THE PROTECTIVE VALUE OF PROTEIDS AND THEIR DECOMPOSITION PRODUCTS ON TRYPSIN.

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It was shown by Falk[1] that the retardation exerted by a small amount of hydrochloric acid upon the amylolytic action of saliva was much diminished if peptone were present. This might be due, he thought, to the peptone combining with the acid. Chittenden and Ely[2] found that peptone would also prevent sodium carbonate from exerting its retarding action upon saliva to a large extent, whilst Langley and Eves[3] confirmed Falk, and concluded with him that in acid solutions the favouring action of the peptone is due mainly to its combining with the acid.

Observations of Langley and Edkins[4] upon pepsin showed that in the case of this ferment likewise the destructive action of sodium carbonate is diminished by the addition of proteids. This was probably due, they thought, to the alkali combining with the proteid, "for the greater the amount of sodium carbonate present, the greater must be the amount of proteid to lessen appreciably the destruction."

As regards the tryptic ferment, Biernacki[5] has found that albumoses, amphopeptone and antipeptone exert a considerable protective value. The trypsin of pancreatic extracts is entirely destroyed when kept for 5 minutes at 50° C. with from ·25 to ·5 % Na_2CO_3, but if albumoses or peptones be added it has to be heated to 60° before undergoing a similar rapid destruction. Salts such as the sulphate, chloride, phosphate or nitrate of ammonium act similarly, but ammonium carbonate and magnesium and sodium sulphates have no protective

[1] Falk. *Virchow's Archiv*, LXXXIV. p. 119. 1881.

[2] Chittenden and Ely. *Amer. Chem. Journ.* IV. p. 107, 1882, and this *Journal*, III. p. 327. 1882.

[3] Langley and Eves. This *Journal*, IV. p. 18. 1883.

[4] Langley and Edkins. This *Journal*, VII. p. 371. 1886.

[5] Biernacki. *Zeit. f. Biol.* XXVIII. p. 49. 1891.

action whatever. Biernacki observed that the trypsin of true pancreatic juice is much more resistant to temperature than that of pancreatic extracts, owing, he supposed, to the proteids present. Finally Bayliss and Starling[1], working with pancreatic juice, have found that the auto-destruction of the trypsin is markedly diminished by the presence of proteids such as egg albumin, caseinogen and commercial peptone.

We may probably conclude, therefore, that most if not all ferments are to some extent protected from the destructive action of acids or alkalies or high temperature by the presence of proteids. The evidence on the whole seems to indicate that such protection is exerted mainly by the proteid combining with the acid or alkali present, and so preventing it from reacting upon the ferment; but it might also be held to argue that some combination or interaction takes place between ferment and proteid. If such be the case, a detailed examination of the conditions and extent of the protective influence might be expected to yield some evidence as to the nature or mode of action of the ferment molecule. The results described below show that the former alternative is in the main the correct one, though they show that in certain cases a combination of ferment and proteid does occur.

The method employed.

The method of experiment employed was the simple one which has been described in a previous paper[2]. It consisted in keeping a small quantity of a glycerin extract of pig's pancreas for 1 hour at 38° C. in the presence of sodium carbonate and from ·1 to 4 % of the proteid or other substance under observation, the total volume of ferment, alkali, and proteid being in all cases made up to 5 c.c. The mixture was then added to a known amount of finely chopped fibrin (previously allowed to swell for an hour at 38° in 1 % Na_2CO_3) in a centrifugal tube. The tube was filled up to the 10 c.c. mark with water or Na_2CO_3, so that the actual digestion of the fibrin always took place in the presence of ·4 % Na_2CO_3 and *half* the percentage of proteid originally mixed with the ferment. From the time of digestion of 80 % of the fibrin the digestive power of the trypsin was determined in the usual way. From a control experiment in which the pancreatic extract and proteid were run directly into a tube of swollen fibrin, it was possible to calculate the

[1] Bayliss and Starling. This *Journal*, xxx. p. 71. 1904.

[2] This *Journal*, xxvi. p. 405. 1901.

destructive effect of exposure to the action of Na_2CO_3 at 38°, and also to determine how far the digestive power of the trypsin was directly affected by the presence of the proteid.

The proteids used in the experiments to be described were obtained from G. Grübler, and most of the other substances used from E. Merck.

The protective influence of Witte's peptone.

