XXV. ACTION OF PEPSIN AND TRYPSIN ON ONE ANOTHER.

(Preliminary Paper.)

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Hitherto only a small amount of work has been done in investigating the action of enzymes on one another, and many of the earlier results are inconclusive or contradictory. It was stated by Corvisart (quoted by Wróblewski) that pepsin and trypsin were mutually destructive. Kühne [1876], on the other hand, found that pepsin digested trypsin, but that the reverse did not hold good. A few years later Engesser [1880] however found that pepsin had practically no action on trypsin, digestion being continued for two hours. Further uncertainty was introduced by the work of later authors.

Langley [1881] tested the action of certain enzymes on one another, taking the precaution to determine the action of acid or alkali of the appropriate strength on the enzymes in question. He found that pepsin was destroyed considerably more rapidly by trypsin in an alkaline solution than by alkali of the same strength acting on the pepsin alone. Langley also found that trypsin lost much of its digestive power when kept for some time in 0.05 % hydrochloric acid before being allowed to act on fibrin in alkaline solution, but he does not appear to have done any experiments himself to show the action of pepsin on trypsin. As the result of a considerable number of experiments on different enzymes, Wróblewski, Bednarski and Wojczynski [1901] conclude that not only does trypsin increase the destructive effect of alkali on pepsin, but also that pepsin increases the destructive effect of acid on trypsin. While different observers have found considerable variations in the range of hydrogen or hydroxyl ion concentration compatible with digestive power on the part of pepsin or trypsin respectively, there seems little doubt that the former enzyme has no appreciable action in an alkaline medium, trypsin on the other hand being active only in presence of alkali. Consequently previous investigations of the action of these enzymes

on one another appear to have been confined to discovering if pepsin would digest trypsin in acid solution, or if trypsin on the other hand would have a destructive action on pepsin in an alkaline medium.

In the light of the older view that enzymes were of a protein nature it was not unreasonable to expect that in such cases the pepsin for example would in an acid medium break up the trypsin into a number of less complex substances, and that thus the specific digestive properties of the trypsin would be lost.

Now that considerable doubt has been thrown on the protein nature of the enzyme molecule, however, such a breakdown of the molecule is by no means certain to take place in every case.

Within the last few years the mode of action of enzymes has been rendered clearer by experiments which appear to show that the enzyme acts on its appropriate substrate by means of different chemical groups or side chains.

Pollak [1905] found that on diluting a trypsin solution with a quantity of the same trypsin solution which had been previously heated to 70° much less digestion took place than in the case where the enzyme was diluted with a corresponding amount of water. Similar results were obtained by Schwartz [1905] in the case of pepsin.

Bayliss [1904] found that trypsin became altered in the above way on being heated, and suggested that the altered enzyme or "zymoid" still retained the power of combining with the substrate, but that the specific digestive power had been destroyed.

Bearn and Cramer [1907] also found that pepsin and rennin when heated to about 60° inhibited the action of the fresh enzyme when excess of the heated enzyme was added. Generally it was found that if the enzyme were heated to 100° this inhibitory power was lost, the reason probably being that the groups which combine with the substrate are themselves destroyed at 100°. Different enzyme preparations, however, varied considerably in their resistance to heat.

Although trypsin is a more energetic proteolytic enzyme than pepsin, nevertheless they are both capable of acting on a large number of proteins with the formation of similar hydrolytic products to a certain stage of digestion. This led to the idea that both enzymes might attack proteins by combining with the same side chains, the specific digestive action of the enzymes, however, being due to dissimilar side chains, the latter in the case of pepsin acting only in acid solution, and in the case of trypsin only in presence of alkali.

If this were the case we should expect that an excess of trypsin would inhibit the action of pepsin in acid solution, and similarly excess of pepsin would inhibit the action of trypsin in alkaline solution. The enzyme present in excess in such cases would thus act in a manner analogous to the zymoid of the other enzyme.

