiming to have proved the individuality of pancreatic rennin and trypsin, suggested that the milk coagulating power might be taken as an accurate measure of the tryptic content of pancreatic juice, because the tryptic and rennetic zymogens of pancreatic juice undergo conversion into the respective enzymes at the same time and probably to the same extent. Further evidence in support of the milk coagulation method as an accurate measure of the tryptic activity of a substance, was obtained from the results of some experiments in which the rennetic and proteoclastic powers of a sample of "Trypsin" (dry, yellowish powder—British Drug Houses), were compared. Using 0.1 % and 0.2 % aqueous solutions of this sample of trypsin, their rennetic power was estimated by the milk coagulation method, while their proteoclastic activity was determined in the following manner.

To each tube, containing 15 cc. of 1 % caseinogen solution (Hammarsten's "Casein") at p_H 6.8, varying amounts of the trypsin solution were added and the volume in each case was made up to a definite mark with water. After incubation for 1 hour at 38° , the undigested caseinogen was completely precipitated with $N/10$ alcoholic acetic acid¹ and colloidal ferric hydroxide, and filtered. The clear filtrate was boiled for two minutes to destroy the enzyme, and then an aliquot portion was used for the estimation of total nitrogen, by the micro-Kjeldahl method. The results of one out of a number of similar experiments are plotted in Fig. 1, and the close resemblance between the two curves clearly indicates a corresponding relationship between the proteoclastic activity of trypsin and its rennetic properties.

¹ The amount of acid added in each case was just enough to bring the solution to p_H 4.6, which is the iso-electric point of caseinogen.

It may also be mentioned that, although Mellanby and Woolley's technique of the milk coagulation method was used during this investigation, the results were calculated on the assumption that the time of coagulation of milk (in minutes) is inversely proportional to the amount of enzyme present. (A coagulation time of one minute was taken as corresponding to 100 units of trypsin.) No attempt was made to calculate the results with reference to a "Standard curve," as advocated by Mellanby and Woolley [1912, 2], because (i) the comparative results contained in this paper correspond mainly to quick coagulation experiments, in which the simple relation of "inverse-proportionality" is known to hold good; (ii) the difference between the calculated and the "Standard curve values," even in the case of slow coagulation experiments—while it may make a slight difference in the absolute values of the results—is too small to affect the comparative significance of these experiments; (iii) the "Standard curve" obtained with certain samples of trypsin and milk cannot be accurately employed in other cases where the trypsin and milk do not exactly correspond to the original standard, or where the experimental conditions have been varied. Under such circumstances the use of a "Standard curve" was not considered to be of much practical value.

Fig. 1. Comparison between the milk-coagulating and casein-digesting powers of trypsin.

THE ACTIVATION OF PANCREATIC JUICE BY ENTEROKINASE.

The rate and extent of activation of pancreatic juice by enterokinase is greatly influenced by a number of factors which were studied during the course of the present work.

1. *Time.* It was found that the generation of trypsin was a function of the time for which enterokinase reacted with the pancreatic juice (Fig. 2). It was further observed (Fig. 2) that in the initial stages of activation the amount of trypsin liberated per unit time was rather small and later on

increased suddenly and went up to a maximum, after which there was a continual fall, due to a decrease in the amount of the substrate and to the destruction of the free trypsin. That the fall in the activation curve was due partly to a decrease in the amount of substrate, was shown by the fact that a "slowed-down" juice exhibited its original maximum activity on a further addition of inactive pancreatic juice, while the addition of more enterokinase made no difference. It was also noticed that the velocity curve for the activation of pancreatic juice (Fig. 2) was fundamentally different from a typical enzyme-action-curve.

Fig. 2. Activation of pancreatic juice by varying amounts of enterokinase at 38° C.

2. *Enterokinase*. The rate of activation of pancreatic juice was found to be accelerated by increasing the concentration of enterokinase in the mixture but, given the necessary time, the maximum amount of trypsin produced from a definite quantity of pancreatic juice was independent of the initial concentration of enterokinase in the mixture. These two facts, illustrated in Fig. 2, lead one to conclude that enterokinase enters into some sort of a temporary combination with trypsinogen, which combination is in turn broken up with the consequent liberation of trypsin and regeneration of enterokinase.

