ON THE RELATION OF ENTEROKINASE TO TRYPSIN.

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At the present time two views are held by physiologists as to the relation of enterokinase to trypsin. According to Pawlow enterokinase is a ferment, and acts on the trypsinogen in the pancreatic juice, converting this into a third substance, trypsin, the active proteolytic ferment of the juice in the intestine. Délézenne and Dastre, on the other hand, regard the nature of the interaction between enterokinase and trypsinogen as analogous to that which exists between the antibody or "amboceptor" and the complement, the action of the enterokinase being to link the trypsinogen on to the proteid molecule. According to this view therefore there is no such body as trypsin, every so-called solution of trypsin consisting of a mixture or combination of the two bodies enterokinase and trypsinogen. In this mixture the trypsinogen would correspond to a "complement," or amboceptor, while the enterokinase would be analogous to an "immune body."

In a previous paper[1] we brought forward evidence, founded mainly on the time relations of the action of enterokinase on trypsinogen, in favour of Pawlow's view, and evidence in the same direction has also been furnished by the experiments of Hamburger and Hekma[2]. In that paper we pointed out that there was no definite quantitative relationship between the amount of enterokinase and the amount of trypsinogen it was able to activate, *minimal* amounts of the enterokinase being able to activate any quantity of inactive pancreatic juice if only sufficient time were allowed for the reaction.

We showed, moreover, that the activating power of an activated pancreatic juice on further portions of juice was directly proportional to the amount of enterokinase it originally contained. We found, namely, that if a juice were activated by the addition of $\frac{1}{10}$ its bulk of enterokinase solution, $\frac{1}{100}$ part of the mixture would have the same

[1] This *Journal*, xxx. p. 61. 1904.

[2] *Arch. f. Physiol.* 1904, p. 343.

activating effect on a fresh portion of pancreatic juice as would be possessed by $\frac{1}{10}$ part of the enterokinase originally added to the first solution. This fact shows that enterokinase is not destroyed in the process of activation of pancreatic juice, and that it is not so firmly bound to the trypsinogen as to be unable to activate a fresh portion of trypsinogen. It seemed to us therefore that a further experimental test might be applied in order to decide between the two opposing views. In the paper above quoted we mentioned the difficulty we had of judging of the continued presence of enterokinase or trypsinogen as distinguished from trypsin in a solution. The only point of difference between trypsin and trypsinogen is their reaction to temperature, trypsin being destroyed at a lower temperature than trypsinogen. The difference, however, is not sufficiently great to enable us to draw any very definite conclusions as to the nature of the substances contained in an activated juice. In the absence of a chemical or physical test which might enable us to identify any of these three bodies in solution we have had recourse to the power possessed by the body of distinguishing minutely between different substances of the nature of proteids or ferments, namely, its power of producing an antibody of any substance belonging to these classes which is injected subcutaneously or into the peritoneal cavity.

It is well known that normal serum possesses an antitryptic quality, which not only preserves the serum from digestion by an active pancreatic juice, but also hinders the action of trypsin when added to a mixture containing trypsin and a digestible proteid. The antitrypsin in this case has been shown by Cathcart[1] to be attached to the albumen fraction of the proteids of the serum. We may therefore say that normal serum contains an antitrypsin. On the theory of Dastre and Délézenne, however, there being no such substance as trypsin, no such substance as antitrypsin can exist, and the so-called antitrypsin of serum must be either antikinase, antitrypsinogen, or a mixture of both substances. Délézenne adopts the view that the antitrypsin of serum is really an antikinase, and it was with a view to testing this hypothesis that this present series of experiments was carried out. If the antitrypsin of serum is really antikinase we should expect by injecting enterokinase into an animal to increase the antikinasic value of its serum and therefore its antitryptic power. If, on the other hand, as we suppose, enterokinase is not a necessary

[1] This *Journal*, xxxi. p. 497. 1904.

constituent of trypsin but has finished its work with the production of trypsin from trypsinogen, an antiserum produced by the injection of enterokinase would have no special inhibiting effect on the action of trypsin, but would only be effective if added to a mixture of trypsinogen and enterokinase before the latter has had time to act upon the trypsinogen. That is to say, on the Pawlow view we may have both antitrypsin and antikinase, whereas on Délézenne's view the two substances must be identical and any increase of antikinasic power must involve a corresponding increase in the antitryptic power.

Experimental methods. For the preparation of an antikinasic serum 10 or 12 c.c. of a strong solution of enterokinase, made as we have already described[1], and sterilized by filtration through a Berkefeld filter, were injected subcutaneously into a rabbit. A week later the rabbit received a second dose, and three days later a third dose of the same volume. A week after the last injection the animal was killed by bleeding into a sterile flask and the serum allowed to separate. At the same time a normal rabbit was killed and its serum collected to serve as a control.

