THE PEPTONE-SPLITTING FERMENTS OF THE PANCREAS AND INTESTINE.

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(*From the Physiological Laboratory, Oxford.*)

CONTENTS.

THE only certain method of distinguishing peptones and true proteids from their decomposition products appears to lie in the biuret test. Hence the quantitative estimation of peptone-splitting ferments rests on the application of this test. Though the method adopted did not give results of as great accuracy as has been attained in the estimation of certain other ferments, this was due to indirect causes rather than to the method itself. This method consisted in comparing the partially digested peptone solution which was to be tested against some of the undigested peptone by means of a colorimeter. Into each of two tall flat-bottomed Nessler tubes were introduced convenient quantities of caustic soda and copper sulphate, and into one of the tubes was run a measured volume of the undigested peptone solution. This tube served as a standard. To the other tube the partially digested peptone solution was gradually added until the mixture showed exactly the same tint as the standard, and supposing, for instance, that just twice as great a volume of the peptone was required as in the standard tube, it would

follow that 50 % of the peptone in the solution had been split up into substances no longer yielding the biuret test. The two Nessler tubes stood on a plate of glass, underneath which a dull white slab was placed at such an angle as to reflect up the tubes a convenient amount of light. The tubes were viewed through a black mask in which had been cut two circular holes 14 mm. in diameter. If care be exercised, it is possible after some practice to make estimations which do not on an average differ from each other by more than 1 %. The amounts of alkali, copper sulphate and peptone solution used can be varied within very wide limits, but those adopted in practically all the observations described below were as follows. Into each tube were run 18 c.c. of caustic soda (containing 4 % by weight of ordinary stick soda) and 2 c.c. of centi-normal copper sulphate solution, and into the standard tube ·40 c.c. of a 2·5 % solution of the peptone which was being used in the digestion experiments. The peptone used in the first part of the experiments was a 5 % solution of Witte's peptone which had been digested 7 days at 38° C. with toluol, ·2 % HCl, and pepsin (2 gm. of Parke, Davis and Co.'s 1—4000 pepsin being added to 1 litre of solution, and further quantities of 1 gm. being added on the 3rd and 5th days of digestion). This solution was neutralised, heated to 90°, and filtered on cooling. As will be shown later, it was not acted on any more rapidly by peptone-splitting ferments than was undigested Witte-peptone solution, hence in the latter part of the observations this solution alone was employed. In that a small portion (less than 3 %) of the peptone does not dissolve in water, it was filtered off, and so this filtered 5 % Witte-peptone solution was ultimately adopted as the standard one. In digestion experiments it was necessary to add small quantities of sodium carbonate and of the ferment extract to be tested, hence to get comparable results water was always added so as to bring the total bulk of digesting liquid up to double that of the peptone taken; *i.e.* the digesting liquid always contained 2·5 % of peptone.

The rate of development of the biuret reaction.

A source of error which has to be carefully guarded against lies in the fact that the development of the maximum tint of the biuret reaction takes an appreciable time. This point does not seem to have been previously noticed, although in the case of native proteids it is obvious to the most superficial examination. Some of the quantitative results obtained are plotted out as curves in the accompanying figure, and from

this we may gather that, as regards Witte-peptone solution, the tint produced immediately the peptone is run into the alkaline copper sulphate is only about 88 % of the maximum tint, whilst after 1 minute

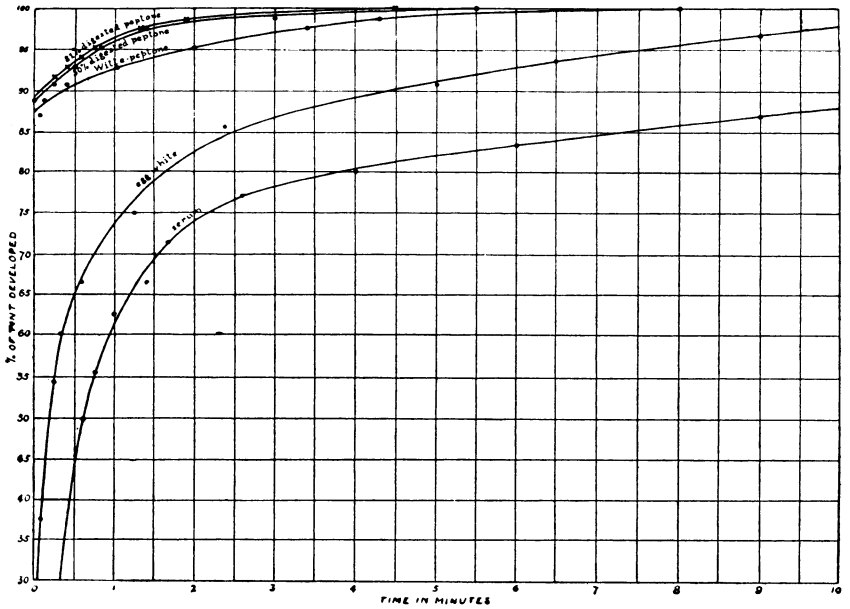

Fig. 1.

it is 93 % of the maximum. This maximum is only attained after about 8 minutes. That is to say, in order to get immediate equality of tint it is necessary to run into one Nessler tube ·455 c.c. of peptone solution, instead of the ·40 c.c. which has been run into the standard tube 10 or more minutes previously. Filtered Witte-peptone solution, and peptone digested a week with pepsin gave practically the same rates of development of tint, but Witte-peptone which had been partially digested with peptone-splitting ferments showed a distinctly more rapid rate of development. Thus in the figure are given the values obtained with a peptone solution 50 % of which had been destroyed by the action of a pancreatic extract, and of a solution 81 % of which had been destroyed by pancreatic and intestinal extracts. This latter solution showed a slightly more rapid rate of development of tint than the former, but the difference is so slight as to be within the limits of experimental error.

In the case of the native proteids the development of the biuret

reaction is remarkably delayed. With filtered egg white we see that 4 seconds after mixing the proteid and alkaline copper sulphate the tint was only about 37% of its ultimate value. After 12 seconds it was 50%, and after 1 minute it was 73% of the maximum, whilst equality of tint was reached after about 12 minutes. From the direction of the curve we see that the proteid could have given no biuret reaction whatever immediately on mixing with the copper sulphate, or it would seem that the reaction can only develop as the proteid becomes gradually decomposed by the action of the caustic alkali. The results obtained with the proteids of blood serum support this view, for we see from the figure that in their case the development of the reaction was still slower than with egg white. In fact about 36 seconds were required for the tint to reach 50% of its final value, and this final value was not attained for 2 hours or more. We must conclude, therefore, that the proteids of serum are decomposed by caustic soda much more slowly than are those of egg white, whilst even Witte-peptone contains certain substances—presumably albumoses—which require to be split up by the alkali before they can yield the maximum biuret reaction. The rate of decomposition of proteids is of course dependent on the strength of alkali used. In all the above observations a solution containing 4% of stick caustic soda was used, but it was found that if 1% NaOH were used instead the reaction developed considerably more slowly, and with ·2% NaOH much more slowly still. In fact with this latter strength of alkali serum proteids gave no appreciable reaction whatever for 2 or 3 minutes, and developed only three-fourths of the full tint in 24 hours, whilst even peptone took some seconds to develop any tint. I hope at some future time to investigate more thoroughly the rate of development of the biuret and of other colour reactions with various pure proteids, as it seems possible that useful information as to the comparative degrees of stability of proteids may thereby be obtained.

It will be noticed that in the above figure no times of arrival at less than 50% of the maximum tint are recorded in the case of serum proteids. The reason of this is that equality of tint was not attainable. As more and more of the serum solution was added, the tint at first developed became more and more pinkish as compared with the standard, till accurate comparison was no longer possible. A similar though not so marked condition of things was observed also with egg white, and to a slight extent even with Witte-peptone. That is to say, the tint at first developed is in all cases more pinkish than that ultimately attained. Now it is well known that peptone gives a pinkish-red biuret

reaction, whilst native proteids give a violet one. Therefore the first portions of the molecule split off by the action of the caustic alkali are —as regards their biuret reaction—comparable to peptone, whilst those split off later react differently.

As far as the few observations made can show, both proteids and peptones yield roughly the same intensity of biuret reaction. For the reason just mentioned, it is impossible to compare accurately solutions of egg white and serum proteids against those of peptone, but it was found that the amounts of proteid required to give distinctly fainter and distinctly deeper tints than that of the standard peptone lay between comparatively narrow limits. Measured volumes of the egg white, the blood serum and the Witte-peptone used in the above observations were dried for 24 hours at 107° C., and taking the percentages of dry solids present as roughly representing their proteid content, it was calculated that 1 part of Witte-peptone gave the same intensity of biuret reaction as ·93 part of egg white, and 1·12 part of serum proteids. It seems probable, therefore, that the intensity of the biuret reaction forms a quantitative measure of the relative numbers of biuret groupings present in proteid and peptone molecules, and also that these relative numbers approach equality.

The law of action of peptone-splitting ferments.

For the quantitative estimation and comparison of the peptone-splitting ferments in various extracts, it is necessary to know their law of action. In order to determine this series of observations were made with intestinal and with pancreatic extracts. Volumes of 5 c.c. (or more) of 5 % Witte-peptone were run into small stoppered bottles, water and sodium carbonate solution added so as to bring up the total volume of liquid to 10 c.c. (less that of the ferment extract to be employed), and the strength of the sodium carbonate to ·1 %. Four drops of toluol were added, and then volumes of extract varying from 1 c.c. down to ·015625 c.c. (or as 64 to 1) were run in. The bottles were kept in an incubator at 38°, and after about 2, 8, 24 and 48 hours small samples of the digesting peptone were withdrawn and tested in the manner above described. In certain cases the digestions were continued further, and were tested after 4 and 8 days, and in certain cases also they were examined twice in the initial stage, viz. after about 1½ and 4 hours, instead of after 2 hours, but for ordinary purposes the four determinations at the above-mentioned hours were found sufficient.

