XI. ON THE RESISTANCE OF TRYPSIN SOLUTIONS TO HEAT.

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The action of heat on aqueous solutions of many enzymes has been studied more or less carefully for many years, and the general conclusion arrived at has been that all enzymes in aqueous solution are destroyed when heated for a short time to about 70° or 75°. So much is this the case that frequently the activity of a substance after its solution has been heated has been taken as proving that the substance in question is not an enzyme (e.g. secretin).

Since trypsin is practically without any digestive action in acid or neutral solution, the effect of heat on this enzyme was at first tested principally in alkaline solution, and it was found that such a solution rapidly became inactive at as low a temperature as 50° or sometimes even at 45° [Biernacki, 1891]. Vernon [1901] also found that fresh preparations of trypsin lost more than half their activity when kept in 0·4 % sodium carbonate at 37° for an hour. Similar results have been found by Vernon in later experiments, and by other observers.

On the other hand Vernon [1904] found that the presence of protein protected trypsin solutions from the effect of heat to a considerable extent, and the same protection was afforded by proteoses or peptone. Bayliss and Starling [1903] had previously noticed the protective effect of proteins or their hydrolytic products on solutions of trypsin.

The action of acids on trypsin, however, has not been so fully studied. Langley [1881] found that trypsin was considerably weakened by warming its solution for 2½ hours with 0·05 % hydrochloric acid. Wróblewski, Bednarski and Wojczynski [1901] found that trypsin when kept at 37° for a few hours in hydrochloric acid of over 0·14 % was considerably affected, and if 0·56 % acid were used the enzyme was sometimes completely destroyed.

The question of the effect of heat on trypsin was investigated later by

Schmidt [1910], who stated that trypsin in a slightly alkaline solution containing 5 % of peptone could be boiled without being destroyed. The same protection against heat was afforded by a 2 % solution of agar or a 10 % solution of gelatin. Schmidt also stated that if trypsin powder were suspended in water-free glycerol it could be heated to 292° without being destroyed or much affected.

Schmidt's work was repeated by de Souza [1911], who however found that 5 % peptone had hardly any effect in protecting trypsin solutions from destruction by heat. It appears from these experiments that an appreciable protection is afforded by 20 % peptone under certain conditions, but if the heating is sufficient to cause complete destruction of the trypsin in pure aqueous solution, then the presence of peptone has only a slight protective effect. De Souza also tried the effect of heat on trypsin in presence of 20 % peptone in acid, neutral and alkaline solutions. The solutions were heated to 80° for five minutes. No difference was observed between the acid and neutral solutions, the activity of these after heating being however slightly greater than that of the alkaline solution. Even in the case of the acid or neutral solution, however, over 85 % of the trypsin was destroyed, and about 90 % in the case of the alkaline solution.

Ohta [1912] also repeated the experiments of Schmidt, but failed to confirm his results.

In a paper just published, Mellanby and Woolley [1913] find that while trypsin is readily destroyed by heat in neutral or alkaline solution, if a solution of trypsin be made slightly acid, say with hydrochloric acid, it can be boiled for five minutes and yet retain considerable digestive power.

According to these observers, trypsin is destroyed in acid, more rapidly than in alkaline or neutral solution up to about 40°, but at higher temperatures the reverse is the case. At 40° there appears to be an optimum protective concentration of acid, above or below which the rate of trypsin destruction is accelerated.

Before the publication of the work of Mellanby and Woolley, and unaware of their experiments, I had tested the effect of heat on trypsin in connection with another research, and obtained results of a similar character. The method of testing the digestive power of the trypsin solutions was that of Hedin [1903]. The trypsin was allowed to act on a solution of caseinogen in presence of toluene and after a definite interval excess of tannic acid was added, to precipitate unaltered protein, meta-protein and proteoses. After standing 12 hours or more the precipitate was filtered off and the nitrogen

determined in a portion of the filtrate by Kjeldahl's method. Controls were also carried out to show the effect, if any, of the alkali used on caseinogen, and the amount of nitrogen not precipitated by tannic acid was also determined in each solution of trypsin used.

The following are the principal results obtained:—

1. Benger's Liquor Pancreaticus used as trypsin solution, 10 cc. of this requiring 1·6 cc. of N/10 sodium hydrate for neutralisation. 2 % caseinogen in normal sodium carbonate was the substrate. A portion of the trypsin was boiled for three minutes and cooled before adding the caseinogen. Digestion was continued at 37° for three hours.

	Digestion in cc. of N/10 nitrogen not ppted by tannic acid
(*a*) 1 cc. trypsin, 20 cc. water, 40 cc. caseinogen	49·8
(*b*) 1 ,, (boiled) ,, ,,	29·7

In this experiment the effect of boiling trypsin in slightly acid solution was to leave 60 % of the original digestive power unimpaired.

2. 10 cc. of the above trypsin solution were neutralised with sodium carbonate and made up to 25 cc. with water. Three portions (*a*, *b* and *c*) were boiled for three minutes in neutral, alkaline and acid solution respectively, cooled, and kept at 37° with 20 cc. of 2 % caseinogen in 2 N sodium carbonate for three hours.

	Digestion in cc. of N/10 nitrogen not ppted by tannic acid
(*a*) 2·5 cc. trypsin, 20 cc. water...	0·2
(*b*) 2·5 cc. trypsin, 19 cc. water, 1 cc. N Na_2CO_3 ...	0·1
(*c*) 2·5 cc. trypsin, 19 cc. water, 1 cc. N HCl ...	20·8
(*d*) 2·5 unboiled trypsin, 20 cc. water	20·9

It may here be mentioned that in all the experiments carried out, any differences in reaction due to the trypsin being boiled in acid etc. were adjusted before the caseinogen was added. Special care was taken also to ensure that none of the trypsin escaped being heated to 100°.