EXPERIMENTAL METHODS.

Various enzyme preparations were used in these experiments, such as Benger's Liquor Pepticus and Liquor Pancreaticus, and the pepsin and trypsin in scales or powder of Merck, Fairchild and Grüber.

The enzyme solutions were put in small flasks and a definite amount of dilute hydrochloric acid or sodium carbonate added. In each flask was then put an equal amount of the protein to be digested and the flasks were kept in a water bath at 37° for a certain time. Any slight accidental variations in temperature would affect all the flasks equally, and thus introduce no difficulty.

Two methods of estimating the amount of digestion were employed. The first, principally used for determining the amount of digestion by pepsin, was that of making the mixture of enzymes act on fibrin for a definite time. The undissolved fibrin was then filtered off and the nitrogen in a portion of the filtrate determined by Kjeldahl's method.

Since the object of these experiments was to determine the activity of one enzyme under different conditions, for example the relative digestive power of pepsin in dilute acid and of pepsin in the same strength of acid containing trypsin in addition, such a method of estimating the amount of digestion seems quite satisfactory, as the stages of digestion must presumably be the same in both cases, and where digestion has gone on for a definite time the amount of dissolved nitrogen must give a true indication of the digestive power of the enzyme under these conditions. The fibrin was always obtained from ox blood, and was minced, thoroughly washed till free from blood, suspended in water and heated to 70° in order to destroy any enzymes present. The water was then pressed out and the fibrin preserved in glycerol. When the fibrin was required for use, the glycerol was washed out with water, the excess of water pressed out and equal weighed amounts of the fibrin taken for experiment.

Control experiments were also carried out with the same amount of fibrin and acid or alkali, but no enzyme. No appreciable amount of fibrin was found to be dissolved in any of the controls.

The amount of nitrogen present in the solutions of enzymes used was also

determined in every case, and that amount deducted from the total nitrogen in solution at the end of the experiment. The difference represented the nitrogen of the protein dissolved by the enzyme.

The other method of determining digestive power employed in these experiments was that described by Hedin [1903]. The enzyme is allowed to act on a solution of caseinogen, and at the end of the period of digestion excess of tannic acid is added. The unchanged protein and proteoses are precipitated, and the nitrogen left in solution is estimated in the filtrate by Kjeldahl's method. This gives a measure of the amount of protein digested to the stage of peptone or amino-acids.

Control solutions of the enzymes and also of the caseinogen used were also treated in the same way with tannic acid, and the nitrogen in the filtrate allowed for in calculating the amount of protein digested. Toluene was added to each flask in order to prevent the action of bacteria. As a rule it was found that the fibrin method was the more suitable when digestion by pepsin was being studied, as pepsin digests caseinogen only very slowly to the stage at which it is not precipitated by tannic acid.

On the other hand, the caseinogen method was found more suitable for digestion by trypsin, the latter enzyme dissolving heated ox fibrin too slowly for convenience in experiment.

EFFECT OF EXCESS OF TRYPSIN ON THE DIGESTIVE POWER OF PEPSIN.

		Digestion in cc. of N/10 nitrogen
1.	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl... ..	11.4
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	26.9
	1.4 g. fibrin added. Digestion 2.5 hours.	
2.	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl... ..	9.0
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	26.3
	1.5 g. fibrin. Digestion 1.5 hours.	
3.	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl... ..	9.9
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	28.8
	1.6 g. fibrin. Digestion 2.25 hours.	
4.	1 cc. pepsin, 10 cc. trypsin, 20 cc. water, 30 cc. N/10 HCl	37.1
	1 cc. pepsin, 20 cc. trypsin, 10 cc. water, 30 cc. N/10 HCl	14.2
	1 cc. pepsin, 30 cc. trypsin, 30 cc. N/10 HCl... ..	5.8
	1 cc. pepsin, 30 cc. water, 30 cc. N/10 HCl	47.6
	2 g. fibrin. Digestion 2.75 hours.	
5.	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl... ..	5.2
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	18.9
	1.7 g. fibrin. Digestion 1.25 hours.	
6.	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl... ..	4.8
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	13.3
	1 g. fibrin. Digestion 2 hours.	