In the experiments made with Witte's peptone the destructive effect of various concentrations of alkali upon trypsin was investigated, and also the effect of water containing no alkali at all. From the data given in the table, we see that when diluted pancreatic extract was kept for an hour at 38° without any alkali or peptone, 25 % of the trypsin was destroyed. In the presence of ·1 % of peptone the amount destroyed was reduced to 12 %, whilst in the presence of 1 % or more of peptone there was no

	Relative tryptic value in presence of *half* the percentage of peptone given	p.c. of ferment destroyed in 1 hour at 38° in presence of			
		water only	·1 p.c. Na_2CO_3	·4 p.c. Na_2CO_3	1 p.c. Na_2CO_3
Pancreatic extract alone	100	25	49	58	67
,, + ·1 p.c. Witte's peptone	99	12	42	53	63
,, ·4 p.c. ,,	97	7	17	44	49
,, 1 p.c. ,,	104	+3	9	25	28
,, 2 p.c. ,,	98	+1	+8	5	13
,, 4 p.c. ,,	88		5	0	+9

destruction whatever. Even a small quantity of alkali greatly affected the stability of the trypsin, for in the presence of ·1 % Na_2CO_3 49 % of the ferment was destroyed. The addition of ·1 % of Witte's peptone now had comparatively little influence on the stability of the trypsin, but the addition of ·4 % reduced the destruction rate to 17 %. Larger quantities of peptone further increased the stability, till with 2 % or less of peptone the ferment underwent no destruction at all. As would be expected, ·4 % and 1 % of Na_2CO_3 destroyed still larger percentages of the trypsin, and the Witte's peptone, though it exerted some protective action even when present in small amount, had to be correspondingly increased in order to protect the ferment absolutely. Thus in presence of 1 % Na_2CO_3 nearly 4 % of peptone was required, and in presence of ·4 % Na_2CO_3 about 2 %.

Though these data show a close parallel to subsist between the relative amounts of alkali and peptone, yet they indicate that the action of the peptone cannot be merely one of chemical neutralisation of the alkali present. As will be shown later, the peptone may roughly

speaking be considered capable of neutralising about half its weight of sodium carbonate, or ·1 % Na_2CO_3 would require ·2 % of peptone for neutralisation, and so on. A solution containing 1 % of Na_2CO_3 and 1 % of peptone would therefore contain ·5 % of free Na_2CO_3, and should accordingly destroy more trypsin than a solution containing ·4 % of Na_2CO_3 and no peptone. As a matter of fact we see that it destroyed only 28 % of the ferment as against 58 %, so the peptone must have continued to exercise a protective influence in spite of its being neutralised. Again, it can be calculated roughly from the above data that in presence of 1 % Na_2CO_3 + 1·8 % of peptone (*i.e.* of ·1 % of free Na_2CO_3) the ferment destroyed would have been about 15 %; in presence of ·4 % Na_2CO_3 + ·6 % peptone about 35 %, whilst in presence of ·1 % Na_2CO_3 and no peptone it was 49 %. Even if it be assumed that the peptone has the power of neutralising an equal weight of sodium carbonate we still find that peptone + excess of alkali has less destructive effect than pure alkali, hence it looks rather as if the peptone combined to some slight extent with the ferment, and so increased its stability.

In the second column of the above table are given the relative digestive powers of the trypsin when acting in the presence of the peptone. Taking the digestive power when no peptone was present as 100, we see that within the limits of experimental error the tryptic value was unaltered by the addition of from ·05 to 1 % of peptone (*i.e.* of ·1 to 2 % of peptone diluted 1 in 2), but that with 2 % of peptone it was diminished by 12 %. The probable explanation of such a very slight retardation of digestion will be given later on.

In a few of the observations made with from 1 to 4 % of peptone it will be seen that the tryptic value was apparently greater after exposure to the action of sodium carbonate at 38° than when not exposed at all. Such discrepancies are due to experimental error. The estimations of tryptic value are liable to an error of about 5 %, and in that the percentages given in the table are dependent on two such estimations, there may be a maximal error in them of 10 %. As a rule, however, the error was much smaller than this.

The protective influence of various proteids.