It was pointed out by Bayliss and Starling [1904, 1905] that the fact that a small amount of enterokinase can activate a very large quantity of pancreatic juice, under suitable conditions, shows that enterokinase is a typical enzyme and that Delezenne's view, regarding the entrance of enterokinase into the composition of trypsin, cannot hold.

It is also obvious that Vernon's view regarding the destructive action of enterokinase on free trypsin is not tenable, since the destruction of trypsin is not increased on increasing the amount of enterokinase in the mixture, even ten times (Fig. 2).

3. *Temperature.* At higher temperatures a marked acceleration in the activation of pancreatic juice and a corresponding increase in the destruction of free trypsin were observed (Fig. 3). It will be noticed that the maximum activity was attained in about one hour at 38° , in about six hours at room temperature, and in more than twelve hours at 3° – 5° . The very rapid destruction of free trypsin at higher temperatures is very clearly illustrated by the lower maximum recorded, and the permanent destruction of the whole of the trypsin after 23 hours at 38° (Fig. 3). It may also be mentioned that after incubation of the pancreatic juice-enterokinase mixture for 23 hours at 38° no trace of trypsinogen, enterokinase or trypsin could be detected in the fluid.

Fig. 3. Activation of pancreatic juice by enterokinase at 38°C ., $14^{\circ}\text{--}16^{\circ}\text{C}$. and $3^{\circ}\text{--}5^{\circ}\text{C}$.

From the above-mentioned considerations it is clear that the activation of pancreatic juice at a comparatively low temperature represents the actual process more exactly than a corresponding activation at a higher temperature, because in the latter case—besides the complications arising out of the simultaneous destruction of trypsin—the velocity of the reaction is too rapid to permit of a thorough study of the intermediate stages of the process. Consequently a large number of the experiments described in this paper were carried out at 3° – 5° and some at 1° .

4. *Dilution.* Vernon [1901, 1] observed the rapid destruction of trypsin on increasing the dilution of the active fluid, especially in the presence of alkalis. This phenomenon was observed very often during the course of this work (Fig. 10, Curve D), and in this connection the protective effect of certain colloids was also noticed.

5. *Spontaneous changes in the reaction of the medium during the activation of native pancreatic juice* (Fig. 4). A mixture of native pancreatic juice (p_{H} 8.8) and enterokinase, on incubation at 38° , invariably became more alkaline (in contrast to the observations of Mellanby and Woolley [1912, 2]). However,

this increase in alkalinity had nothing to do with the interaction between pancreatic juice and enterokinase, since pancreatic juice alone, under the same conditions, showed a similar rise in alkalinity (compare Curves B and D, Fig. 4) without exhibiting any tryptic activity (Curve C).

Again, a mixture of pancreatic juice (previously neutralised with HCl to p_H 5.6) and enterokinase gave the normal type of activation curve (not shown in Fig. 4) without showing any appreciable change in the alkalinity of the medium (Curve G).

These facts show that the rise in alkalinity of pancreatic juice—which is due to the decomposition of bicarbonate present in it, and the consequent expulsion of CO_2 —under the conditions mentioned above, does not alter the course of its activation, though it affects the velocity of that reaction, as is shown later on in this paper.

Fig. 4. Changes in the reaction of the medium during activation of pancreatic juice.

6. *Influence of the reaction of the medium on the activation of pancreatic juice.* According to Vernon [1913], a slight alkalinity of the medium is most favourable for the activation of pancreatic juice, while Mellanby and Woolley [1912, 2] maintain that pancreatic juice is best activated in a neutral medium.

In view of these conflicting statements, a series of experiments, extending over a wide range of H-ion concentration were carried out. The general procedure adopted was to incubate suitable mixtures of pancreatic juice and enterokinase containing varying amounts of HCl or Na_2CO_3 , and to estimate their tryptic activity at intervals, by the milk coagulation method. In each series of experiments, all conditions, excepting the p_H of the mixtures, were kept constant.