The intestinal extracts used, which contain the peptone-splitting ferment erepsin, recently discovered by Cohnheim[1], were prepared by washing out the intestine thoroughly with a stream of water, allowing the water to drain off for half-an-hour, and then, with a scalpel, scraping away the mucous membrane and some of the muscularis mucosæ. To each gram of tissue were then added 2 c.c. of glycerin, or of 30 % methylated spirit. Small portions of the clear extract were filtered off for testing as required. The results obtained with a glycerin extract of cat's intestine—acting upon peptone previously digested a week with pepsin—are indicated in the accompanying figure. Here

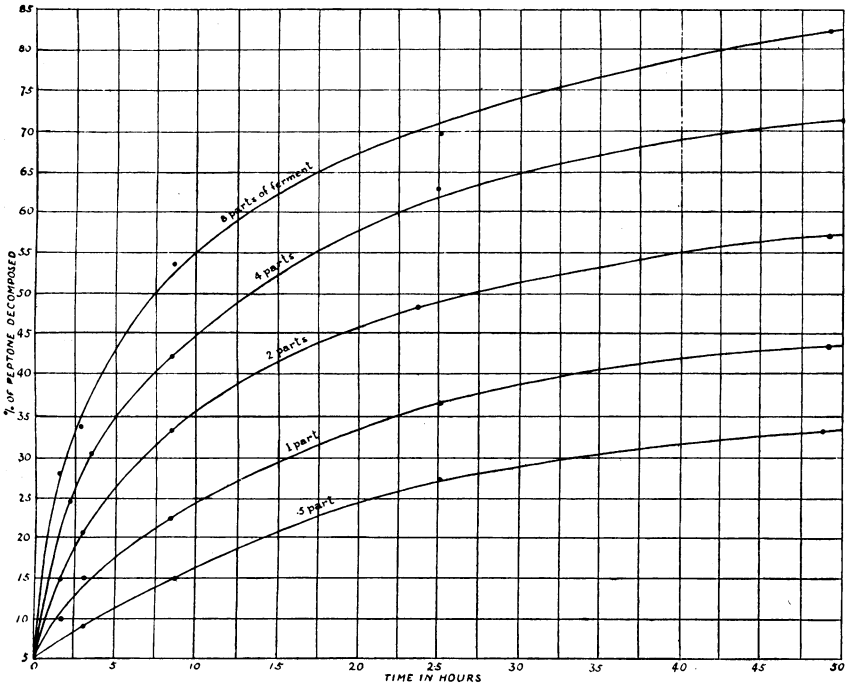

Fig. 2.

ordinates represent the percentages of peptone destroyed, and abscissæ the time in hours. Through the experimentally determined values, represented by dots, have been drawn smoothed curves. It will be seen that the percentage of peptone decomposed increases regularly with the duration of the digestion and with the amount of ferment present. The rate of digestion is most readily studied by reading off from these curves

[1] *Zeit. f. physiol. Chem.* xxxiii. p. 451, and xxxv. p. 134.

the times required to split up definite amounts of peptone, such as 20, 30, and 40 %. The values so obtained are given in the table, and from them we see that the time varied in inverse ratio to the quantity

Parts of ferment	Hours required to digest				Time × quantity of ferment			
	20 p.c.	30 p.c.	40 p.c.	50 p.c.	20 p.c.	30 p.c.	40 p.c.	50 p.c.
8	·7	1·9	4·2	7·4	5·6	15·2	34	59
4	1·4	3·4	7·4	13·4	5·6	13·6	30	54
2	2·8	6·8	13·8	27	5·6	13·6	28	54
1	6·6	15·7	33		6·6	15·7	33	
·5	13·8	33			6·9	16·5		

of ferment. For instance, 8 parts of ferment split up 30 % of the peptone in 1·9 hours, whilst 4 parts of ferment required 3·4 hours, and 2 parts 6·8 hours. In the right half of the table are given the products of quantity of ferment into time in hours, and it will be seen that the law of inverse proportion holds with fair approximation in every case. Close agreement could not be expected, as small errors in the peptone estimations produce considerably larger ones in the digestion times. For instance the proportions of peptone destroyed after 24 hours by ·5 and 1 part of ferment were respectively 27 and 36 %, or differed by 9 %. An error of 1 % in either of the peptone estimations would thus represent $\frac{1}{9}$th of the difference produced by doubling the amount of ferment added, or would become magnified to an error of 11 % in the determination of the digestion time.

The times of digestion of more than 50 % of the peptone have not been recorded, as it is difficult to estimate them with much accuracy. As digestion proceeds, the tint of the biuret reaction gradually becomes more and more reddish pink, but until more than 50 % or so of the peptone has been split up, the change of tint is so slight as to diminish very little the exactness of the colour comparison. The change of tint was generally more marked in pancreatic digestions than in intestinal. In some of these latter, indeed, there was no change of tint whatever, even when 60 % or more of the peptone had been decomposed.

It will be seen that even when the maximum amount of intestinal extract was used (1 c.c. in 10 c.c. of 2·5 % peptone) only about 83 % of the peptone was split up in two days. None of the other extracts, either intestinal or pancreatic, effected even as much decomposition as this; hence the disintegration of the peptone was never rapid[1].

[1] Weinland (*Zeitschr. f. Biol.* XLV. p. 292. 1903) likewise found that the intestinal ferment acted somewhat slowly.

Cohnheim obtained extracts which caused the disappearance of the biuret reaction in a much shorter time, but he added much larger quantities of extract. For instance he found that 15 c.c. of extract almost completely decomposed ·06 gm. of peptone in 45 minutes. He ground up the mucous membrane with sand previous to extraction, but I did not find that this treatment yielded more active extracts, and therefore I discontinued it.

In the above figure the curves of digestion have not been drawn from zero, but from a point indicating that 5 % of the peptone had already been split up. This splitting was due to the direct action of the ·1 % Na_2CO_3 in which the digestions were carried out. That is to say, if peptone be kept at 38° with the alkali and no ferment whatever, it is found that within an hour or two nearly 5 % of it is decomposed, whilst after two days the amount has risen to from 5 to 6 %. It might be thought that this apparent decomposition was due merely to some error incidental to the method of ferment estimation. Probably, however, it is genuine, as no decomposition at all is effected if no alkali be added, and conversely with stronger alkali the decomposition is greater. Thus with ·4 % Na_2CO_3 12 % was decomposed after 2 days, and with ·8 % Na_2CO_3, 16 %.

The next table shows the values obtained with a dilute alcoholic extract of the mucous membrane of pig's intestine. This extract was a weak one, the rate of decomposition of the peptone being only about half as great as before. The products of digestion time into quantity of ferment show a moderately close agreement, except the 50 % values. The digestions were continued for 8 days, but even then only 68 % of the peptone was decomposed by the maximum amount of ferment.

Parts of ferment	Hours required to digest				Time × quantity of ferment			
	20 p.c.	30 p.c.	40 p.c.	50 p.c.	20 p.c.	30 p.c.	40 p.c.	50 p.c.
8	1·3	4·9	14·8	39	10·4	39	118	310
4	2·7	10·2	28·7	62	10·8	41	115	250
2	5·6	20·0	49	110	11·2	40	98	220
1	12·0	40	119		12·0	40	119	
·5	24·4	104			12·2	52		

The law of action of the pancreatic peptone-splitting ferments is not so simple as that of intestinal erepsin. As will be proved later on, the peptone-splitting is due only in part to the trypsin, more than half of it being effected by an entirely different ferment, which may be termed pancreatic erepsin. Probably these two ferments interfere with each other's action, for it was found that as a rule the digestion times did not

decrease in proportion to the increase of ferment. In Fig. 3 are plotted out the values obtained with a dilute alcoholic extract of pig's pancreas (1 part of minced gland substance to 1 of methylated spirit and 3 of water) acting upon filtered Witte-peptone solution. Volumes of extract varying as 64 to 1 were used, and the digestions were continued for 8 days. It will be seen that the experimental values are in fair agree-

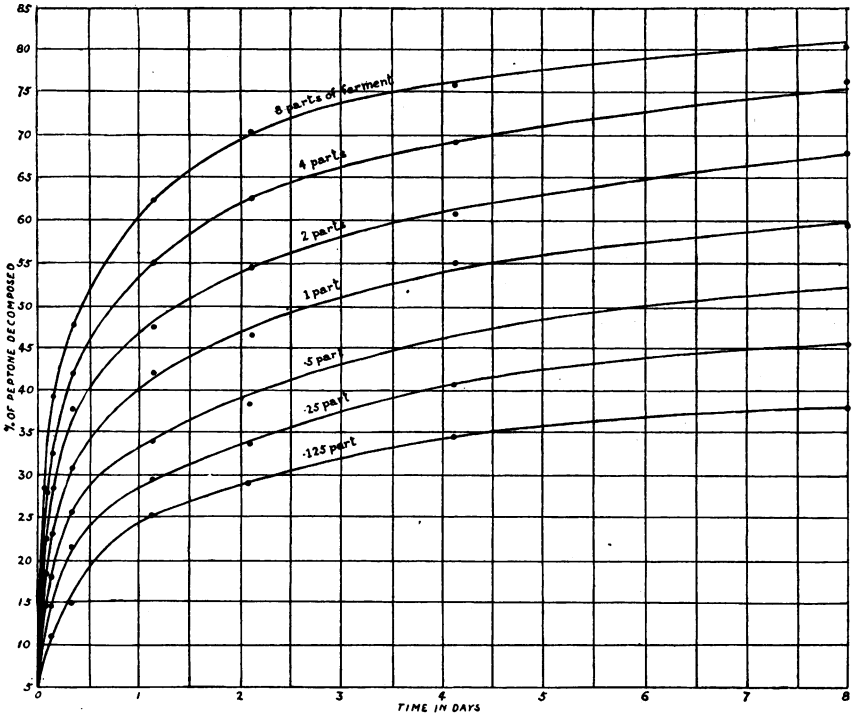

Fig. 3.

ment with the smoothed curves. The digestion mixture containing ·5 part of ferment underwent putrefaction after 2 days, but for the sake of completeness the curve has been continued about midway between the curves on either side. In the table are given the times of digestion of various percentages of peptone, together with the products of time into quantity of ferment. It will be seen that some of these latter values by no means approach equality. Especially is this the case as regards the 20 % digestion values. With 8 parts of ferment the product is nearly four times greater than with ·25 part, or in other words the rate of digestion was relatively only a quarter as rapid. The 30 % digestion

time values show a much greater resemblance, and except in the case of the values obtained with 8 and 4 parts of ferment are nearly equal. The 40, 50 and 60 % values all correspond fairly closely, so that within certain limits the law of inverse proportion holds for pancreatic ferments almost as well as it does for intestinal erepsin.

Parts of ferment	Hours required to digest					Time × quantity of ferment				
	20 p.c.	30 p.c.	40 p.c.	50 p.c.	60 p.c.	20 p.c.	30 p.c.	40 p.c.	50 p.c.	60 p.c.
8	·75	1·7	3·7	9·8	23·3	6·0	13·6	30	78	186
4	1·2	2·5	6·6	18·0	41	4·8	10·0	26	72	164
2	1·6	3·8	11·4	33	87	3·2	7·6	23	66	174
1	2·3	7·2	23·6	64	192	2·3	7·2	24	64	192
·5	3·8	14·4	52			1·9	7·2	26		
·25	6·7	30	92			1·7	7·5	23		
·125	13·3	56				1·7	7·0			

In the next table are given the products of digestion time into parts of ferment obtained in two different series of experiments, one with a glycerin extract of pig's pancreas, and the other with a glycerin extract of sheep's pancreas. Peptone previously digested for a week with pepsin was used in both instances. The law of inverse proportion

Parts of ferment	Pig's pancreas: Digestion time × ferment					Sheep's pancreas: Digestion time × ferment			
	20 p.c.	30 p.c.	40 p.c.	50 p.c.	60 p.c.	20 p.c.	30 p.c.	40 p.c.	50 p.c.
8	4·8	10·4	26·4	74	296	3·2	9·4	30	165
4	3·5	8·6	20·0	59	230	2·6	7·6	29	240
2	2·3	5·3	13·6	61	320	1·7	6·5	34	380
1	1·5	4·6	14·5	62		1·5	7·0	57	
·5	1·3	3·6	14·3	60		1·1	10·2		
·25						1·4	14·0		
·125						1·5	13·3		

does not hold so well as in the previous series, for in the case of the pig's pancreas it is only the 50 % digestion values which show much approach to equality. The last three of the 40 % values correspond, but the 30 % values, as well as the 20 %, diminish steadily with diminishing quantities of ferment. The values obtained with glycerin extracts of sheep's pancreas are the most irregular of all, and they show a feature not exhibited by the two other series. Thus they for a time steadily diminish with diminishing quantities of ferment, and then begin to increase again.

The maximum amount of peptone split up by the alcoholic extract of pig's pancreas after 8 days' digestion was 80·5 %, and by the glycerin extract of pig's pancreas after 4 days' digestion was 67·8 %. The

glycerin extract of sheep's pancreas split up only 63·6 % after 8 days, and this in spite of the fact that the initial stages of the digestion were more rapid than with either of the other extracts. There was in fact a much greater slowing down of the later stages of digestion than usual. This was probably due to a more rapid destruction of the ferment, but, as will be shown later, there are many and varying factors upon which the rate of progress of digestion depends, and hence this rate is subject to wide variations in different cases. With one extract it may take five times as long to split up 30 % of the peptone as to split up 20 %, and with another only 2 to 2½ times as long. Also, as can be gathered from some of the above quoted data, the relative rate of progress of digestion is greatly influenced by the quantity of ferment used. Hence really accurate comparison of the ferment content of various pancreatic extracts is almost out of the question, however exactly the rate of peptone-splitting be determined. Still it will be seen that by taking suitable precautions, it is possible in certain cases to attain all the accuracy that is essential.