With these two sera three sets of experiments were made. In the first set the enterokinase was added to the pancreatic juice, allowed to stand for half an hour for the conversion of trypsinogen into trypsin to take place, and the serum was then added. In the second set the serum was added to a solution of enterokinase, allowed to stand for half an hour, and the two then added to a given volume of fresh pancreatic juice. In the third set of experiments the actions of the two sera were compared on the digestive powers of a solution of Grübler's trypsin. In each series a control experiment was also made, using normal salt solution instead of serum. The results of these experiments are given below.

June 23, 1904. Pancreatic juice obtained from a dog by injection of secretin, and mixed with an equal volume of 2% NaF. This mixture is spoken of throughout the experiments as pancreatic juice or simply 'juice.' The following mixtures were made:

A_1. 3 c.c. juice + 0·1 c.c. enterokinase, 30 mins. at 38° C., then added 1 c.c. *normal* serum.

A_2. Same mixture, 30 mins. at 38° C., then added 1 c.c. *antikinasic* serum.

A_3. Same mixture, 30 mins. at 38° C., then added 1 c.c. *normal saline* solution.

B_1. 0·1 c.c. enterokinase + 1 c.c. *normal serum*, 30 mins. at 38° C., then added 3 c.c. juice.

B_2. 0·1 c.c. enterokinase + 1 c.c. *antikinasic* serum, 30 mins. at 38° C., then added 3 c.c. juice.

B_3. 0·1 c.c. enterokinase + 1 c.c. saline, 30 mins. at 38° C., then added 3 c.c. juice.

[1] This *Journal*, xxx. p. 80. 1904.

C_1.	5 c.c. 1 % Grübler's	trypsin	+ 1 c.c. *normal* serum.
C_2.	,,	,,	,,	+ 1 c.c. *anti*serum.
C_3.	,,	,,	,,	+ 3 c.c. *normal* serum.
C_4.	,,	,,	,,	+ 3 c.c. antiserum.

All the mixtures were cooled to room temperature, gelatin tubes (18 to 20 mm. long) were inserted and left at room temperature (about 18° C.) for 24 hrs.

The amount of digestion, in millimetres, at the end of this time was as follows:

Serum + Activated juice		Serum + Enterokinase		Serum + Trypsin	
A_1.	All (more than 9)	B_1.	7·5	C_1.	8
A_2.	7·5	B_2.	0	C_2.	8
A_3.	All	B_3.	All	C_3.	1
				C_4.	0

It will be seen from these experiments that whereas both sera have a slight, and almost equal, inhibitory power on the action both of activated pancreatic juice, and of trypsin on the gelatin tubes, there is a marked difference in their effects on the enterokinase. When added to the latter body, before the mixture is added to the pancreatic juice, the antikinase serum diminishes or stops altogether the activating power of the enterokinase, whereas the normal serum has very little effect.

This result is perfectly natural on the hypothesis that enterokinase acts as a ferment converting trypsinogen into trypsin. It is evident that if the antiferment be added to the mixture after the conversion is far advanced, it will not inhibit the digestive powers of the product of the reaction, namely, trypsin. If, on the other hand, the digestive power of trypsin is due simply to the coexistence in it and the co-operation of the two bodies enterokinase and trypsinogen, any increase in the antikinasic power of the serum should be accompanied with a corresponding increase in its antitryptic power, and it should be a matter of indifference to the result whether the antiserum be added to the enterokinase first, or to the mixture of enterokinase and pancreatic juice. It has been objected to our experiments that we might conceivably imagine that the antikinase serum, though active in neutralising enterokinase when added to it alone, would be ineffectual when added to a solution containing enterokinase in combination with trypsinogen. That this objection is groundless is shown by the fact mentioned above, viz. that enterokinase can be still detected in an activated pancreatic juice, by its power of activating fresh portions of juice. Moreover, normal serum is acknowledged to possess an anti-digestive power on trypsin or on activated juice. This power is ascribed by Délézenne to the existence in the serum of antikinase.

Any increase in the antikinase present, such as is attained by the injection of enterokinase, ought therefore to increase the antitryptic power of the serum; such however is not the case. There seems to be entire independence between the antikinasic and antitryptic powers of any given serum.