Out of a large number of experiments carried out at 38° and at 3° – 5° , a few are illustrated in Figs. 5, 6, 7 and 8, from which the following conclusions are derived:

(i) That native pancreatic juice with a p_H of about 8.8, does not, by any means, present the most favourable conditions for its own activation by enterokinase (Figs. 5, 6, 7 and 8).

Fig. 5. Effect of p_H on the activation of pancreatic juice by enterokinase at 38° C.

(ii) That the region of quick activation lies between p_H 6.0 and 7.0 (Fig. 7). (Note. It was found by a blank experiment that the amount of acid added in the above experiments did not, by itself, coagulate milk even in two hours, though a grainy appearance was imparted to the milk in about half an hour, which, however, never resulted in coagulation. So that the observed acceleration in the activation of pancreatic juice, after treatment with HCl, cannot be ascribed to the direct action of the acid on the coagulation of milk.)

Fig. 6. Effect of p_H on the activation of pancreatic juice by enterokinase at 3°-5° C.

(iii) That between p_H 7.8 and 9.8, the rate of activation is markedly low (Fig. 7).

(iv) That in the vicinity of p_H 10.0, there is another region, in which the rate of activation is comparatively high, though it always falls short of the

corresponding rate of activation in the acid region (Figs. 6 and 7), presumably owing to the destructive action of alkali on free trypsin. The significance of these two regions of high activity, is discussed later (p. 1410).

(v) That on increasing the p_H far beyond 10.0, the rate of activation again falls low (Fig. 7).

Fig. 7. Effect of p_H on the activation of pancreatic juice by enterokinase at 38° C.

(vi) That on decreasing the p_H below 5.4, the fluid develops no tryptic activity at all. (This was found to be due to the complete destruction of enterokinase and an almost entire destruction of trypsinogen under those conditions.)

(vii) That although a variation in the reaction of the medium affects the rate of activation of pancreatic juice, the maximum activity attained in each case is almost the same, except in very alkaline media, where the maximum is lower, presumably due to the quicker and greater destruction of trypsin (Fig. 8).

7. *Influence of albumin on the activation of pancreatic juice.* The addition of a small quantity of egg albumin to a mixture of pancreatic juice and enterokinase, produces a very marked retardation in the activation process—apparently not unlike the one produced by small quantities of alkali.

Some samples of pancreatic juice were activated by enterokinase at 38°, with and without small quantities of egg albumin, at an initial p_H 6.8. The milk coagulating capacity of the samples was determined at intervals and at the same time the extent of proteolysis in each sample was estimated by Sørensen's formaldehyde-titration method. The milk coagulation results of the above experiments (Fig. 9) show that while pancreatic juice alone attained its maximum activity in about three hours, in the presence of some albumin

the activity attained in that time was almost nil. It is clear, however (Fig. 9), that the extent of proteolysis in both the cases was very much the same, markedly in contrast to the widely divergent results obtained by the milk coagulation method. These observations led the author to conclude that the albumin influenced the activation process, primarily, by sorbing most of the trypsin as soon as it was elaborated, and thus preventing the latter from showing its full capacity for coagulating milk.

Fig. 8. Effect of p_H on the maximum activation of pancreatic juice by enterokinase at 38°C .

Another interesting point brought out by the above-mentioned observations is that the albumin affords a considerable measure of protection to trypsin against destruction by heat, etc. (Fig. 9).

Fig. 9. Influence of albumin on the activation of pancreatic juice at 38°C .

* $V = \text{c.c. } N/200 \text{ NaOH required for formaldehyde titration of } 0.4 \text{ c.c. of fluid.}$

Significance of the two maxima in the activation-curve. As has been stated before, a high rate of activation of pancreatic juice was observed in two distinct regions, one on either side of the neutral point. This phenomenon may either be explained by assuming the existence of two separate enzymes, each activating trypsinogen at a different p_H , or it may be ascribed to other causes which may tend to produce two such regions of high activity in the same enzyme. It is suggested that only one enzyme is responsible for both the maxima, obtained at such widely different p_H values, and that these two points correspond to those two positions where the difference between the electrical charges on the enzyme and the substrate is the maximum. The arguments for this view are derived mainly from the work of Loeb on amphoteric colloids [1918, 1922] and that of Northrop [1921, 1922, 1924] on the digestion of proteins by pepsin and trypsin.