		Digestion in cc. of N/10 nitrogen
7.	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl... ..	8.9
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	22.7
	1.1 g. fibrin. Digestion 1.5 hours.	
8.	1 cc. pepsin, 10 cc. trypsin, 20 cc. water, 30 cc. N/10 HCl	7.2
	1 cc. pepsin, 20 cc. trypsin, 10 cc. water, 30 cc. N/10 HCl	5.1
	1 cc. pepsin, 30 cc. trypsin, 30 cc. N/10 HCl... ..	4.2
	1 cc. pepsin, 30 cc. water, 30 cc. N/10 HCl	18.5
	1.8 g. fibrin. Digestion 1.5 hours.	

Of these experiments Nos. 1 to 4 were carried out with Benger's preparations, the others being with Merck's pepsin and trypsin (1 % solutions). As Liquor Pancreaticus is slightly acid, it was first neutralised, and a corresponding small amount of sodium chloride added to the control flask though this was probably not at all necessary.

It may be mentioned that exactly similar results were obtained by using Grubler's pepsin and trypsin in 1 % solution, and that many other experiments carried out with Benger's and Merck's preparations confirmed in every respect those described above, but those quoted are sufficient to show very clearly the effect of an excess of trypsin in inhibiting the digestive action of pepsin solutions. Experiments 4 and 8 also show that this inhibition varies with the proportion of trypsin present. If the amount of the latter enzyme be very large, the action of the pepsin is almost stopped entirely. This is what was to be expected if we assume that trypsin can combine with protein in acid solution.

The objection might be raised that the action of the trypsin was really due to the presence of protein or the products of protein digestion in the trypsin solutions used. That such is not the case, however, is shown by the following experiments.

		Digestion in cc. of N/10 nitrogen
9.	1 cc. pepsin, 20 cc. 2.5 % albumin, 30 cc. N/10 HCl	26.3
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	25.4
	1 g. fibrin. Digestion 1.5 hours.	
10.	1 cc. pepsin, 20 cc. 2 % caseinogen, 20 cc. water, 10 cc. N/10 HCl	29.7
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	24.9
	1 g. fibrin. Digestion 2 hours. (The caseinogen was dissolved in N/10 HCl.)	
11.	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl	31.9
	1 cc. pepsin, 20 cc. water, 30 cc. caseinogen	30.7
	1 cc. pepsin, 20 cc. water, 30 cc. albumin	37.6
	(The caseinogen and albumin were 1.5 % in N/10 HCl.) 1.2 g. fibrin. Digestion 1 hour.	

In these three experiments Liquor Pepticus was used.

			Digestion in cc. of N/10 nitrogen
12.	1 cc. pepsin, 20 cc. water, 20 cc. N/10 HCl ...	...	25.8
	1 cc. pepsin, 20 cc. water, 20 cc. caseinogen ...	...	24.4
	1 cc. pepsin, 20 cc. water, 20 cc. albumin ...	...	26.1
	1.1 g. fibrin. Digestion 1.75 hours.		
	(The caseinogen and albumin were 1 % in N/10 HCl.)		
Merck's pepsin (0.1 %) was used in this experiment.			
13.	1 cc. pepsin, 20 cc. water, 20 cc. N/10 HCl ...	...	32.3
	1 cc. pepsin, 20 cc. water, 20 cc. caseinogen ...	...	32.6
	1 cc. pepsin, 20 cc. water, 20 cc. albumin ...	...	31.7
	1.2 g. fibrin. Digestion 0.75 hour.		
	(Caseinogen and albumin 1 % in N/10 HCl.)		
Grübler's pepsin (1 %) was used in this experiment.			