In the above experiment it will be seen that after being boiled in acid solution for three minutes, the trypsin still retained all its power of digesting caseinogen, while boiling in alkaline or neutral solution had completely destroyed the enzyme.

The digestive power of this trypsin before and after being boiled as above was also tested on boiled ox fibrin, the amount of nitrogen in the filtrate from the undissolved fibrin at the end of the digestion being taken as the measure of the action of the enzyme. It was found that on such fibrin trypsin acts

only slowly, producing much less effect in a given time than when acting on caseinogen. Nevertheless the trypsin boiled in acid dissolved as much fibrin as the unboiled trypsin, while that boiled in neutral or alkaline solution again had no digestive power.

3. Merck's trypsin used. A weak solution of this trypsin was dialysed against running water for 18 hours and filtered. The solution was neutral. The trypsin contained 0·02 % nitrogen. Three portions were boiled for three minutes (*a*, *b* and *c*) and then allowed to act on 20 cc. of 2 % caseinogen in 0·4 N Na_2CO_3 at 37° for three hours.

	Digestion in cc. of N/10 nitrogen not ppted by tannic acid
(*a*) 25 cc. trypsin, 1 cc. N Na_2CO_3	0
(*b*) 25 cc. trypsin, 1 cc. water	0·2
(*c*) 25 cc. trypsin, 1 cc. N HCl	21·4
(*d*) 25 cc. unboiled trypsin	28·9

In this experiment 75 % of the original digestive power remains after boiling the trypsin in acid solution, but the trypsin is destroyed in neutral or alkaline solution.

For the rest of the experiments the trypsin used was prepared in the method described by Hedin [1905]. An ox pancreas was minced and allowed to undergo autolysis at 37° in presence of water and toluene for a day and filtered. The filtrate was again kept at 37° for two days, dialysed against running water for two days, filtered, and kept with a little toluene.

This trypsin solution was neutral, contained less than 0·01 % nitrogen, and gave practically no biuret reaction.

4. Three portions of this trypsin were boiled for three minutes, and cooled before adding 20 cc. of caseinogen (same as in last experiment). Digestion lasted three hours.

	Digestion in cc. of N/10 nitrogen
(*a*) 25 cc. trypsin, 0·5 cc. N/10 Na_2CO_3	0·2
(*b*) 25 cc. trypsin, 0·5 cc. water...	0·2
(*c*) 25 cc. trypsin, 0·5 cc. N HCl	6·5
(*d*) 25 cc. unboiled trypsin	10·5
(*e*) Control (water + caseinogen)	0

In this experiment we see that over 60 % of the original digestive power of the trypsin survives after the acid solution has been boiled, but none in the case of the neutral or alkaline solutions, the 0·2 cc. being within the limits of experimental error.

5. In order to see to what extent this trypsin would survive more prolonged heating, 25 cc. of the solution together with 5 cc. of N/10 HCl

were brought to boiling in a flask and then put in a steriliser for 20 minutes. During the whole of this time the temperature throughout the interior of the steriliser was 100°. The contents of the flask were then cooled and neutralised.

To a fresh portion of 25 cc. trypsin 5 cc. of N/10 HCl were added, and immediately neutralised. Then to both flasks were added 20 cc. of the usual caseinogen solution.

25 cc. of water were treated in exactly the same way as the fresh portion of trypsin, and the flasks were kept at 37° for 4·25 hours.

		Digestion in cc. of N/10 nitrogen
(*a*)	Boiled trypsin	4·2
(*b*)	Fresh trypsin	16·7
(*c*)	Control	0

The amount of nitrogen not precipitated by tannic acid, which was contained in the 25 cc. trypsin used, was also estimated, and allowed for in the above results. It corresponded only to 1 cc. of N/10 nitrogen.

From this experiment it appears that under suitable conditions a solution of trypsin can be heated to 100° for 20 minutes and yet retain 25 % of its original digestive power.

6. I have repeated one of the experiments described by Mellanby and Woolley to test the effect of varying concentrations of acid on trypsin. My experiment was carried out at 45°, at which temperature the acid solutions were kept for 15 minutes. 20 cc. of the usual caseinogen solution were added and digestion continued at 37° for three hours.

		Digestion in cc. of N/10 nitrogen
(*a*)	25 cc. trypsin, 0·2 cc. N HCl	8·0
(*b*)	25 cc. ,, 0·4 ,, ...	8·9
(*c*)	25 cc. ,, 0·6 ,, ...	8·9
(*d*)	25 cc. ,, 0·8 ,, ...	9·3
(*e*)	25 cc. ,, 1·0 ,, ...	8·9
(*f*)	25 cc. unboiled trypsin	9·8

In the above series the trypsin seems to be the least protected by the weakest acid (0·008 N), each of the other concentrations of acid having much the same effect. In all cases at least 80 % of the original digestive power remains after heating.

SUMMARY.

Solutions of trypsin when neutral or alkaline are rendered completely inactive by boiling.

Acid solutions of trypsin, on the other hand, after being boiled retain a considerable power to digest caseinogen. In some cases there is no destruction of this digestive power at all.

The power to digest caseinogen appears to be less affected by heat than the power to coagulate calcified milk, this being taken as the measure of the activity of trypsin by Mellanby and Woolley.

It may be that these two evidences of the action of trypsin are due to different sets of groupings of the trypsin molecule, and that the groupings to which the digestion of caseinogen are due are more thermostable than the others.

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