It must be understood, however, that although two regions of maximum activity are observed under laboratory conditions, there is no suggestion that the maximum activity in the alkaline region is ever attained *in vivo* under normal conditions, for the simple reason that such a high concentration of OH^- is not maintained in the intestines. Further, in view of the fact that the optimum reaction for the activation of pancreatic juice has been found during the course of this investigation to lie between p_H 6.0 and 7.0, it is of interest to note that the reaction of the chyme is often feebly acid to litmus for a considerable distance along the intestine [Mathews, 1920].

AUTOCATALYSIS IN THE ACTIVATION OF TRYPSINOGEN.

As has been pointed out already, the velocity of activation of trypsinogen varies very widely under different conditions, though the general form of the activation curve remains unaltered. Again, the shape of the activation-velocity-curve is fundamentally different from that of a typical mono-molecular-reaction curve—a fact which aroused much interest among a number of previous investigators, who explained it in a variety of ways. Mellanby and Woolley [1912, 2], believing the activation of trypsinogen to be a true enzyme action, sought to discover those extra factors which caused the activation curve to deviate so markedly from the course of a typical enzyme-action-curve, but without success.

Having failed to discover any external cause for the peculiar shape of the activation-velocity-curve, Mellanby and Woolley [1912, 2] concluded that the curve represented the true course of the reaction, and that it was not thrown into that form by any disturbing influences.

On the basis of the above conclusion, they put forward a hypothesis to explain the observed facts. They suggested that “Enterokinase is a proteolytic ferment and trypsinogen contains a protein moiety with which the trypsin is combined, the nature of the combination being such that the proteolytic properties of trypsin are masked. The generation of trypsin from trypsinogen by enterokinase is effected by the adsorption of enterokinase by the protein

moiety of trypsinogen, digestion of this protein moiety by enterokinase and the subsequent liberation of trypsin." They further assumed that the adsorption of enterokinase by the protein moiety is a slow process, as compared to the subsequent digestion of the latter, and, therefore, owing to an increase during the process of activation in the proportion of enterokinase, relative to trypsinogen, the adsorption stage is shortened and consequently the activation is accelerated.

Vernon, in a series of papers [1901, 1, 2, 1902, 1913], put forward the view that the activation of pancreatic juice was initiated in the intestines by enterokinase, but it was completed almost entirely by the trypsin produced during the process. Later, Vernon [1914] modified his views and suggested that the trypsin liberated from trypsinogen by enterokinase gradually produces an enzyme—"deuterase"—from a precursor which is present in pancreatic juice, and that this deuterase is mainly responsible for the later stages of the activation process.

In connection with the view at that time held by Vernon regarding the activation of trypsinogen by trypsin, Bayliss and Starling [1904] state that "Although such a process would not be without analogy in other catalytic processes known to chemists, it would be quite a unique case among ferments that a product of a reaction should itself be the catalyser of the reaction. Such a reaction would necessarily proceed with an ever increasing velocity." Bayliss and Starling [1904] tried and failed to activate pancreatic juice with Grubler's powdered "Trypsin" or with a sample of "active" pancreatic extract. Thus they concluded that trypsin had no power of activating inactive pancreatic juice and that the slight activation observed in these cases was due to the small quantity of enterokinase present in the fluid.

As has been mentioned before (p. 1406, Fig. 4), a marked increase in the alkalinity of the juice occurs during the process of activation, so that the question of the acceleration of activation towards the end, due to a partial neutralisation of the alkalinity, does not arise at all. Again, it has been shown in this paper (p. 1408) that the proteins present in pancreatic juice do influence the normal course of activation by adsorbing the freshly produced trypsin and also by protecting the trypsinogen and enterokinase molecules from mutual attacks. However, as is shown in Fig. 9, there is an initial period of slow reaction, even as determined by the Sørensen method, which suggests that the influence of the proteins of pancreatic juice is not wholly responsible for the observed deviation of the activation curve from the path of a typical mono-molecular-reaction curve. Evidently, then, there is yet another factor involved which causes a slow production of trypsin in the earlier stages of activation, and which also accounts for the initial slow rate of proteolysis of the proteins of pancreatic juice (Fig. 9).