The protective influence of various native and hydrated proteids upon trypsin was determined in a similar manner, the pancreatic extract being kept for an hour at 38° with ·4 % Na_2CO_3 and from ·4 to 4 % of

the proteid under observation. The percentages of trypsin destroyed are given in the right half of the table, and from them it may be gathered that all proteids have roughly speaking the same protective power. In presence of ·4 % of proteid, whether albumin or a globulin,

Substance	Relative tryptic value in presence of				p.c. of ferment destroyed in 1 hour at 38° in presence of ·4 p.c. Na_2CO_3 and			
	·2 p.c.	·5 p.c.	1 p.c.	2 p.c.	·4 p.c.	1 p.c.	2 p.c.	4 p.c.
Serum albumin	94	73	63	44	41	34	17	13
Paraglobulin	91	89	75	43	42	27	11	6
Crystallin	98	87	87	78	47	29	16	3
Vitellin (vegetable)	102	101	90	86	48	30	15	8
Caseinogen	105	103	97	84	41	21	13	11
Syntonin (muscle)	95	95	92	78	45	32	13	11
Protoalbumose	95	100	86	82	49	29	10	4
Deuteroalbumose	95	103	98	79	50	29	9	6
Antipeptone (Kühne)	102	100	97	84	48	19	8	10
Witte's peptone	97	104	98	88	44	25	5	0
Witte's peptone 81 p.c. hydrolysed	101	105	100	81	39	21	10	8

caseinogen or syntonin, or of a hydrated proteid such as an albumose or peptone, we find that from 40 to 50 % of the trypsin was in all cases destroyed, as against the 56 % destroyed when no proteid at all was present. In the presence of 1 % of the proteid 27 % of the ferment was destroyed on an average; in the presence of 2 % of proteid about 12 %, and in presence of 4 % of proteid, about 7 %. Probably the albumoses and peptones have a slightly greater protective power than the native proteids, for the mean amounts of trypsin destroyed in the presence of ·4, 1, 2 and 4 % of the former bodies were 46·0, 24·6, 8·4 and 5·6 % respectively, whilst the mean amounts destroyed in the presence of similar percentages of albumin, paraglobulin, crystallin, vitellin, caseinogen and syntonin were 44·0, 28·8, 14·2 and 8·7 % respectively. Again, in the last line of the table are given the values obtained with a solution of Witte's peptone of which 81 % had been hydrolysed into products no longer yielding the biuret test by the prolonged action of trypsin and of intestinal erepsin. These values show that proteid decomposition products exert if anything a greater protective action upon the ferment than either hydrated or native proteids.

In the left half of the table are shown the relative digestive powers of the trypsin when acting in the presence of from ·2 to 2 % of the various proteids. Except in the case of serum albumin and paraglobulin very little retardation was exerted. The mean of the tryptic values obtained in the presence of ·2 and ·5 % of all the proteids except

the two mentioned is 99·3, that obtained when no proteid was present being 100. In presence of 1 % of proteid the mean value fell to 93·9, and in presence of 2 % to 82·2, or showed some distinct retardation. The departures from these mean numbers shown by the various values in the table are doubtless due in chief part to experimental error, and so the percentages of ferment destroyed by the ·4 % Na_2CO_3 in the presence of the various proteids were calculated against the mean values, and not the individual ones.

The considerably greater retardation exerted by serum albumin and paraglobulin may have been due to their being contaminated with small quantities of the antiferments which are known to be present in blood serum. As will be mentioned later on, however, there is another proteid, *viz.* egg albumin, which undoubtedly possesses a most marked antitryptic action.

The protective influence of proteid decomposition products.

It has already been pointed out that peptone when hydrolysed into decomposition products seemed to exert even more protective action upon trypsin than when undecomposed. Observations were made in addition upon the protective value of various single decomposition products, as well as of other substances, and the results obtained are collected in the table. As regards proteid decomposition products, we see that aspartic acid and glycocoll had a greater protective value than proteids, the amounts of ferment destroyed in presence of 1 and 2 % of the former body being 16 and 4 % respectively, and in presence of the latter 27 and 7 %, whilst the corresponding mean values for proteids were 27 and 12 % respectively. The protective value of leucin was somewhat smaller than that of proteids, the amounts of ferment destroyed in its presence being 35 and 18 % respectively, whilst urea had no protective value whatever, the amounts of ferment destroyed being 53 and 50 %. The explanation of these marked differences lies in the fact that the protective value which any proteid, proteid decomposition product or other substance exerts upon trypsin depends almost solely on its power of combining with and neutralising the alkali acting upon the ferment. In the second column of the table are given the numbers of parts of sodium carbonate which can combine with 100 parts of each substance, and so be prevented from acting on the ferment. It will be seen that each of the first four substances recorded in the table, *viz.* ammonium chloride, aspartic acid, alloxan and glycocoll,

has a considerable combining power, and so exerts a correspondingly great protective action upon the trypsin. The next five substances, which have only about half as great a combining power, possess on an