The differentiation of trypsin and pancreatic erepsin.

It has always been assumed that the whole of the proteolytic changes brought about by the action of pancreatic juice or extracts are due to a single ferment, for until Cohnheim's discovery of erepsin no ferment was known which would attack the products of proteid hydrolysis, but not the proteid itself. However the assumption is incorrect, for it was found that the fibrin-digesting and peptone-splitting powers of pancreatic extracts do not run in the least degree parallel. Fresh pancreatic extracts, containing almost all the trypsin in the form of zymogen, may have as great a peptone-splitting power as when the whole of the zymogen has undergone its conversion into enzyme. In Fig. 4 are shown the curves of peptone digestion obtained with a glycerin extract of sheep's pancreas which had been mixed with a third of its volume of water. This permitted a fairly rapid conversion of tryptic zymogen into enzyme, and we see from the table that the tryptic value increased

Sheep's pancreas extract diluted with water	Tryptic value	Hours required to digest				Peptone-splitting value	
		20 p.c.	30 p.c.	40 p.c.	50 p.c.	20 p.c.	20 + 30 p.c.
0 day	13·9	1·6	3·8	7·7	24	17	19
2 days	38·1	1·0	2·8	8·6	38	27	28
6 ,,	71·5	·7	2·1	7·3	59	38	38
23 ,,	95·4	1·9	4·8	12·8	59	14	15

from 13·9 to 38·1 in the first two days, and then to 71·5 in the next four days; *i.e.* the rate of digestion of fibrin was increased about three- and five-fold respectively. The rates of digestion of 20 and 30 % of the

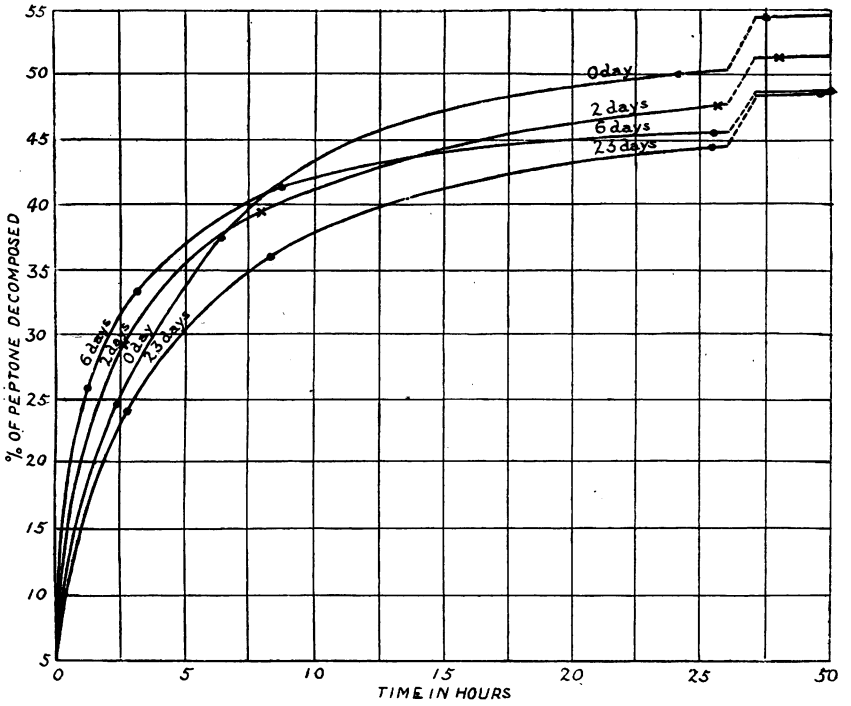

Fig. 4.

peptone were likewise increased, but in nothing like the same proportion, whilst the rate of digestion of 40 % of the peptone was almost unchanged, and that of 50 % of peptone was considerably prolonged. Also the rate of peptone digestion after 23 days, when the extract had attained its full tryptic value, was at all stages considerably slower than at first. These apparently contradictory results were obtained in every case in which the tryptic and peptone-splitting powers of a zymogen extract were determined during the course of liberation of the enzyme, and hence there is no doubt as to their validity. The explanation of them seems to be as follows. Pancreatic extracts contain two independent ferments, viz. pancreatic erepsin—which does not exist in the form of a soluble zymogen—and trypsin—which does. As the trypsinogen gradually undergoes its conversion into enzyme, it is able to assist the

erepsin to some extent in digesting peptone, but it likewise begins to attack and destroy the ereptic ferment itself. In the presence of much free trypsin, therefore, the initial stages of peptone-splitting may be accomplished more rapidly than when none is present, but the later stages become relatively slower and slower owing to the increased destruction of erepsin. Finally the peptone-splitting becomes slower than when little or no free trypsin is present, or erepsin alone is able to effect more decomposition than trypsin + (still undestroyed) erepsin. As is suggested by evidence to be adduced later, this is probably due to the tryptic ferment being able to attack the first part of the peptone with ease, but the other parts with greater and greater difficulty, whilst the ereptic ferment is more even in its action. In kept extracts the erepsin seems to undergo a slow destruction by the trypsin even at room temperature, and hence the peptone-splitting value steadily deteriorates, though the tryptic value may be almost unchanged.

In order to calculate roughly the relative amounts of peptone-splitting ferment present, we can make use of the law of inverse proportion between time and ferment arrived at in the previous section. But obviously we should get very different results according as we took the times of digestion of 20 % of the peptone, or of a higher amount. The 20 % values are theoretically the best as they afford the best measure of the original peptone-splitting power of the extracts before the trypsin has had much time to destroy the erepsin. Unfortunately the actual times are so short that it is not possible to read them off from the curves with much accuracy. Still they must needs serve, and the peptone-splitting value has been calculated from them by means of the formula:

$$\text{P-S} = \frac{10}{\text{volume of extract in c.c.} \times \text{digestion time}}.$$

In the present observations, the volume of diluted extract used was ·5 c.c., or the equivalent of ·375 c.c. of undiluted extract (this being added to 5 c.c. of 5 % peptone and made up to a total volume of 10 c.c.). The values are given in the last column but one of the above table, whilst in the last column are given the values calculated from both 20 % and 30 % digestion times. It was found that as a rule, and roughly speaking, the 30 % digestion time was about three times as great as the 20 % digestion time. Hence the 30 % times given in the table have been divided by 3, and a mean taken between them and the 20 % times. The peptone-splitting values derived from these means are

very similar to the others, and hence either series may be accepted as representing the peptone-splitting values of the extract.

Other series of observations upon the tryptic and peptone-splitting powers of zymogen extracts have been made in the case of pig's and cat's pancreas. The accompanying table shows the results obtained with a 75 % glycerin extract of pig's pancreas which had been diluted with a quarter of its volume of water. The tryptic power of the extract increased sevenfold in seven days, and meanwhile the peptone-splitting power—as judged by the 20 % digestion times—had increased by a

Pig's pancreas extract diluted with water	Tryptic value	Peptone-splitting value	Hours required to digest			
			20 p.c.	30 p.c.	40 p.c.	50 p.c.
0 day	7·9	18	2·2	5·5	12·0	32
1 day	11·9	17	2·3	5·5	11·9	32
5 days	44·4	22	1·8	5·2	12·4	31
7 ,,	56·6	27	1·5	5·0	13·0	38
12 ,,	71·3	21	1·9	5·9	16·0	45
22 ,,	68·0	22	1·8	5·5	19·0	47

half. Judged by the 30 % digestion times, there was practically no alteration of peptone-splitting power throughout. Even the 50 % digestion times kept constant for 5 days, but they then began to increase rapidly.

The next table shows the results obtained with a concentrated glycerin extract of pig's pancreas which had been mixed with an equal volume of water. This greater dilution caused the tryptic power to develop more rapidly. The peptone-splitting power also increased

Pig's pancreas extract diluted with water	Tryptic value	Peptone-splitting value	Hours required to digest			
			20 p.c.	30 p.c.	40 p.c.	50 p.c.
0 day	8·2	13	3·0	8·2	17·2	31
3 days	33·4	20	2·1	6·0	14·3	30
6 ,,	64·6	27	1·5	5·2	14·3	36
9 ,,	66·8	29	1·4	5·0	13.6	37

more rapidly than before, and after 9 days had more than doubled its original value. The rates of digestion both of 30 % and of 40 % also increased with the tryptic power, but that of 50 % slowly diminished as in other cases.

In the three series of observations thus far recorded, the extracts used contained a fair amount of free trypsin from the very outset, hence it might be thought that this trypsin was mainly responsible for the initial peptone-splitting. The next table shows the data obtained with a glycerin extract of cat's pancreas, which had a moderate peptone-

splitting power, but which contained practically no free trypsin at the beginning, and indeed very little at any time. In order to bring about the liberation of the enzyme, a third of a volume of water was added, together with 2 % of an intestinal extract containing enterokinase. This extract of course contained erepsin in addition, but this had very

Cat's pancreas extract	Tryptic value	Peptone-splitting value	Hours required to digest 20 p.c.	30 p.c.	40 p.c.
0 day + water only	—	7·0	3·8	11·6	64
0 day + water + intestinal ext.	·8	7·4	3·6	8·3	24
1 day ,, ,, ,,	2·3	7·8	3·4	7·7	22
3 days ,, ,, ,,	3·5	10·3	2·6	6·7	17
11 ,, ,, ,, ,,	6·4	7·4	3·6	9·3	32

little effect on the 20 % digestion time. The maximum peptone-splitting power of this extract was about a third that of the previous extracts, whilst its maximum tryptic power was not a tenth as great. In other words, it contained a moderate amount of erepsin, but very little trypsin.

It is not possible, from the various data given above, to determine with any exactness how much of the peptone-splitting power of an extract is due to trypsin, and how much to erepsin. In the first series, during the liberation of trypsin from trypsinogen, the 20 % peptone-splitting values increased from 19 to 38; in the second, from 18 to 27; and in the third, from 13 to 29. On an average, therefore, they became nearly doubled, and hence one may conclude that roughly half of the initial peptone-splitting power of a comparatively fresh pancreatic extract is due to the trypsin, and half to the erepsin. We have seen, however, that the final amounts of peptone split up are almost always smaller in the presence of the free trypsin than with erepsin only. Hence it is practically impossible in any given case to say how much of the digestive action must be ascribed to one ferment, and how much to the other. It depends on the stage of digestion reached, and the further advanced this stage the relatively greater the proportion of peptone-splitting accomplished by erepsin and the relatively less by trypsin.