It is clear from these experiments that digestion of a solid protein such as fibrin is not less rapid when there is also present in solution a protein such as caseinogen or egg albumin. The action of the pepsin on the fibrin seems to begin immediately in such a case, there being no interval during which the protein in solution is first broken down into less complex substances.

The action of the trypsin is not an actual destruction of the pepsin. This is at once seen if the amounts of fibrin used vary considerably. It has been shown by Hedin [1905] that the amount of caseinogen digested in a given time by a certain amount of trypsin increases with the concentration of the substrate, until a maximum is reached when sufficient substrate is present to unite with the whole of the trypsin.

Similar results are obtained in the digestion of fibrin by pepsin.

			Digestion in cc. of N/10 nitrogen
14.	1 cc. pepsin, 20 cc. trypsin, 20 cc. N/10 HCl, 0.9 g. fibrin		9.9
	1 cc. pepsin, 20 cc. trypsin, 20 cc. N/10 HCl, 2.7 g. fibrin		28.8
	1 cc. pepsin, 20 cc. water, 20 cc. N/10 HCl, 0.9 g. fibrin...		20.7
	1 cc. pepsin, 20 cc. water, 20 cc. N/10 HCl, 2.7 g. fibrin...		65.6
	Digestion 1 hour.		
15.	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl, 0.9 g. fibrin		10.9
	1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl, 3.4 g. fibrin		42.2
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl, 0.9 g. fibrin...		25.5
	1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl, 3.4 g. fibrin...		94.1
	Digestion 0.75 hour.		
In these two experiments Bengel's preparations were used.			
16.	0.8 cc. pepsin, 25 cc. trypsin, 30 cc. N/10 HCl, 1 g. fibrin		7.9
	(a) 0.8 cc. pepsin, 25 cc. trypsin, 30 cc. N/10 HCl, 3.5 g. fibrin		17.1
	(b) 0.8 cc. pepsin, 25 cc. water, 30 cc. N/10 HCl, 1 g. fibrin		22.4
	0.8 cc. pepsin, 25 cc. water, 30 cc. N/10 HCl, 3.5 g. fibrin		84.5
	Digestion 0.75 hour.		
17.	0.8 cc. pepsin, 25 cc. trypsin, 30 cc. N/10 HCl, 0.7 g. fibrin		4.9
	(a) 0.8 cc. pepsin, 25 cc. trypsin, 30 cc. N/10 HCl, 5 g. fibrin		12.7
	(b) 0.8 cc. pepsin, 25 cc. water, 30 cc. N/10 HCl, 0.7 g. fibrin		18.5
	0.8 cc. pepsin, 25 cc. water, 30 cc. N/10 HCl, 5 g. fibrin...		121.9
	Digestion 0.75 hour.		

	Digestion in cc. of N/10 nitrogen
18. 0.8 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl, 0.5 g. fibrin	4.7
0.8 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl, 1.5 g. fibrin	12.6
0.8 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl, 7 g. fibrin	13.1
0.8 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl, 0.5 g. fibrin	10.5
Digestion 0.5 hour.	

0.5 % pepsin and 1 % trypsin (Grübler) were used in these three experiments.

Those experiments show well the effect of varying the quantity of fibrin added to the enzyme solutions. It is clear that the effect of adding an excess of trypsin to a given amount of pepsin can be apparently counteracted by increasing the amount of fibrin. The reason evidently is that when the fibrin is increased in amount a larger number of pepsin molecules can carry on their digestive action, although in those experiments at least the amount of digestion by pepsin + trypsin relative to that by pepsin alone is no greater when much fibrin is present than when a smaller amount of the substrate only is used.