Substance	Parts of Na_2CO_3 neutralised by 100 parts of substance	Relative tryptic value in presence of		p.c. of ferment destroyed in 1 hour at 38° with ·4 p.c. Na_2CO_3 and		Mean p.c. of ferment destroyed in 1 hour at 38° with ·4 p.c. Na_2CO_3 and	
		·5 p.c.	1 p.c.	1 p.c.	2 p.c.	1 p.c.	2 p.c.
Ammonium chloride	99	104	82	15	5		
Aspartic acid	80	95	31	16	4		
Alloxan	75	90	28	19	15	19	8
Glycocoll	71	111	94	27	7		
Taurin	42	105	96	20	11		
Asparagin	40	103	97	23	11		
Leucin	40	97	103	35	18	25	11
Hippuric acid	30	103	88	20	10		
Glucosamin hydrochloride	25	112	74	27	4		
Cholalic acid	13	100	111	49	25		
Glycocholic acid	11	104	110	47	23	47	24
Taurocholic acid	10	88	79	44	25		
Creatin		82	90	44	56		
Urea		101	89	53	50		
Sodium acetate		89	81	49	53		
Sodium tartrate		87	84	43	35		
Sodium chloride		96	84	55	41	50	49
Lactose		97	88	51	47		
Maltose		97	89	50	52		
Dextrose		97	100	56	56		

average a distinctly smaller protective action, whilst the next three substances, with only about a seventh the combining power, have a much smaller protective action still. Finally the remaining eight substances in the table, which have no combining power whatever, have correspondingly no protective action. Thus in their presence 50 % of the trypsin was destroyed, whilst when no substance was present at all 53 % was destroyed. The combining or neutralising power of a substance does not necessitate its having an acid reaction, for we see that neutral bodies such as ammonium chloride, glycocoll and leucin acted just as well as the markedly acid aspartic acid or hippuric acid. All these bodies, however, can react with sodium carbonate to form stable sodium salts and free carbonic acid (or in the case of ammonium chloride, ammonium carbonate, and of glucosamin hydrochloride, free glucosamin), but these substances do not destroy the ferment or affect its course of action to any extent.

In the last two columns of the table are given the means of the

percentages of trypsin destroyed in the presence of the various substances. These have been split up arbitrarily into four groups, according to their combining power for sodium carbonate. It will be seen that the protective power of the second group, including such bodies as leucin and hippuric acid, is about the same as the mean protective power of similar amounts of proteids (25 and 11 % of ferment being destroyed in presence of 1 and 2 % respectively of substance, as against the corresponding values of 27 and 12 % for proteids). Now on hydrolysis, proteids are found, according to Cohn[1], to yield about 40 to 50 % of their weight of leucin, whilst casein yields 4·5 % of tyrosin and 30 % of glutaminic acid. Certain of the products of hydrolysis such as glucosamin and ammonia do not react with the alkali at all, and hence we may probably conclude that the total combining power of all the products of hydrolysis of a proteid is somewhat greater than that of leucin and tyrosin, but much smaller than that of glutaminic and aspartic acids. Now 100 parts of the former bodies combine with 40 and 29 parts respectively of sodium carbonate, and of the latter bodies with 72 and 80 parts respectively of sodium carbonate. Hence probably the whole proteid decomposition products have the power of combining with about 50 parts or less of sodium carbonate.

We saw above that the protective value of hydrated proteids was slightly greater than that of native proteids, whilst that of more or less hydrolysed peptone was somewhat greater still. Nevertheless the increase was only slight, and so it would seem as if most of the COOH groupings which are present in the products of proteid decomposition are likewise present as such when forming part of the complex proteid molecule, and in either condition are capable of combining with any molecules of alkali brought in their neighbourhood. This conclusion is at variance with that of Hofmeister[2], who considers that the amido-acid nuclei in the proteid molecule are linked together by an NH_2 grouping of one nucleus uniting with a COOH grouping of another to form a $\rangle CH—NH—CO—$ grouping. If such were the case, then more than half of the COOH groupings would be required for this purpose, and so lose their power of combining with alkali. Accordingly the neutralising power of proteids should be less than half as great as that of their hydrolytic decomposition products.