A source of error which has to be carefully investigated and defined in experiments with zymogen extracts lies in the fact that a certain amount of the zymogen undergoes conversion into enzyme during the course of digestion. The estimation of the tryptic value of an extract by fibrin digestion takes only about half-an-hour, and hence there is not sufficient time for the liberation of much enzyme. Peptone

digestions on the other hand extend over two days, or a sufficient time for the whole of the enzyme to be liberated. It was therefore necessary to determine the actual rate of liberation of tryptic enzyme during the course of peptone digestion by a zymogen extract. Some of the concentrated glycerin extract of pig's pancreas mentioned above was used, and some of the same extract after it had been kept for 9 days with an equal volume of water, and had in consequence attained its full tryptic power. The tryptic value of the extract was always determined in the presence of equal amounts of peptone solution, which to some extent retarded its action upon fibrin. From the table we see that the

Pig's pancreas	Tryptic value of zymogen extract	Tryptic value of enzyme extract
Originally	6·6	63·9
After 2 hours at 38° with peptone	13·8	61·6
After 25 „ „ „	27·2	51·2

zymogen extract had doubled its tryptic value after it had been kept for 2 hours at 38° with the peptone solution, whilst after 25 hours it had quadrupled it. Still even after this period the tryptic value was only half as great as that of the liberated enzyme extract kept under similar conditions with peptone. This enzyme solution had, in fact, undergone comparatively little deterioration, the actual loss of tryptic value being only 3·6 % after 2 hours, and 20 % after 25 hours. This result seems to contradict previous experience[1], which showed that in the presence of ·4 % Na_2CO_3 about 70 % of the enzyme might be destroyed in an hour, and in the presence of water only, about 50 %. The explanation is that the peptone exerts a marked protective influence over the enzyme. The protective action of proteids and peptones upon trypsin has been pointed out by Biernacki[2], and more recently by Bayliss and Starling[3]. I hope to discuss the matter more fully in a subsequent paper.

The rates of digestion of the peptone by the zymogen and enzyme extracts, which are given herewith, show that though the earlier stages

	Hours required to digest				
	20 p.c.	30 p.c.	40 p.c.	50 p.c.	60 p.c.
Zymogen extract	1·3	3·1	6·8	13·1	23·0
Enzyme extract	·7	1·7	4·8	12·3	28·5

of digestion by enzyme were more rapid, yet the final stage was distinctly slower in spite of there being twice as much free enzyme

[1] This *Journal*, xxvii. p. 283. 1901.
[2] *Zeit. f. Biol.* xxviii. p. 55. 1891.
[3] This *Journal*, xxx. p. 71. 1903.

present. Presumably, therefore, a large amount of the erepsin had been destroyed by the trypsin, though it is possible that some of the retardation of the digestion was due merely to the collection of greater quantities of digestion products, produced by the activity of the greater amount of trypsin. It should be mentioned that the very rapid rates of digestion of the peptone in these experiments were due to the fact that four times as much extract was added as before. This was done in order to render the determination of the tryptic value of the digesting mixtures more easy.

A similar series of observations was carried out in the case of the glycerin extract of sheep's pancreas already referred to. By the time that the portion of extract mixed with water had attained its full tryptic power (or after 20 days) the undiluted extract had spontaneously attained nearly double its original tryptic value. Hence the conditions of experiment were somewhat different from those obtaining in the original series of comparisons of tryptic and peptone-splitting powers, and they differed also in that the digestions were carried out with filtered Witte-peptone, and not peptic peptone. From the figures given in the table, we see that the zymogen extract had increased

Sheep's pancreas	Tryptic value of zymogen extract	Tryptic value of enzyme extract
Originally	18·8	81·1
After 2 hours at 38° with peptone	32·1	75·5
After 25 ,, ,, ,,	23·5	27·2

in value by about 70 % after being 2 hours at 38° with the peptone, but it then diminished. As compared with the pig's pancreas observations, there was a very rapid destruction of ferment. Thus the enzyme extract had lost 66 % of its tryptic power at the end of 25 hours, and in fact possessed only a slightly higher tryptic value than the zymogen extract. The rates of digestion of the peptone show the same kind of relationship as the previous series, the enzyme extract digesting more rapidly at first, and less rapidly afterwards.

	Hours required to digest				
	20 p.c.	30 p.c.	40 p.c.	50 p.c.	60 p.c.
Zymogen extract	·6	1·9	5·2	11·5	21·0
Enzyme extract	·5	1·7	4·6	11·3	25·0

The tryptic and peptone-splitting powers of kept extracts.

It has been shown in previous papers[1] that if pancreatic extracts be kept for days or months and tested from time to time, the tryptic and rennetic powers, being independent of each other, often both increase and diminish at very different rates. The same thing holds as regards the tryptic and peptone-splitting powers. Some minced pig's pancreas was divided into two portions, and one portion was mixed with 3 parts of glycerin and 1 of water, and the other with 3 of water and 1 of methylated spirit. From time to time small samples of the extracting liquids were filtered off, and tested in respect of both tryptic and peptone-splitting powers. The glycerin extract showed comparatively little variation of peptone-splitting power throughout, although the tryptic power gradually increased from 7·9 to 100. The dilute alcohol

Time of extraction (glycerin)	Tryptic value	Peptone-splitting value (20 + 30 p.c.)	Hours required to digest			
			20 p.c.	30 p.c.	40 p.c.	50 p.c.
3 days	7·9	20	2·2	5·4	12·5	31
13 ,,	30·7	23	1·9	4·7	10·7	25
20 ,,	58·3	30	1·4	3·8	9·3	24
36 ,,	100	26	1·6	4·3	11·1	29

extract, on the other hand, reached almost its full tryptic power after 2 days, the actual maximum—attained after 6 days—being only half as great as that of the glycerin extract. The peptone-splitting power varied almost *pari passu* with the tryptic, and reached a value half as great again as the maximum attained by the glycerin extract. The final ratio between tryptic and peptone-splitting powers was thus very different,

Time of extraction (alcohol)	Tryptic value	Peptone-splitting value (20 + 30 p.c.)	Hours required to digest			
			20 p.c.	30 p.c.	40 p.c.	50 p.c.
16 hours	21·3	26	1·8	4·0	10·1	24
2 days	43·1	35	1·1	3·6	8·5	20
6 ,,	46·9	41	·9	3·1	9·6	24
12 ,,	43·2	44	·8	3·0	9·8	25
18 ,,	35·3	26	1·6	4·4	12·4	40
28 ,,	27·0	26	1·4	5·0	17·8	43
39 ,,	23·7	24	1·4	5·7	21·0	49

it being as 3·8 to 1 in the glycerin extract, and as 1·0 to 1 in the alcoholic. Glycerin therefore proved to be the best preservative of the tryptic ferment, and dilute alcohol of the peptone-splitting ferment. Observations with other extracts gave a similar result. It was found that

[1] This *Journal*, XXVII. p. 269, 1901; and XXVIII. p. 448, 1902.

generally speaking the longer a glycerin extract were kept, especially if in contact with the gland substance, the higher and higher became the ratio between tryptic value and peptone-splitting value. The accompanying table gives the ferment values of various glycerin extracts of pig's

Time of contact with gland substance	Tryptic value	Peptone-splitting value (20 + 30 p.c.)	T ÷ P-S
4 days	69·6	21	3·3
13 ,,	65·7	29	2·3
36 ,,	100	26	3·8
5 months	156	45	3·5
9 ,,	160	30	5·3
11 ,,	145	30	4·8

pancreas which were filtered off from the gland substance at the specified periods, and, if they still contained tryptic zymogen, allowed to undergo complete conversion into enzyme before testing.

As regards the preservative action of alcohol on the peptone-splitting ferment, several extracts of pig's pancreas which had been filtered off from their gland substance and kept at room temperature for 15 months were tested. Two of the extracts, which had lost 68 and 66 % of their tryptic power, had ratios of tryptic value to peptone-splitting value of 1·4 and 2·1 respectively. Two other extracts, which had lost 78 and 84 % of their tryptic power, had ratios of ·8 and ·7 respectively. The tryptic power of these extracts had therefore diminished at a greater rate than the peptone-splitting power.

This seemingly destructive action of glycerin and preservative action of alcohol upon the peptone-splitting ferment is probably connected with the presence of the trypsin, for no such relationship holds as regards the intestinal peptone-splitting ferment. As in the case of the tryptic, rennetic, and diastatic ferments of the pancreas, glycerin was found to be a better preservative than dilute alcohol. The following data were obtained with mucous membrane scraped off from pig's intestine,

Extracting liquid	Days of extraction	Peptone-splitting value	Hours required to digest		
			20 p.c.	30 p.c.	40 p.c.
Glycerin	3 days	18	1·9	7·7	22
,,	11 ,,	21	1·9	6·0	32
,,	24 ,,	17	2·1	8·1	32
,,	41 ,,	12	2·7	12·0	35
30 % alcohol	16 hours	10	3·9	11·9	28
,,	2 days	16	2·3	8·5	22
,,	4 ,,	13	3·1	9·9	22
,,	14 ,,	8·5	4·6	14·4	35
,,	28 ,,	8·3	4·3	16·0	36

one portion of which was mixed with two parts of pure glycerin, and another with two parts of 30 % alcohol. Small samples were filtered off and tested from time to time as in the case of the pancreatic extracts. The glycerin extract reached its maximum peptone-splitting power after 11 days, and then slowly deteriorated, whilst the alcohol extract reached its maximum after 2 days, and then deteriorated somewhat quickly. It will be seen that these data afford no evidence of the existence of a soluble zymogen of the peptone-splitting ferment comparable to trypsinogen. The increase of ferment power shown just at first is due either to physical causes (*i.e.* the slow soaking out of the ferment from the tissue substance) or more probably to the ferment quickly splitting off from an insoluble zymogen, in some such manner as does the diastatic ferment of the pancreas[1]. In some other cases the extracting liquid was filtered off from the intestinal mucous membrane after a day or two, and tested from time to time, but it was never found to show any increase of peptone-splitting power. Hence probably intestinal erepsin has no soluble zymogen. It has been assumed that the same is true of pancreatic erepsin, and that the whole of the increase of peptone-splitting power observed in kept extracts is due to the liberation of trypsin. Possibly this view is erroneous, but absolute proof in either direction is almost impossible. Certainly the data quoted fall most readily into line with the explanation given.

The comparative stability of trypsin and of peptone-splitting ferments.

The differences in the relative stabilities of the trypsin and erepsin of pancreatic extracts were examined by a method frequently used in previous work. This consists in keeping some of the extract with ·4 % sodium carbonate at 38°, and determining the amounts of ferment remaining undestroyed at the end of one or more hours. In order as far as possible to avoid error in the estimation of the peptone-splitting power, larger and larger quantities of the extract were used according as more and more of the ferment present was destroyed, so that the digestion rates of the peptone were in all cases nearly the same. Also, in order to avoid the disturbing effects which increasing quantities of extract might exert by reason of their containing digestion products and glycerin, the amount of extract added was always small. It was, for instance, ·02 c.c. in the case of the original extract, and ·20 c.c. after the extract had been kept for 24 hours with Na_2CO_3 at 38°. The

[1] Cf. this *Journal*, XXVIII. p. 137. 1902.

times of digestion of even 20 % of the peptone accordingly extended to from 5·0 to 7·3 hours. From the data given in the table we see

Time of exposure to ·4 p.c. Na_2CO_3 at 38°	Tryptic value	Per cent. of trypsin destroyed per hour	Peptone-splitting value	P.c. of peptone-splitting ferments destroyed per hour	T ÷ P-S
0 hour	135·5		69		2·0
		61		40	
1 „	53·1		41		1·3
		25		21	
2¾ hours	30·3		26		1·2
		6·6		4·2	
9¼ „	17·3		19		·9
		2·9		4·6	
24 „	9·8		6·2		1·6

that of the trypsin 61 % was destroyed in the first hour, but of the peptone-splitting ferments, only 40 %. During the next 1¾ hours the tryptic ferment was destroyed at the rate of 25 % per hour (the destruction rate being calculated on the tryptic value of the extract at the end of the first hour), and the peptone-splitting ferments at the rate of 21 % per hour; during the next 6½ hours, the tryptic ferment at 6·6 % per hour, and the peptone-splitting at 4·2 %. The rapid increase in the stability of the trypsin still remaining undestroyed, which has been discussed at length in previous papers, is thus found to hold likewise in the case of pancreatic erepsin. It is to be noticed, however, that during the first 9 hours the peptone-splitting ferment always suffered less destruction than the trypsin. During the final 15 hours it was destroyed more rapidly than the trypsin, so that ultimately there was not much more peptone-splitting ferment left than trypsin. The ratios between the two ferments are given in the last column of the table, and we see that after 9 hours' treatment with Na_2CO_3 so much trypsin was destroyed that the extract contained—relative to peptone-splitting ferment—less than half its original proportion of trypsin. The final rise in the ratio is curious and unexpected, but I believe it is none the less genuine. Thus a repetition of the experiment with another glycerin extract of pig's pancreas gave an almost identical result.