In experiments 16 and 17 it will be noticed that the amount of digestion in (a) is still less than that in (b) in spite of the fact that the amount of fibrin in the former is considerably greater than in the latter case. In these two experiments the proportion of trypsin to pepsin is probably higher than in experiments 14 and 15, consequently the proportion of fibrin should have been correspondingly higher in (a) than in (b) to produce the same effect. By using still smaller amounts of fibrin as in No. 18 this difficulty is got over, and in this last experiment we see again that by employing suitable proportions of fibrin it is possible to get a greater amount of digestion by the pepsin + trypsin than by the pepsin alone.

Since it has been clearly shown that the inhibition of pepsin by excess of trypsin is not due to the presence of soluble protein in the trypsin solution, also that it is not due to the pepsin being destroyed by the trypsin, it seems reasonable to suppose that this inhibition is due to the trypsin itself. If this were the case, we should expect that such an inhibition would no longer occur if the trypsin were destroyed.

The simplest way appeared to be by boiling the solution of trypsin before adding it to the pepsin. This, however, proved somewhat ineffective as the following show.

	Digestion in cc. of N/10 nitrogen
19. 1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl ...	9.9
1 cc. pepsin, 20 cc. boiled trypsin, 30 cc. N/10 HCl ...	16.8
1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl ...	28.8
1.6 g. fibrin. Digestion 2.25 hours.	
20. 1 cc. pepsin, 20 cc. trypsin, 30 cc. N/10 HCl ...	15.4
1 cc. pepsin, 20 cc. boiled trypsin, 30 cc. N/10 HCl ...	20.7
1 cc. pepsin, 20 cc. water, 30 cc. N/10 HCl ...	40.2
1.7 g. fibrin. Digestion 1.5 hours.	

In these two experiments Liquor Pepticus and Liquor Pancreaticus were used. The latter was boiled under a reflux condenser for about 30 minutes and filtered. The precipitate was found to be entirely and readily soluble in the same concentration of pepsin as was used for the main experiment. As mentioned in a previous paper by me [1914] this trypsin preparation is slightly acid and retains a considerable proportion of its digestive power even after being boiled. In the last two experiments therefore, the digestive power of the trypsin was probably not entirely destroyed, or at least the power of the trypsin to unite with its substrate. In this case it is easy to understand why boiled trypsin still inhibits the action of pepsin on fibrin.

It may be mentioned here that it was through these unexpected results that I was led to investigate the effect of heat on trypsin more fully. As it has been shown in the paper referred to and by other workers that trypsin loses its digestive power readily when boiled in neutral or alkaline solution, I tried the effect of trypsin, which had been thus boiled, on pepsin, but found that it was still effective in inhibiting the digestion of fibrin by pepsin.

There is reason to believe, from preliminary experiments, that it is much harder completely to inactivate the trypsin molecule than the pepsin molecule, so that the zymoid modification of trypsin would still inhibit the action of pepsin even after prolonged heating of the trypsin solution. Different trypsin preparations also are found to behave differently in this respect. It was also found by Bearn and Cramer that different preparations of pepsin varied considerably in their behaviour to heat. The results with trypsin will be described in a later paper on this subject.

Trypsin was found to inhibit the action of pepsin on caseinogen solutions also, but the effect of pepsin on this substrate is slight, so far, at least, as rendering the nitrogen non-precipitable by tannic acid goes.

		Digestion in cc. of N/10 nitrogen not pptd. by tannic acid
21.	1 cc. pepsin, 20 cc. trypsin, 15 cc. N/10 HCl...	3.1
	1 cc. pepsin, 20 cc. water, 15 cc. N/10 HCl ...	6.2
	20 cc. of 1 % caseinogen in N/20 HCl added. Digestion 4 hours.	
22.	1 cc. pepsin, 20 cc. trypsin, 30 cc. 2 % caseinogen in N/10 HCl ...	9.8
	1 cc. pepsin, 20 cc. water, 30 cc. 2 % caseinogen in N/10 HCl	14.3
Liquor Pepticus and Liquor Pancreaticus used in these two experiments.		