The above considerations, coupled with the undisputed enzyme-nature of enterokinase, and supported by the further experimental evidence cited below, have led the author to conclude that the activation of trypsinogen is essentially

an autocatalytic process, and that the activation-velocity-curve represents the actual reaction faithfully and is not a deviated form of a mono-molecular-reaction curve.

In order to get a direct proof of the auto-catalytic nature of the activation of trypsinogen, it was decided to observe if trypsin itself could induce such activation in inactive pancreatic juice. With this object in view, some experiments were conducted, with suitable controls, at 3°–5°. When a mixture of inactive pancreatic juice (2.0 cc.), enterokinase (0.2 cc. of 1 : 10 solution) and water (0.8 cc.) had attained its maximum activity on incubating at 3°–5° for four days, and was fairly steady in its tryptic content, it was used as the source of trypsin (i) in these experiments. The following combinations were kept under toluene, at 3°–5°, and their tryptic activity was determined at suitable intervals:

Serial no.	Pancreatic juice cc.	Other additions	Water cc.
(ii)	1.0	0.3 cc. of (i)	0.2
(iii)	1.0	(i) (Heated for 10 mins. at 60° and cooled) 0.3 cc.	0.2
(iv)	nil	0.3 cc. of (i)	1.2
(v)	1.0	Enterokinase (1 : 100) 0.2 cc. ¹	0.3
(vi)	1.0	Enterokinase (1:100) (Heated for 10 mins. at 60° and cooled) 0.2 cc.	0.3

¹ This is equivalent to the amount of enterokinase present in (ii).

From the results of Exp. (ii) (Curve B, Fig. 10), it is clear that "activated-pancreatic juice" (i) has a marked capacity for activating inactive pancreatic juice. This activating power of "activated-pancreatic juice" (i) may be due either to the trypsin or to the small amount of enterokinase present in it. That the latter factor is not involved is seen very clearly from a comparison of Exps. (ii) and (v) (see Curves B and E, Fig. 10). The fundamental difference between Curves B and E indicates that the activating agent in (ii) is a factor more potent than a corresponding quantity of enterokinase. Again, the great rise in Curve B cannot be ascribed to an increase in the activity of the inoculated fluid (i) itself, since the corresponding control experiments (i) and (iv) (see Curves A and D) show practically no rise at all. Further, the effect (on milk coagulation) of the trypsin present initially in the inoculated fluid, is shown in Curve B by the high position of the curve at the point of origin, and therefore the very rapid rise in Curve B, after 24 hours' incubation, cannot be ascribed to the direct influence of the trypsin present in the inoculated fluid. From the above-mentioned considerations it is concluded that "activated-pancreatic juice" possesses the power of activating further quantities of inactive pancreatic juice, mainly by virtue of its trypsin-content. In other words auto-catalysis during the activation of trypsinogen is a very prominent feature of the process.

It is suggested that the failure of some of the previous investigators [Bayliss and Starling, 1904; Mellanby and Woolley, 1912, 2] to discriminate

between the activation of pancreatic juice by "activated-pancreatic juice" and by enterokinase, may have been due to the comparatively high temperatures employed by them, in which case, the intermediate stages of the two reactions would be difficult to observe, owing to the high velocity of the reactions and the very rapid destruction of trypsin at those temperatures.