Series of observations were likewise made with intestinal extracts. The destructive effect of ·4 % Na_2CO_3 at 38° upon the ferment was much slighter than in the case of the pancreatic ferments, as may be gathered from the data given in the table. These were obtained with a glycerin extract of the mucous membrane of pig's intestine. During the first hour 33 % of the ferment was destroyed, or not much less than in the case of the pancreatic extract; and in the next two

hours, 13 % per hour. After that, however, the ferment remained almost unchanged, only 10 % of it being destroyed in 21 hours. The final peptone-splitting value of the extract after 24 hours was 45 % of its original amount, whilst in the pancreatic extract above mentioned it was only 9 %. The very much greater destruction of ferment

Intestinal extract	Time of exposure to ·4 p.c. Na_2CO_3 at 38°	Peptone-splitting value	Per cent. of ferment destroyed per hour
Pig	0 hour	16·1	
			33
	1 ,,	10·8	
			13
	3 hours	8·0	
			1·9
	9 ,,	7·1	
			0
	24 ,,	7·2	
Cat	0 hour	10·0	
			38
	1 ,,	6·2	
			4·7
	3 hours	5·8	

observed with the pancreatic extract must almost certainly be due in large part to the digestive action of the trypsin upon the erepsin.

In the lower half of the table are given the results obtained with a glycerin extract of cat's intestine. So far as the incomplete data can show, the stability of the undestroyed ferment increased even more rapidly than before.

The precipitability of trypsin and pancreatic erepsin by alcohol.

An attempt was made to separate the trypsin and erepsin of pancreatic extracts by means of fractional alcohol precipitation. Measured quantities of a 75 % glycerin extract of pig's pancreas were mixed with 1, 1·5, 2 and 3 volumes of absolute alcohol, and after standing 24 hours the precipitates were filtered off, and the filter papers containing them placed in known quantities of water. The precipitates completely dissolved in 2 hours, and at the end of this period the tryptic and peptone-splitting powers of the solutions were determined. Care was taken to use such quantities of solution as gave almost equal rates of digestion of peptone, each determination being performed in duplicate with in the one case twice as much ferment solution as in the other. The curves of peptone digestion obtained corresponded very closely, and so the data given in the right half of the table represent the relative peptone-splitting powers of the solutions of the precipitates with

considerable accuracy. It will be seen that they increase regularly with the strength of alcohol used for precipitation, just as do the tryptic

	Filtrate			Precipitate dissolved in water		
	Tryptic value	Peptone-splitting value	T ÷ P-S	Tryptic value	Peptone-splitting value	T ÷ P-S
Glycerin ext. (+·67 c.c. alcohol)	87·7	69	1·3			
1 of ext. to 1 of alcohol	72·8	21·0	3·5	15·3	8·8	1·7
1 ,, 1·5 ,,	41·3	9·1	4·5	37·6	20·2	1·9
1 ,, 2 ,,	10·9	1·6	6·8	69·0	31·7	2·2
1 ,, 3 ,,	0			83·3	32·4	2·6
Glycerin ext. alone				135·5	61	2·2

values, though the ratios between the two ferments are not quite constant. In the left half of the table are given the tryptic and peptone-splitting powers of the filtrates. In determining these values, care was taken that the digestions should always be carried out in the presence of equal quantities of alcohol. The fibrin digestions were made in the presence of ·67 c.c. of alcohol, and this reduced the tryptic value by 35 %. The peptone digestions contained only ·2 c.c. of alcohol, and as this had scarcely any effect on the digestion rate, the apparent ratio of tryptic value to peptone-splitting value became considerably lowered. We see that in the filtrates the ratio between the two ferments underwent a very great and progressive rise. Compared with the original extract, the filtrate from a mixture of equal quantities of extract and alcohol contained relatively nearly three times more trypsin than peptone-splitting ferments, and that from a mixture of extract with twice its volume of alcohol five times more. The actual differences of precipitability of trypsin and erepsin must be much greater than this, for it is to be remembered that about half of the initial peptone-splitting power of an extract is due to the trypsin alone. Perhaps, therefore, the filtrates from extract and 1·5 or 2 volumes of alcohol contained no erepsin whatever, but owed the whole of the little peptone-splitting power they possessed to trypsin. As pancreatic erepsin is so much more readily precipitated by alcohol than trypsin, we should expect the solutions of the precipitates to contain relatively more erepsin than trypsin. The ratio of trypsin to peptone-splitting ferment in the original extract was 2·2, and the ratios in the solutions of the 1 : 1 and 1 : 1·5 precipitates were respectively 1·7 and 1·9. In their case, therefore, a relatively greater amount of erepsin was present. However the solutions of the 1 : 2 and 1 : 3 precipitates had ratios of 2·2 and 2·6 respectively, or contained relatively more trypsin than erepsin.

This discrepancy of result may perhaps be due to erepsin being a less stable ferment than trypsin when precipitated by alcohol and redissolved in water. As has been shown in a previous paper[1], and as may be gathered from the contents of the above table, only about 60 % of the trypsin precipitated is subsequently recovered. In the present instance it appears that of the peptone-splitting ferment only about 54 % or less was recovered. In any case, the differences in the relative amounts of trypsin and erepsin present in the alcohol filtrates from those in the original extract are so striking that there can be no doubt as to the difference in the precipitability of the two ferments. It was found by Cohnheim[2] that intestinal erepsin was much more readily precipitated by ammonium sulphate than was trypsin, so the two erepsins probably have similar precipitabilities.

A few observations were made with a glycerin extract of sheep's pancreas, and it exhibited just as great a difference in the precipitability of its two ferments as had the pig's pancreas extract. The filtrates from mixtures of 1 part of extract with 1·5 and 2 parts of alcohol both had five times more tryptic power than peptone-splitting power, as compared with the original extract.

The differences of action of pancreatic and intestinal peptone-splitting ferments.

Judged by the course of action of pancreatic and intestinal extracts upon partially digested peptone, pancreatic and intestinal erepsin must be regarded as entirely distinct ferments. It has already been mentioned that some of the digestions were carried out upon filtered Witte-peptone solution, and others upon peptone which had previously been digested for a week with pepsin and ·2 % HCl. This treatment had very little effect upon the biuret test yielding groups, less than 5 % of them being split up; but it seemed to have converted most of the albumoses present into peptone. Thus the addition of five volumes of saturated ammonium sulphate solution caused only a marked opalescence, of about the same density as was produced by the addition of half a volume of the sulphate to the undigested peptone. Comparative experiments upon these two samples of peptone with various pancreatic and intestinal extracts showed that the undigested peptone was most readily attacked by pancreatic extracts, and the digested, by intestinal

[1] This *Journal*, xxix. p. 314. 1903.

[2] *Zeit. f. physiol. Chem.* xxxiii. p. 438.

extracts. From the data given in the table, we see that pancreatic extract digested the Witte-peptone solution most rapidly, filtered Witte-

Extract	Peptone acted upon	Hours required to digest 20 p.c	30 p.c.	40 p.c.	50 p.c.
Alcohol ext., pig's pancreas	Witte	1·7	4·3	9·7	30·8
	filtered Witte	1·8	4·6	12·3	40·1
	peptic	1·9	5·2	21·5	58·5
Glycerin ext., cat's intestine	Witte	3·4	8·5	24·0	52·0
	filtered Witte	4·0	9·3	30·0	70·0
	peptic	5·0	12·0	25·2	41·6

peptone next, and peptic peptone the least rapidly. It is not known why the filtration of the Witte-peptone solution should have diminished its digestibility, but perhaps the small quantity of solid matter exerted a protective influence on the ferment. Intestinal extract likewise digested unfiltered Witte-peptone solution more rapidly than filtered. In the initial stages it digested peptic peptone most slowly of all, but in the later stages most quickly of all. Some other extracts showed more striking differences of digestion rate than those indicated in the table. For instance an extract of sheep's intestine after 48 hours had digested 51 % of peptic peptone, and only 39·2 % of filtered Witte-peptone, whilst on the other hand extract of sheep's pancreas had digested only 45·4 % of peptic peptone, but 58·1 % of filtered Witte-peptone.

The difference between the two peptone-splitting ferments is brought out much better by comparing their rates of digestion of peptone already partially digested. Portions of the 5 % filtered Witte-peptone solution were kept for days or even weeks at 38° (in the presence of toluol) with small quantities of glycerin extract of pig's pancreas, and of cat's intestine. After about 50 % of the peptone had been split up in each case, samples were withdrawn and heated to boiling so as to destroy the ferment. The remainders were still further digested until 72 % of the peptone was destroyed, when they too were heated to boiling. Still another portion of the Witte-peptone was digested for a week with pancreatic extract, whereby 42 % of the peptone was destroyed, and then for a fortnight with some intestinal extract, whereby another 39 % of the peptone was destroyed, or 81 % in all. The rates of digestion of these various samples of partially digested peptone by a glycerin extract of pig's pancreas are shown in the accompanying figure. The same proportion of extract to peptone solution was used in each case, and the ferment always acted in the presence of ·1 % Na_2CO_3. In estimating

the rates of digestion, each sample of peptone constituted its own standard, so that these curves represent the percentage rates of destruction of the peptone on that present at the beginning of the digestions. We see that, as compared with normal undigested Witte-peptone

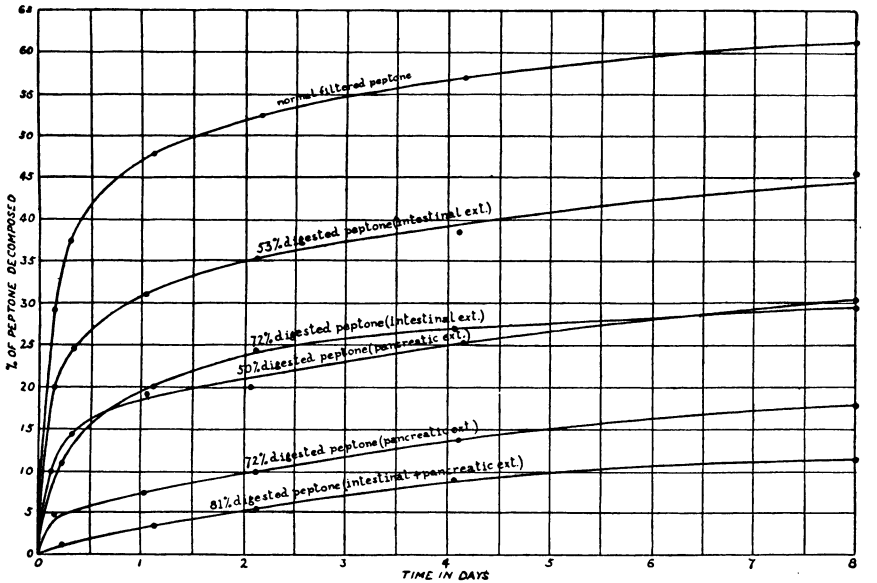

Fig. 5. Action of pancreatic extract.

solution, peptone of which 53 % had been previously split up by intestinal extract was attacked very much more slowly. Thus to digest 40 % of it eleven times as long a period was required. Peptone of which 72 % had previously been digested by intestinal extract was acted on very much more slowly still, as can be seen from the curve. Slowest of all, however, was the digestive action of pancreatic extract upon peptone already partially digested by pancreatic ferments. Peptone of which 50 % had been previously digested by pancreatic extract was not acted

Nature of peptone	Hours required to digest				Relative digestion rates
	10 p.c.	20 p.c.	30 p.c.	40 p.c.	
Filtered Witte	·5	1·6	3·6	9·5	1
53 p.c. destroyed by intestinal ext.	1·0	3·4	21	105	2 to 11
72 ,, ,, ,,	4·1	26			8 to 16
50 ,, ,, pancreatic ext.	2·8	37	182		5·6 to 51
72 ,, ,, ,,	51				102
81 p.c. destroyed by pancreatic + intestinal ext.	122				244

upon more rapidly than peptone of which 72 % had been digested by intestinal extract. The peptone of which 42 % had previously been digested by pancreatic extract and 39 % by intestinal extract was acted upon with extreme slowness, and even after 8 days had suffered a loss of only 11·6 %. The times of digestion of various percentages of peptone are given in the table, and from them have been calculated the relative rates of digestion of the various samples of peptone. The times of digestion of 10 %, etc., of the normal Witte-peptone were in all cases taken as unity, and the extreme variations in the rates of digestion calculated in relation to them.