The fact that normal serum contains an antitrypsin does not exclude the possibility of its containing also an antikinase. Since we must ascribe the formation of antitrypsin in serum to the slight entry of trypsin from the alimentary canal into the circulating blood, it would be only natural to imagine that there would be a similar slight leakage of enterokinase from the intestinal cavity, and any such leakage into the blood-stream would cause the formation of antikinase in the serum. The following observations show that normal serum may at times possess antikinasic in addition to antitryptic qualities. Antikinase is not, however, a constant constituent of the blood serum, and in many of our experiments normal serum had no more influence on enterokinase than a corresponding quantity of normal salt solution.

The following mixtures were made. (The enterokinase solution was rather weaker than in the first experiment.)

A. 3 c.c. pancreatic juice + 1 c.c. enterokinase allowed to stand at 20° C. for 1 hour, then added 2 c.c. normal serum.

B. 1 c.c. enterokinase + 2 c.c. normal serum, 1 hour at 20° C., then added 3 c.c. pancreatic juice.

C. 3 c.c. of 2 % solution of trypsin (Grübler's) + 2 c.c. normal serum.

D. 3 c.c. of 2 % solution of trypsin + 2 c.c. normal saline.

Gelatin tubes added to all and left overnight.

The next morning the following amounts were digested:

A. All (more than 11). B. 1 mm. C. All. D. All.

It occurred to us as a possibility that the antitryptic power of serum might be explained as due to a coexistence in this fluid of two antibodies, namely, antikinase and antitrypsinogen, and that the failure to affect fully formed trypsin by the addition of antikinase might be determined by the fact that we were only adding an antibody to one-half the molecule. Such a view would introduce a somewhat new phenomenon, and would be difficult to picture according to the prevailing ideas on the interaction of immune bodies and complements with their antibodies. To test this view we made a series of experiments on three rabbits, injecting one with enterokinase, the second with inactive pancreatic juice containing trypsinogen, and the third with solutions of Merck's trypsin. Three injections were given of each substance, as in the experiment related earlier in the paper. A week after the last injection all three animals were killed, together with

a control rabbit, and their blood sera collected. A series of experiments conducted with these sera is given below.

Series A. ? Antibody to "trypsin."

(1) Juice 3 c.c. + enterokinase 1 c.c., 30 mins. at 30° C., then + 2 c.c. normal serum.
(2) ,, ,, ,, ,, 2 c.c. "antikinase" serum.
(3) ,, ,, ,, ,, 2 c.c. "antitryptic" serum.
(4) ,, ,, ,, ,, 2 c.c. "antitrypsinogen" serum.
(5) ,, ,, ,, ,, 2 c.c. normal saline.

Gelatin tubes added to all.

Next morning amounts of digestion were as follows:

A_1. All. A_2. 8 mm. A_3. All. A_4. 5·5. A_5. All.

In this case the serum from the rabbit treated by injections of inactive juice had the strongest retarding effect on the digestion. The injections of the juice had in contradistinction to the other injections excited a certain amount of local necrosis, and it seemed possible that the juice had slowly undergone activation in the tissues and had really produced an antitryptic serum. This view received some confirmation by the comparison of the retarding action of the various sera on the digestive power of solutions of Grübler's trypsin. The two following series of experiments show that the "antitrypsinogen" serum had no special neutralising or 'anti' effect either on enterokinase or on trypsinogen.

Series B. ? Antibody to enterokinase.

(1) Enterokinase 2 c.c. + 2 c.c. normal serum.
(2) ,, ,, + 2 c.c. "antikinase" serum.
(3) ,, ,, + 2 c.c. "antitryptic" serum.
(4) ,, ,, + 2 c.c. "antitrypsinogen" serum.
(5) ,, ,, + 2 c.c. normal saline.

All warmed for 10 mins. to 40° C. and then 3 c.c. pancreatic juice and gelatin tubes added to each.

Digestion on following day:

(1) All (more than 14). (2) 6. (3) All. (4) All. (5) All digested.

Series C. ? Antibody to trypsinogen.

(1) Pancreatic juice 3 c.c. + 2 c.c. normal serum.
(2) ,, ,, + 2 c.c. "antikinase" serum.
(3) ,, ,, + 2 c.c. "antitryptic" serum.
(4) ,, ,, + 2 c.c. "antitrypsinogen" serum.
(5) ,, ,, + 2 c.c. normal saline.

All warmed to 40° C. for 10 mins., then cooled and 1 c.c. enterokinase and gelatin tubes added to each.

Digestion 20 hours later:

(1) All (more than 11). (2) 4·5 mm. (3) All. (4) 10. (5) All.

Series D. ? Antibody to "trypsin" (Grübler's).