In No. 22, digestion lasted 18 hours, so that while these experiments show that trypsin again inhibits pepsin, they show still more clearly that this method is unsuitable for estimating the digestive activity of pepsin solutions.

EFFECT OF EXCESS OF PEPSIN ON THE DIGESTIVE ACTION OF TRYPSIN.

The experiments were carried out similarly to those already described. When Liquor Pepticus was used as the pepsin solution, a considerable allowance had to be made for the acidity of this solution, the control solution having sodium chloride added in amount corresponding to that in the neutralised pepsin solution.

		Digestion in cc. of N/10 nitrogen
23.	1 cc. trypsin, 20 cc. pepsin, 40 cc. 1 % Na_2CO_3 ...	6.7
	1 cc. trypsin, 20 cc. water, 40 cc. 1 % Na_2CO_3 ...	12.2
	1 g. fibrin. Digestion 2 hours.	
24.	1 cc. trypsin, 20 cc. pepsin, 40 cc. 1 % Na_2CO_3 ...	7.9
	1 cc. trypsin, 20 cc. water, 40 cc. 1 % Na_2CO_3 ...	18.6
	1.2 g. fibrin. Digestion 2 hours.	
25.	1 cc. trypsin, 10 cc. pepsin, 20 cc. water, 35 cc. 1 % Na_2CO_3 ...	26.4
	1 cc. trypsin, 20 cc. pepsin, 10 cc. water, 35 cc. 1 % Na_2CO_3 ...	21.1
	1 cc. trypsin, 30 cc. pepsin, 35 cc. 1 % Na_2CO_3 ...	12.8
	1 cc. trypsin, 30 cc. water, 35 cc. 1 % Na_2CO_3 ...	33.3
	2 g. fibrin. Digestion 1.5 hours.	

Liquor Pancreaticus and Liquor Pepticus were used in these experiments.

It will be seen from the above that an excess of pepsin inhibits the action of trypsin on fibrin, the amount of inhibition varying with the amount of pepsin added.

On the whole it was found that much more digestion was effected when caseinogen was used as the substrate, and the nitrogen estimated in the filtrate from the tannic acid precipitate.

		Digestion in cc. of N/10 nitrogen not pptd. by tannic acid
26.	1 cc. trypsin, 20 cc. pepsin, 40 cc. caseinogen ...	20.3
	1 cc. trypsin, 20 cc. water, 40 cc. caseinogen ...	46.9
	Caseinogen was 2 % in 1 % Na_2CO_3 . Digestion 3 hours.	
27.	1 cc. trypsin, 20 cc. pepsin, 40 cc. caseinogen ...	28.2
	1 cc. trypsin, 20 cc. water, 40 cc. caseinogen ...	53.6
	Caseinogen as above. Digestion 4 hours.	

The effect of a soluble protein such as caseinogen on the digestion of fibrin by trypsin was tested by two experiments.

		Digestion in cc. of N/10 nitrogen
28.	1 cc. trypsin, 20 cc. caseinogen, 20 cc. N/10 Na_2CO_3 ...	19.3
	1 cc. trypsin, 40 cc. N/10 Na_2CO_3 ...	16.3
	1 g. fibrin. Digestion 2 hours.	
	The caseinogen was 2 % in N/10 Na_2CO_3 .	
29.	1 cc. trypsin, 20 cc. caseinogen, 20 cc. N/10 Na_2CO_3 ...	17.9
	1 cc. trypsin, 40 cc. N/10 Na_2CO_3 ...	16.8
	0.8 fibrin. Digestion 2 hours.	
	The caseinogen was 2 % in N/10 Na_2CO_3 .	

These two experiments show that the presence of excess of a soluble protein such as caseinogen does not delay the digestion of fibrin by trypsin. Egg albumin, as is well known, inhibits the action of trypsin, and I found that only a small amount of fibrin was digested if albumin was added in place of the caseinogen in these two experiments. The above results show, however, that the inhibition of trypsin by excess of pepsin is not due to the trypsin digesting the proteins in solution before attacking the fibrin.