The results obtained in the corresponding digestions with intestinal extract are shown in the next figure. Here it will be seen that so far from partially digested peptone being attacked more slowly, it was in some cases broken down more readily than the undigested peptone. Peptone of which 50 % or 72 % had previously been split up by pancreatic extract was further decomposed to the extent of about 61 % in

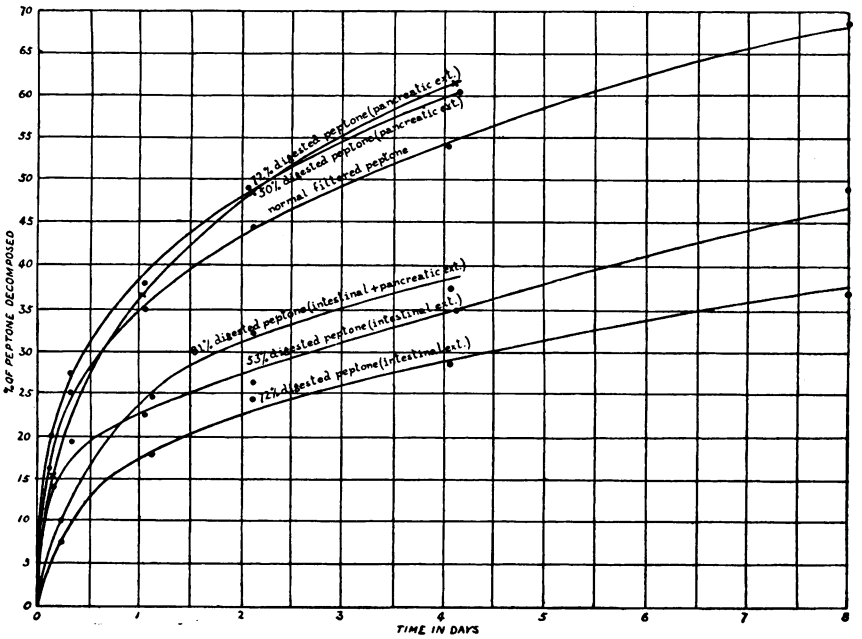

Fig. 6. Action of intestinal extract.

four days, whilst of the previously undigested peptone only 54 % was split up in the same period. The contrast between the action of the two peptone-splitting ferments is thus very marked. Intestinal erepsin

holds all peptones alike, and digests every portion of them with equal ease, provided no products of its own action have accumulated. The pancreatic peptone-splitting ferments, on the other hand, have a differential action, and digest the first half of the peptones 2 to 11 times faster than the second half, and 8 to 16 times faster than the last quarter. Kühne's well-known hypothesis of the existence of hemi- and anti-peptone in pancreatic digestion is thus to some extent supported, though as far as my observations can show the peptones are not sharply divisible into two halves. Siegfried and his pupils[1] have recently isolated and analysed several different peptones produced by peptic and tryptic digestion, and found them to differ in composition. Doubtless they vary also in their powers of resistance to the action of the pancreatic ferments, and probably form a regular ascending series of stability. To intestinal erepsin, however, we must suppose they would all react alike.

The slight increase in the digestibility of the partially digested peptone which the results obtained with intestinal extract indicate, is probably dependent on the conversion of the primary albumoses present in the Witte-peptone into deutero-albumoses and peptone. As Cohnheim has shown, deutero-albumoses are much more readily attacked by erepsin than are primary albumoses, whilst peptone is the most readily attacked of all. A repetition of this experiment with pepsin-digested peptone gave a very similar result, so there can be little doubt of its validity. Thus samples of peptone of which respectively 40 and 54 % had been previously split up by the action of pancreatic extract were found to undergo digestion by intestinal extract at almost identically the same rates, these rates being moreover distinctly quicker than that observed with the peptic peptone itself.

As regards the remaining curves in the above figure, we see that peptone previously acted upon by intestinal extract was digested by the fresh intestinal extract at a much slower rate than the undigested peptone. This is only what previous experience would lead one to expect, the retardation being no doubt due to the accumulation of the products of action of the ferment. Thus we see that the more such products had accumulated, the greater the delay. The 72 % digested peptone was acted upon more slowly than the 53 % digested peptone, but the 81 % digested peptone, in that only 39 % or less of it had been split up by the previous action of intestinal erepsin, was acted upon more rapidly

[1] *Zeit. f. physiol. Chem.* xxxv. p. 164, 1902; and xxxviii. p. 259, 1903.

than the 53 % digested peptone. For some unknown reason, the experimental values obtained with the 53 % digested peptone were distinctly irregular, but as the true digestion-rate curve must almost certainly have resembled the other curves in general contour, the curve in the figure has been drawn upon this hypothesis, and not in strict agreement with the experimental values.

The times of digestion of various percentages of the peptones are given in the table, and it is instructive to compare them with the

Nature of peptone	Hours required to digest						Relative digestion rates
	10 p.c.	20 p.c.	30 p.c.	40 p.c.	50 p.c.	60 p.c.	
Filtered Witte	1·1	4·0	15	37	75	128	1
50 p.c. destroyed by pancreatic ext.	·8	2·9	11	27	55	97	·7 to ·8
72 ,, ,, ,, ,,	1·8	5·8	15	31	55	91	·7 to 1·6
81 p.c. destroyed by pancreatic + intestinal ext.	5·1	17	43				2·9 to 4·6
53 p.c. destroyed by intestinal ext.	1·9	14	65	135			1·7 to 4·3
72 ,, ,, ,, ,,	8·3	34	106				7·1 to 8·5

corresponding values obtained in the pancreatic digestions. The most striking difference of all was obtained with the 81 % digested peptone, for of this 37·5 % was digested in 4 days by intestinal extract, as against 11·6 % in 8 days by pancreatic extract, whilst the 10 % digestion rate was about 24 times greater in the latter case than in the former.

If the general contour of the curves obtained with pancreatic and intestinal extracts be compared, it will be seen that most of the former show a somewhat sharp rise during the first 8 or 10 hours, and then for the remainder of the 8 days a very slow ascent. The intestinal extract curves, on the other hand, show a much more gradual initial rise, followed by a moderately rapid ascent throughout the digestion period. This is no doubt due in large part to the essential differences in the action of the ferments, but it is to be remembered that pancreatic extracts contain two independent peptone-splitting ferments. As already suggested, it seems probable that trypsin can act with rapidity upon the first part of the peptone only. The subsequent hydrolysis would therefore be chiefly attributable to the pancreatic erepsin. Still this ferment likewise acts better upon the first portions of the peptone than upon the last, and so in this respect is probably intermediate between trypsin on the one hand and intestinal erepsin on the other.

Almost as complete a series of observations as that described above was made with pepsin-digested peptone, of which samples were previously

split up with pancreatic extract to the extent of 40 and 54 %, and with intestinal extract to the extent of 40 and 62 %. The results obtained were in complete agreement with those recorded, and so need not be dwelt upon further.

The effects of alkalinity and other conditions on peptone digestion.

Cohnheim states that intestinal erepsin decomposes peptone best in neutral or feebly alkaline solutions, but does not act in feebly acid ones. I have not examined the influence of acids, but have determined the effects of various degrees of alkalinity upon the action of the pancreatic and intestinal ferments. The results obtained with pancreatic extract are given in the left half of the table. It will be seen that the digestion

Medium in which ferment was acting	Glycerin extract of pig's pancreas					Glycerin extract of cat's intestine				
	Hours required to digest			Relative times of digestion of		Hours required to digest			Relative times of digestion of	
	20 p.c.	30 p.c.	40 p.c.	30 p.c.	40 p.c.	20 p.c.	30 p.c.	40 p.c.	30 p.c.	40 p.c.
Water	1·7	5·5	14·6	3·2	8·6	13·0	23·8	41·5	1·8	3·2
·05 p.c. Na_2CO_3	1·4	3·4	10·1	2·4	7·2	6·6	13·2	23·5	2·0	3·6
·1 ,,	1·0	2·4	8·3	2·4	8·3	4·4	9·5	22	2·2	5·0
·2 ,,	·7	1·9	5·6	2·7	8·0	1·5	5·0	22	3·3	15
·4 ,,	·5	1·6	3·8	3·2	7·6	·9	3·5	20	3·9	22
·8 ,,		·8	2·7			·7	4·0	32	5·7	46
1·2 ,,		·4	1·4			·5	3·6		7·2	
2·0 ,,		·5	1·7				3·0			

rate became more and more rapid as the alkalinity increased up to 1·2 % Na_2CO_3. With 2·0 % of Na_2CO_3, however, there was a retardation of the digestion, doubtless produced by excessive destruction of ferment. The final amounts of peptone digested after 2 days in the presence of ·05, ·1, ·2, ·4, ·8 and 1·2 % of Na_2CO_3 were respectively 50·9, 51·6, 52·9, 52·1, 53·5 and 51·2 %, or practically the same whatever the alkalinity. In the presence of 2·0 % Na_2CO_3, however, it was only 48·0 %.

The right half of the table shows the effect of alkalinity upon the action of intestinal extract. Though the digestion rate was favourably influenced by increased alkalinity up to a certain point, yet it appears that even from the first the alkali exerted a destructive influence upon the ferment. At the extreme right of the table are given the relative times of digestion of 30 % and 40 % of the peptone, as compared with the times of digestion of 20 % taken as unity. From these ratios it will be seen that the relative time required to digest 30 % of the peptone became steadily longer and longer as the alkalinity increased, whilst

that required to digest 40 % became longer and longer in still more rapid proportion. That is to say, increasing alkalinity acts in two opposing senses. It more and more accelerates the action of the ferment, but it also more and more rapidly destroys the ferment. Over a certain range of alkalinity these two opposite influences nearly neutralise each other, and we see that 30 % of the peptone was digested in nearly equal periods of time (3 to 4 hours) though the alkalinity ranged from ·4 to 2·0 % Na_2CO_3, and 40 % in nearly equal periods (20 to 23·5 hours) though it ranged from ·05 to ·4 % Na_2CO_3. With an alkalinity of more than ·4 % Na_2CO_3, the final percentage of peptone digested was considerably reduced. Thus after 2 days' digestion with from ·05 to ·4 % Na_2CO_3 the amount split up varied from 50·6 to 51·2 %, but with ·8 % Na_2CO_3 it was only 40·3 %; with 1·2 % Na_2CO_3 only 31·6 %, and with 2·0 % Na_2CO_3, 30·4 %. In the presence of 1·2 % Na_2CO_3, in fact, all digestion ceased after 7½ hours, and in 2 % Na_2CO_3, after 3½ hours. That is to say, in these short periods the whole of the ferment was destroyed.