(1) 3 c.c. of 2 % trypsin (in 1 % NaF) + 2 c.c. normal serum.
(2) ,, ,, ,, + 2 c.c. "antikinase" serum.
(3) ,, ,, ,, + 2 c.c. "antitryptic" serum.
(4) ,, ,, ,, + 2 c.c. "antitrypsinogen" serum.
(5) ,, ,, ,, + 2 c.c. normal saline.

Gelatin tubes added to each.

Digestion 20 hours later:

(1) 8. (2) 6. (3) 8. (4) 6·5. (5) All (more than 10 mm.).

It will be seen that whereas in this series the antikinasic serum acted as in the first experiment described, we entirely failed to procure any antibody to trypsinogen. It will be noticed, moreover, that the serum from the animal treated with injections of trypsin was very little, if at all, superior in its antitryptic qualities to normal serum; and we came to the conclusion that our injections were too small, in comparison with the normal absorption of trypsin from the intestine, to have had any appreciable result on the animal. The failure to produce an anti-trypsinogen by the injection of pancreatic juice in large quantities, shows that antitrypsin cannot be regarded as consisting of a mixture of antienterokinase and antitrypsinogen.

These results therefore tend only to confirm the view put forward by Pawlow and sustained by us, namely, that trypsin is not a mixture of two substances, but is a specific single substance formed by the action of a ferment, enterokinase, on a precursor or prozymin, trypsinogen.

In a recent paper by Dastre and Stassano[1] these observers have collected together a number of experiments, some of which they had previously published, in favour of the view that trypsin is merely a mixture (or rather combination) of trypsinogen and enterokinase. In this paper they study the effects of different proportions of enterokinase on trypsinogen, and point out that there is an optimum amount of enterokinase which must be added to a given quantity of trypsinogen in order to evoke its full action, and, representing their results in the form of curves, speak of the "plateau" which represents this optimum amount. It is remarkable that these observers, in trying to come to a conclusion as to the reputed ferment-like action of enterokinase, take no account at all of the time-relations of the activation. It is evident that when enterokinase is added to trypsinogen and produces a substance, trypsin, which rapidly undergoes auto-destruction, the optimum amount of trypsinogen will be that which converts, within a few minutes, the whole of the trypsinogen into trypsin. Anything below this amount will convert, as we showed, the trypsinogen more gradually, so that there is time for the ready formed trypsin to be destroyed, while fresh amounts of trypsin are being formed from trypsinogen. One might indeed prophesy from the Pawlow theory that such curves as those given by Dastre and Stassano would be obtained. On the other hand they distinctly state that they took no account of the small initial variations in the development of tryptic power, variations which are of all importance in deciding the question at issue. One set of experiments

[1] *Archives internationales de Physiologie*, I. p. 86. 1904.

which they quote without attempting to give an explanation furnishes in fact strong support to the Pawlow view. They show, namely, that dilution of the whole mixture shifts the plateau upwards, a result we should expect from the dilution of the ferment enterokinase, and the consequent retardation of its action. If before the dilution $\frac{1}{10}$th of a c.c. was sufficient to rapidly activate 10 c.c. of pancreatic juice, it is evident that after dilution some of the trypsin formed would have time to be destroyed before the rest of the trypsinogen was converted, and more enterokinase would therefore have to be added before the optimum activity of the activated juice was attained.

These observers also investigated the antitryptic power of intestinal worms, which was first described by Weinland, and attributed by him to the presence of an antitrypsin. Dastre and Stassano give experiments to show that the extract of the worms contains not antitrypsin but antikinase, and some of their experiments certainly present similarities to the observations recorded in this paper on antikinase serum. The results, however, on intestinal worms do not affect the main question at issue, namely, as to the nature of the interaction between enterokinase and trypsinogen, and we propose therefore to deal with the question of the protection of intestinal worms against the digestive action of the intestinal juices in a subsequent paper.

Summary of results.

1. Normal rabbit's serum, besides its known antitryptic qualities, may sometimes possess the power of neutralising or destroying enterokinase.

2. This power when absent may be always evoked by the repeated injection of solutions of enterokinase either subcutaneously or intraperitoneally.

3. The production of an "antikinase" in the serum does not increase the antitryptic powers of the serum.

4. Injections of trypsinogen subcutaneously do not give rise to the production of any "antitrypsinogen" in the blood serum.

5. The antitryptic qualities of normal serum are therefore not due, as stated by Délézenne, to the presence of antikinase.

6. There is no evidence that a solution of trypsin is equivalent to a combination of kinase and trypsinogen. Trypsin is a new substance, differing from trypsinogen, and produced from the latter by the ferment-like action of enterokinase. There is no evidence that the enterokinase is essential to or takes any part in the proteolytic activities of trypsin.