Delezenne and Ledebt [1922] observed that "Inactive pancreatic-juice to which a suitable quantity of succus entericus has been added, becomes activated at 0°, and the quantity of trypsin which appears is even greater than at 39°, but the time necessary for the process is very much longer. Further, when maximum activity is attained, it is possible by adding some of this juice, to activate a fresh specimen, and at 0° this activation can be transmitted in

Fig. 10. Activation of trypsinogen by "active trypsin" at 3°-5° C.

series, from juice to juice, indefinitely." In view of the important bearing of Delezenne and Ledebt's observations on the theory of the auto-catalytic activation of trypsinogen, some experiments were carried out by the author at 1°, in which the activating power of fully "activated-pancreatic juice" (p_H 8.8) was successfully transmitted in series to fresh specimens of native pancreatic juice (p_H 8.8) (Fig. 11). At the same time a number of control experiments were conducted at 1°, in which the activating-agent employed was enterokinase in quantities exactly similar to those existing in a certain volume of the "activated-juice" which was used in a corresponding experiment. The results of the above experiments (Fig. 11, Curves B and B', C and C') show with an unmistakable precision that the activation of pancreatic juice by "activated-juice" cannot be said to be due entirely or even principally—as suggested by Bayliss and Starling [1904] and Mellanby and Woolley

[1912, 2]—to the activating power possessed by the small amount of enterokinase in it. “Activated-pancreatic juice” has been shown to contain a substance, viz. trypsin, which is a more potent activator of trypsinogen than a corresponding amount of enterokinase, and which is therefore, presumably, the principal activator of trypsinogen *in vivo*.

Fig. 11. Activation of trypsinogen by “activated-pancreatic juice” and by enterokinase, at 1° C.

It was noticed, however, that the marked alkalinity of native pancreatic juice (p_H 8.8) had a destructive effect on trypsin, so that the activating power of the “activated-juice” was appreciably reduced at the third stage in the series (Fig. 11). Later on, by using partially neutralised pancreatic juice for the purpose, the transmission of the activating power of “activated-juice” could be carried almost undiminished over a much longer series (Fig. 12). However, it must be mentioned that it is not possible to transmit in series the activating power of “activated-juice” indefinitely, as stated by Delezenne and Ledebt [1922], unless means are devised to prevent the slightest destruction of trypsin during the whole course of the experiment. Delezenne and Ledebt’s statement, while it is theoretically sound, appears to be difficult to realise in practice on account of the extreme instability of trypsin to a large number of influences like temperature, dilution and variations in the reaction of the medium, etc.

It is a very significant fact to note that, during the transmission in series of the activating power of “activated-pancreatic juice” (Fig. 12), the amount

of enterokinase was considerably reduced at each successive stage, till it might be supposed, for all practical purposes, to have been completely eliminated from the activation system. Thus the activation—particularly in the latter part of the series—may be taken as being due entirely to the activating power

Fig. 12. Activation of trypsinogen in series by "activated-pancreatic juice."

of trypsin. These observations demonstrate that trypsin, under suitable conditions, is capable of activating trypsinogen much faster than, and quite independently of, enterokinase. These facts leave very little doubt about the activation of trypsinogen being mainly an auto-catalytic process, and enterokinase being chiefly an initiator of the reaction.

CONCLUSIONS.

From the evidence brought forward in this paper the following general conclusions have been drawn:

(i) That trypsin, as it exists in inactive pancreatic juice, is associated with a protective substance of a colloidal nature, the combination being of such a type as to mask the characteristic properties of trypsin, viz. proteoclastic power, extreme instability to heat, acids and alkalis.

(ii) That enterokinase is a specialised proteoclastic enzyme with a range of activity limited to react with that particular type of grouping only which exists in trypsinogen between a trypsin molecule and its associated protective colloidal covering.

(iii) That enterokinase acts primarily as a stimulus to the activation of trypsinogen, inasmuch as it initiates the reaction, but takes no important part in the later stages of the process.

(iv) That after the initiation of activation by enterokinase, the process is carried to completion, almost entirely by the trypsin produced during the reaction.

(v) That the functions of enterokinase and trypsin—as far as the activation of trypsinogen is concerned—are essentially similar. Both of them activate trypsinogen by digesting the protective part in the trypsinogen molecule, and thus liberating trypsin, which in turn attacks a further quantity of trypsinogen and produces more trypsin. This process, under favourable conditions, continues till the substrate is completely activated.

(vi) That the activation of trypsinogen is essentially an auto-catalytic process, except in the initial stages of the interaction between trypsinogen and enterokinase, where it may presumably be a true enzyme action of the mono-molecular type.

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