As compared with the relative digestion times of the intestinal ferment, those of the pancreatic are very instructive. We see that within the limits of experimental error they are nearly constant whatever the alkalinity. In their reaction to alkalinity, therefore, the pancreatic and intestinal peptone-splitting ferments once again seem to prove themselves entirely distinct bodies. However, it should be mentioned that Weinland[1], as the result of a few qualitative observations, came to the conclusion that the two ferments were affected by alkalinity in the same manner, the action of both of them being delayed by the presence of ·4 to 1·2 % Na_2CO_3. This discrepancy of result may be due to the fact that Weinland took the time of disappearance of the biuret reaction as a criterion of ferment activity, and did not estimate the initial rates of peptone-splitting.

It is a curious fact regarding the action of pancreatic extract, that within certain limits the ferment splits up almost exactly the same percentage of peptone whatever the alkalinity of the medium in which it is acting. A few data illustrating this point have been quoted incidentally above, but others were obtained which are still better adapted to show it. These are reproduced in the accompanying figure. Here we see that 8 parts of ferment (a glycerin extract of pig's pancreas) had split up almost equal proportions of the peptone after 2 days, whether acting in water, or in ·1, ·4, or 1·2 % Na_2CO_3. After 4 and 8 days, the

[1] *ibid.*

correspondence in the amounts split up was even closer as regards the action in water and in ·1 to ·4 % Na_2CO_3, but in 1·2 % Na_2CO_3 there

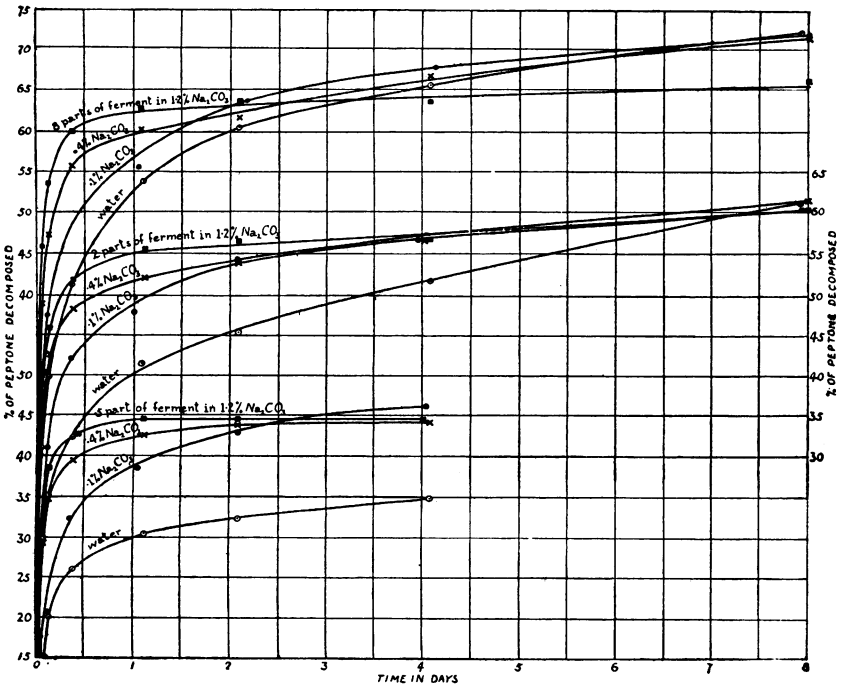

Fig. 7.

was a retardation, due presumably to the destructive action of the alkali on the ferment. The correspondence in the amounts of peptone split up by 2 parts of ferment was closest of all, the actual values obtained after 8 days varying only from 60·4 to 61·5 %. The observations with ·5 part of ferment were continued only for 4 days. This period was sufficient to bring about an equality of peptone-splitting in the digestion mixtures containing ·1 to 1·2 % Na_2CO_3, but in water only the decomposition was still far behind. Presumably the amount of peptone split up in this latter medium would ultimately have coincided with that split up in the alkali, but a very long time would have been required. Thus with 8 parts of ferment the curves of digestion almost coincided after 2 days' action, but with 2 parts, only after 8 days' action. With ·5 part, perhaps 32 days would have been necessary.

All the digestions thus far described were carried out upon pepsin-

digested peptone. Another series made with filtered Witte-peptone gave a like result. Thus 2 parts of ferment after 4 days' action in water, in ·1 % Na_2CO_3 and ·4 % Na_2CO_3, had split up respectively 56·0, 57·0 and 58·3 % of the peptone, and after 8 days' action respectively 61·1, 61·2 and 63·3 % of the peptone, or nearly identical amounts. There can be little doubt, therefore, that under the particular conditions of experiment described the ultimate proportion of peptone split up is within limits dependent solely upon the amount of ferment present. Though in 1·2 % Na_2CO_3 the initial rate of peptone-splitting is some 14 times more rapid than in water, yet we find that after 2 or 8 or more days the actual amount of decomposition effected by the ferment is constant. This is not because the ferment has been destroyed by the more powerful alkali (provided this be not above ·4 % Na_2CO_3 in strength), for we see from the above curves that with 8 parts of ferment the peptone-splitting continued to progress at nearly the same rate from 2 days onwards, whether it was in water, in ·1 % Na_2CO_3 or ·4 % Na_2CO_3. These results on the face of them seem to indicate that the ferment undergoes some definite chemical reaction when hydrolysing the peptone, and in so doing is destroyed. Thus they offer a direct contradiction to the well-known and generally accepted hypothesis that a ferment is a catalytic agent capable of acting indefinitely provided that the products of its action are removed when formed. It is to be remembered, however, that owing to the extreme instability of ferments this hypothesis has never been submitted to adequate proof, and though I do not wish to deny its probable validity, yet I think that the above-quoted results give one some right to question it. It may be pointed out that these digestions were carried out under circumstances which were very favourable for testing the point at issue. The excess of peptone present affords a medium in which the peptone-splitting ferments can exist in a peculiarly stable condition; for, as already recorded, it was found that even after 25 hours at 38° only 20 % of the trypsin was destroyed. Arguing from analogy, the total amount destroyed after 8 days would have been only about 40 %.

It may be said that if the decomposition effected by a ferment is strictly proportional to its amount, then the ·5, 2 and 8 parts of ferment used in the above experiments ought to have split up corresponding proportions of peptone. As it was, they had split up about 45, 57 and 67 % respectively after 4 days' action. We saw, however, in a previous section that all peptones do not hold themselves alike in their reaction to the ferment. The first portion is easily and quickly split up, but

the subsequent portions with ever increasing difficulty. Again, the conditions of action of the ferment in the later stages of digestion are never the same as in the earlier stages, because of the greater and greater accumulation of the products of action. Only under very special conditions, therefore, could there possibly be any numerical correspondence between amount of ferment and degree of action.

As regards the intestinal ferment, no close correspondence between the amounts of peptone split up in various media could be expected, because of the destructive action of the alkali. For instance, after 8 days' digestion of Witte-peptone by extract of cat's intestine, it was found that in ·1 % Na_2CO_3, 67·8 % of the peptone had been split up, but in ·4 % Na_2CO_3, only 48·7 %.

Temperature. All the digestions thus far recorded were carried out at 38°C., but a few observations were made especially to test the effect of temperature. The results obtained with a glycerin extract of pig's pancreas, acting in ·1 % Na_2CO_3 on pepsin-digested peptone, are recorded in the figure. The temperatures given are correct to within ·5°. It

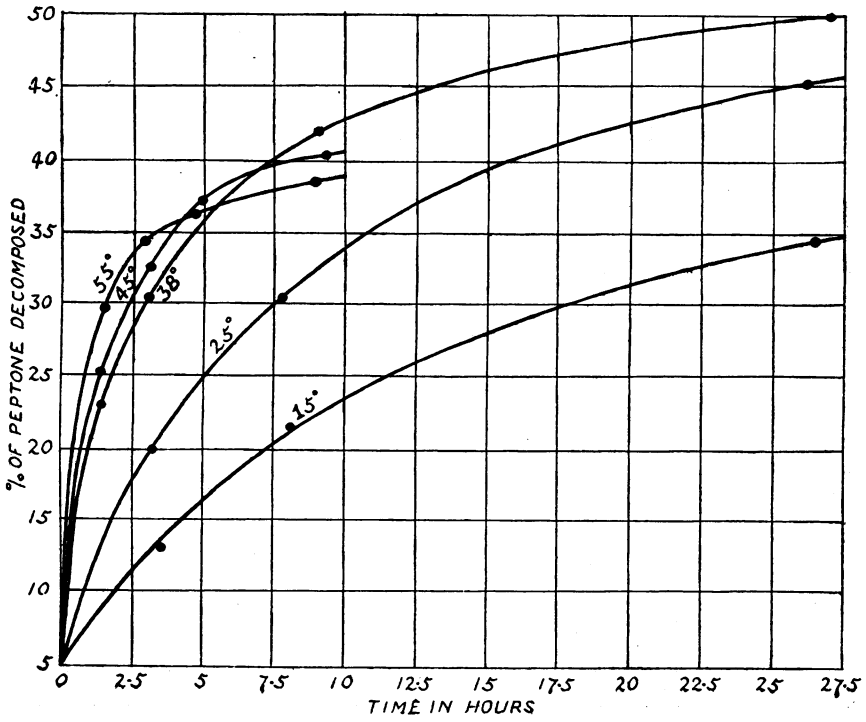

Fig. 8,

will be seen that the rate of digestion increases with the temperature, rapidly from 15° up to 38°, and then more slowly. However, temperatures above 38° act destructively upon the ferment, for we see that after 6 hours' digestion the peptone began to undergo more rapid hydrolysis at 38° than at 45°, the two curves of digestion cutting one another at this point. Again, the 55° curve cut the 45° one after 4·4 hours, and the 38° one after 5·2 hours.

Temperature was found to have practically the same influence on the action of the intestinal ferment as on that of the pancreatic. For the sake of clearness the curves obtained are not given in the figure, but the actual times of digestion at the various temperatures are given in the table. The 20 % digestion times vary from 1·7 to 16·4 hours, or as 1 to 9·6, whilst the 30 % times vary as 1 to 9·5. Both the 45° and the 55° curves cut the 38° one after about 9 hours' digestion. The

	Hours required to digest			Relative times of digestion of 30 p.c. of peptone by	
Temperature	20 p.c.	30 p.c.	40 p.c.	Intestinal extract	Pancreatic extract
15° C.	16·4	31·5		6·8	6·1
25°	5·8	11·8	21·2	2·6	2·7
38°	2·3	4·6	9·2	1·0	1·0
45°	2·0	3·7	9·7	·80	·79
55°	1·7	3·3	9·0	·72	·54

relative times of digestion of 30 % of the peptone by the two ferment extracts are given in the last two columns of the table. Taking the times of digestion at 38° as unity, we see that for all temperatures up to 45° the correspondence is almost perfect. At 55°, however, there is a considerable difference, but as experimental errors are likewise increased at this high temperature, it cannot with certainty be accepted as genuine.