Several experiments were carried out to see if pepsin solutions which had been boiled lost their power of inhibiting the action of trypsin. Here again, as in the corresponding experiments with boiled trypsin in excess acting on pepsin, the results varied considerably.

		Digestion in cc. of N/10 nitrogen not pptd. by tannic acid
30.	1 cc. trypsin, 20 cc. pepsin, 40 cc. caseinogen	14.9
	1 cc. trypsin, 20 cc. boiled pepsin, 40 cc. caseinogen	40.3
	1 cc. trypsin, 20 cc. water, 40 cc. caseinogen	52.8
	The caseinogen was 2 % in N/10 Na_2CO_3 . Digestion 3 hours.	

Liquor Pepticus and Liquor Pancreaticus were used in this experiment.

This shows that pepsin by being boiled loses most of its power to inhibit trypsin. In this case the pepsin had been boiled for about 10 minutes. Probably it still retained some of its zymoid properties, which would have been destroyed by more prolonged heating.

Although I had previously noticed that boiled trypsin still inhibited the action of pepsin, it was in the course of this last experiment that I found, in one of the controls containing boiled trypsin and caseinogen, that the boiled enzyme had brought about a considerable amount of digestion of the protein. I was led from that to consider the question of the resistance of trypsin to heat, and since then have been unable to get clear evidence of the destruction of the inhibitory power of pepsin by heat. As was shown by Bearn and Cramer, different preparations of pepsin vary widely in their resistance to heat, and it may be that in my experiments the solutions have always retained their power of combining with the substrate. This question is being now investigated, and the results will be published in a later paper.

It may be mentioned that Bayliss found that he could not always produce the zymoid modification of trypsin, or at least demonstrate the action of the zymoid, and it is evident from the experiments described in this preliminary paper that a number of points have still to be settled in regard to the varying behaviour of different enzyme solutions after being heated.

SUMMARY.

Excess of trypsin inhibits the digestive action of pepsin in acid solution. Such inhibition is not due simply to the presence of protein associated with the trypsin. Nor is it due to the pepsin being destroyed by the trypsin.

The power of trypsin to inhibit pepsin is to some extent lost if the trypsin solution is boiled before being added to the pepsin.

It is suggested that trypsin can combine with protein in acid solution, so that although unable to digest such protein, an excess of trypsin would prevent pepsin from combining fully with the protein.

Similarly an excess of pepsin inhibits the digestive action of trypsin in alkaline solution. The amount of inhibition in this case is usually less than that caused by an excess of trypsin on the digestive power of pepsin in acid solution.

This inhibition again is not simply due to the presence of protein associated with the pepsin. Heating the pepsin solution destroys to a considerable extent its power to inhibit trypsin.

In this case it is suggested that the pepsin acts by combining with the protein in alkaline solution, thus preventing the digestion of the protein by the trypsin.

In both cases the amount of inhibition produced depends on the relative proportions of the enzymes present. Thus, the greater the amount of pepsin, the less digestion will be effected by a given amount of trypsin acting in alkaline solution.

REFERENCES.

- Bayliss (1904), *Arch. Sci. Biol.* 11, *Supplement*, 261.
Bearn and Cramer (1907), *Biochem. J.* 2, 174.
Edie (1914), *Biochem. J.* 8, 84.
Engesser (1880), *Maly's Jahresbericht*, 297.
Hedin (1903), *J. Physiol.* 30, 155.
— (1905), *J. Physiol.* 32, 468.
Kühne (1876), *Maly's Jahresbericht*.
Langley (1881), *J. Physiol.* 3, 246.
Pollak (1905), *Beiträge*, 6, 95.
Schwartz (1905), *Beiträge*, 6, 524.
Wróblewski, Bednarski and Wojczynski (1901), *Beiträge*, 1, 289.