Concentration of peptone. As already mentioned, all the digestions were made with 5 % peptone solution diluted up to twice its volume with alkali and ferment extract. The few observations made with other concentrations of peptone did not seem to indicate the existence of any simple relationship between concentration and digestion time, so they were not continued. For instance, with pancreatic extract the times of digestion of 30 % of the peptone in solutions containing ·625, 1·25, 2·5 and 4·5 % of peptone were respectively 1·4, 2·3, 3·0 and 9·7 hours. Digestion was thus relatively the most rapid in 2·5 % peptone, or the normal concentration, and proportionately slower at lesser or greater concentrations than this. Intestinal extract, on the other hand, seemed to act best in 1·25 % peptone.

Antiseptics. All digestions were carried out in the presence of a small quantity of toluol. In the table are recorded the results of a few observations—made with pancreatic extract—in which chloroform and 1 % sodium fluoride were used instead of toluol. Though the earlier stages of digestion were but little affected, yet in the later stages the chloroform exerted a distinct retardation, and the sodium fluoride a still more considerable one.

	Hours required to digest			
Antiseptic	20 p.c.	30 p.c.	40 p.c.	50 p.c.
Toluol	1·0	3·5	9·2	17·5
Chloroform	1·0	3·0	11·5	26
1 p.c. sodium fluoride	1·0	4·0	26	42

The action of peptone-splitting ferments on native proteids.

Cohnheim states that intestinal erepsin has no action upon proteids such as fibrin, vitellin and serum proteids, but Kutscher and Seemann[1] found that the mucous membrane of carefully washed dog's intestine could digest itself slowly. After keeping some for two months in chloroform water, they found that 53 % of the solid constituents had passed into solution. However, this auto-digestion may have been effected by enzymes derived from the leucocytes present in the tissues, or by traces of trypsin which had been soaked up by the mucous membrane, and not removed by the subsequent washing. Of the extracts used by me, those of cat's and sheep's intestine had no appreciable action on swollen fibrin in 24 hours, whilst that of pig's intestine had a slight action. In the observations to be described extract of cat's intestine was always used, as there was less chance of its being contaminated with trypsin than in the case of the other extracts. Thus the cat's pancreas did not contain a tenth the proportion of trypsin present in sheep and pig's pancreas.

In order to obtain roughly comparable results, equal proportions of glycerin extract of cat's intestine were allowed to act at 38° upon 2·5 % Witte-peptone solution, 3·0 % egg-white, and 2·0 % serum (from ox blood). Digestions were carried out in the presence of water only, of ·1 % Na_2CO_3, and of ·4 % Na_2CO_3, and were continued for 8 days. The amounts of proteid split up were determined colorimetrically, care being taken to keep the mixtures of alkaline copper sulphate, and proteid for about two hours before making the final colour comparison,

[1] *Zeit. f. physiol. Chem.* xxxv. p. 432.

so as to be certain that the maximum biuret tint had been reached. Some of the results obtained are reproduced in the figure. The curves obtained for the digestion of peptone in water have been introduced for the sake of comparison, and it will be seen that they indicate a much more rapid peptone-splitting than occurred with the proteids of blood serum or of egg-white. For the first day or so the action of pancreatic extract upon both serum and egg-white was assisted by the presence of ·1 % Na_2CO_3, but subsequently digestion took place much more rapidly in the absence of alkali. This must have been due to the destructive action of the alkali on the ferment, for in the presence of ·4 % Na_2CO_3 digestion entirely ceased after a few hours. Thus of the serum proteids, 17·0 % was split up in 3 hours in the presence of ·4 % Na_2CO_3, but

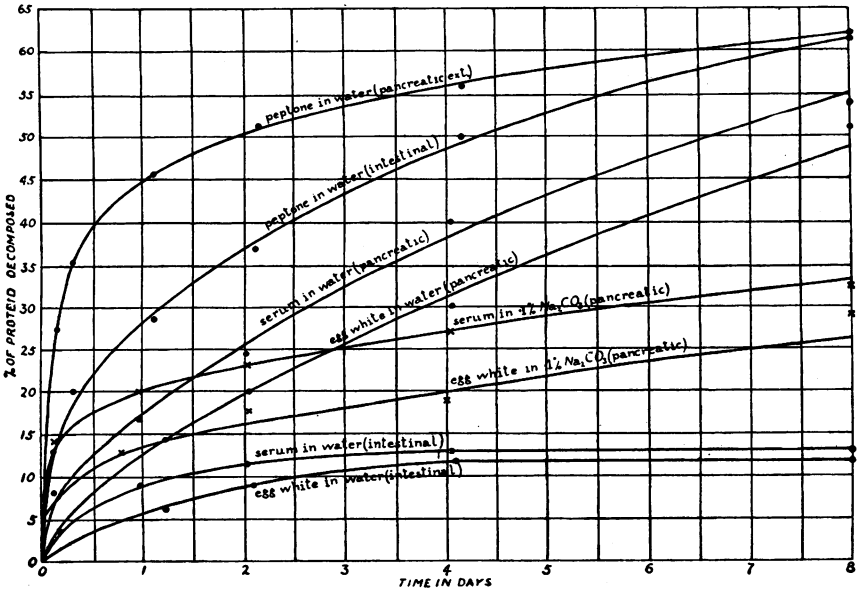

Fig. 9.

only 19·0 % after 4 days, whilst of the egg-white proteids, 9·0 % was split up in 3 hours, and only 10·4 % after 4 days. Even this slight decomposition was effected largely by the alkali, for ·4 % Na_2CO_3 when acting without any ferment whatever split up 6·2 % of the serum proteids, and likewise 6·2 % of the egg-white proteids.

Of the corresponding digestions carried out with intestinal extract, only two are represented in the figure. These show that in the presence of water only, the extract had the power of very slowly

digesting both serum and egg-white proteids. After 4 days' digestion 13·0 % of the serum proteids was split up, and 11·8 % of the egg-white proteids, but then decomposition ceased. In the presence of alkali the biuret-test-yielding groups were split up more readily at first, but digestion entirely ceased after 24 hours. Thus in ·1 % Na_2CO_3 9·2 % of the serum proteids was split up in 4 hours, and 16·0 % in 24 hours, and then digestion ceased, whilst in ·4 % Na_2CO_3 9·1 % was split up in 4 hours, and then digestion ceased. Of these small amounts of decomposition, 3·1 % was due to the ·1 % Na_2CO_3 alone, and, as already mentioned, 6·2 % to the ·4 % Na_2CO_3 alone, so the ferment effected practically nothing when acting in ·4 % Na_2CO_3. Upon egg-white it had no action at all in ·4 % Na_2CO_3, the decomposition being no greater than that effected by the alkali alone. In ·1 % Na_2CO_3 it decomposed 11·8 % of the proteids in 3 hours, and then ceased to act, but of this amount of decomposition 5·4 % was due to the alkali alone.

These observations, so far as they go, may be held to support Cohnheim's view that erepsin does not act upon native proteids; for at best the action is an extremely slow one, and such as it is may be due to other ferments. They also indicate that even the pancreatic ferments act somewhat slowly on native proteids, as compared with their action on Witte-peptone. In fact they show that the complete splitting up of proteids is best effected by the cycle of processes which occurs in the normal animal body; *i.e.* first a hydrolysis to the albumose and peptone stage by pepsin, and then a further hydrolysis to the crystalline decomposition product stage by trypsin, pancreatic erepsin, and intestinal erepsin.

Summary.

The peptone-splitting power of ferments can be estimated colorimetrically by means of the biuret test. If, for instance, twice as great a volume of the partially digested peptone-solution as of the undigested peptone is needed to give the same tint with alkaline copper sulphate when observed in a colorimeter, then 50 % of the peptone must have been split up by ferment action.

It was found that the biuret reaction took an appreciable time to develop its maximum tint. Immediately after adding peptone to copper sulphate and 4 % caustic soda, the tint is only 88 % of the maximum, and it takes 5 to 8 minutes to reach this maximum. With egg-white proteids 12 seconds are required for the tint to reach half its full

value, and with serum proteids 36 seconds are required, whilst the actual maxima are reached only after 12 minutes and 2 hours respectively.

The law of action of the peptone-splitting ferments of the intestine and pancreas was determined by acting upon a 2·5 % solution of Witte's peptone (in some cases previously digested for a week with pepsin) with from 1 to 64 parts of ferment extract for 8 days at 38°, and estimating the amount of peptone destruction from time to time. It was found that the time required to split up any given percentage of the peptone varied inversely as the quantity of ferment; *e.g.* 8, 4, 2 and 1 parts of ferment split up 30 % of peptone in 4·9, 10·2, 20 and 40 hours respectively.

Of the peptone-splitting effected by pancreatic extracts, the larger part is due to pancreatic erepsin, a ferment entirely distinct from trypsin. Thus extracts which have little or no fibrin-digesting power—owing to the existence of the trypsin in the zymogen form—have a considerable peptone-splitting action. When the trypsin has become liberated from the trypsinogen, the extract may have twice as rapid an initial action on peptone, but the amount of peptone-splitting ultimately accomplished by it is always smaller than that by the zymogen extract. This is presumably due to the free trypsin destroying the erepsin.

Neither pancreatic nor intestinal erepsin exists in a soluble zymogen form.

The peptone-splitting and fibrin-digesting powers of kept pancreatic extracts vary independently, trypsin being more stable in glycerin extracts, and pancreatic erepsin in alcoholic. When kept at 38° with ·4 % Na_2CO_3, the trypsin is for the first 9 hours destroyed at a much more rapid rate than the erepsin. However, pancreatic erepsin is destroyed more rapidly than intestinal erepsin, owing to the destructive action of the trypsin on it.

Pancreatic erepsin is much more readily precipitable by alcohol than trypsin. Thus the filtrate from a mixture of extract with 2 volumes of absolute alcohol contained—relative to trypsin—only a fifth as much peptone-splitting ferment as the original extract.

Pancreatic erepsin is a different ferment from intestinal erepsin. Thus when pancreatic extract was allowed to act upon Witte-peptone of which 53 to 72 % had already been split up by the action of intestinal extract, its digestion rate was 2 to 16 times slower than with undigested peptone, and when acting upon peptone of which 50 to 72 % had previously been split up by pancreatic extract, 6 to 100 times slower.

Intestinal erepsin, on the other hand, acted somewhat more rapidly upon peptone of which 50 to 72 % had previously been split up by pancreatic extract than upon undigested peptone, whilst it acted only 1·7 to 8·5 times more slowly on peptone of which 53 to 72 % had previously been split up by intestinal extract. In each case, therefore, the ferment was retarded by the products of its own action, but except for this intestinal erepsin acted equally well upon all portions of the peptone, whilst pancreatic erepsin acted more and more slowly according as less and less of the peptone still remained to be split up.

The action both of intestinal and of pancreatic extracts is accelerated by increasing alkalinity up to ·4 to 1·2 % Na_2CO_3, but the intestinal ferment—in contradistinction to the pancreatic—is at the same time more and more rapidly destroyed. Though the initial rate of peptone-splitting by pancreatic extracts is 14 times more rapid in 1·2 % Na_2CO_3 than in water, yet the final amount of peptone decomposed is almost constant whatever the alkalinity, and seems to depend solely upon the amount of ferment present. Thus 8 parts of ferment acting for 8 days in water, in ·1, ·4 and 1·2 % Na_2CO_3, split up respectively 72·3, 72·0, 71·4 and 66·1 % of the peptone, whilst 2 parts of ferment acting in the same media split up respectively 61·3, 60·4, 61·5 and 60·4 % of peptone.

In confirmation of Cohnheim, it was found that extracts of intestinal mucous membrane had little or no action on fibrin, and very little on egg-white and serum proteids. Even pancreatic extracts had a much slower hydrolysing action on native proteids than upon Witte-peptone.

The expenses of this research were defrayed by a grant from the Government Grant Committee of the Royal Society.