Probably the protective action of proteids upon ferments is almost

[1] Cohn. *Zeit. f. physiol. Chem.* XXII. p. 153, 1896 and XXVI. p. 395. 1899.

[2] *Ergebnisse der Physiologie*, I. p. 787. 1902.

entirely one of mass action. The ferment molecules presumably have similar structural relationships and affinities to the proteid molecules, and hence when alkali is added to a mixture of proteid and ferment, it attaches itself to these substances in proportion to their relative masses. If a large quantity of proteid is present, then the ferment is unable to annex more than a very small proportion of the alkali, and *vice versa* if only a small quantity of proteid is present. Presumably once the alkali has combined with the ferment it begins to destroy it, the rate of destruction being proportional to the amount of alkali combined.

In order to render more convincing the proof that the protective value of a substance depends on its combining power with alkali, some experiments were made in which the ferment was mixed with protective substance *plus* the amount of sodium carbonate necessary to neutralise it completely. In such a case, as can be seen from the data given in the accompanying table, practically no protective action was exerted. Glycocoll, of which 1 and 2 % previously reduced the amounts of ferment destroyed by sodium carbonate to 27 and 7 % respectively, now permitted the destruction of 54 and 52 % respectively. The other substances investigated showed a similar loss of protective power. Instead of adding ammonium chloride + sodium carbonate, the simpler procedure of adding free ammonia was adopted. The sodium carbonate now caused the destruction of 65 and 62 % of the trypsin, so probably the free ammonia of itself exerted some destructive influence. As can be seen from the data given in the left half of the table, it distinctly

Substance (neutralised)	Relative tryptic value in presence of		p.c. of ferment destroyed in 1 hour at 38° in presence of ·4 p.c. Na_2CO_3 and	
	·5 p.c.	1 p.c.	1 p.c.	2 p.c.
Glycocoll	78	71	54	52
Leucin	78	65	54	41
Hippuric acid	76	72	39	39
Glucosamin hydrochloride	87	85	43	42
(Ammonium hydrate)	90	65	65	62

retarded the digestive action of the ferment. Thus in presence of 1 % of free ammonia the tryptic value was reduced to 65 % of its normal amount. In the case of the other substances there was likewise a considerable retardation. This was probably due in chief part to the greater alkalinity of the medium in which the ferment was now acting, for as can be seen from the data given in the previous table, there was very little retardation exerted when no extra alkali was added. More-

over it has been shown in a former paper[1] that as a rule the digestive power of trypsin is considerably diminished by increased alkalinity and increased by diminished alkalinity, the optimum alkalinity being generally about ·05 % Na_2CO_3. We find, accordingly, that the addition of substances with considerable neutralising power may distinctly increase the digestive activity of the trypsin. Thus glycocoll, glucosamin hydrochloride and bile acids all enhanced the activity of the trypsin when present in suitable proportions, whilst on the contrary the addition of 1 % of aspartic acid or of alloxan not only completely neutralised the alkali, but rendered the digestion mixture distinctly acid, and so reduced the digestive power of the trypsin to less than a third its normal value. On the other hand none of the substances which possessed no neutralising power had any accelerating or special retarding influence upon the trypsin. In ·5 % solution they retarded the ferment slightly, and in 1 % solution rather more considerably. We may presume, therefore, that proteids likewise tend to delay the digestive action of trypsin upon fibrin, but owing to their power of neutralising the sodium carbonate, this tendency is more or less masked. Had the digestions been carried out in more dilute alkali, doubtless their retarding influence would have been much more apparent.

The antitryptic action of egg albumin.

We have seen that of the eleven proteids mentioned above only two, viz. serum albumin and paraglobulin, even moderately retarded the digestive action of trypsin on fibrin. Egg albumin, however, forms a remarkable exception to this rule. Experiments were made both with filtered egg white and with crystalline egg albumin, and in each case the retardation was extremely marked. As can be seen from the data given in the table, the digestive action of the trypsin fell to half its

	Tryptic value in presence of egg white	p.c. of ferment destroyed in 1 hour at 38° with ·4 p.c. Na_2CO_3 and egg white (× 2)	Tryptic value in presence of egg albumin	p.c. of ferment destroyed in 1 hour at 38° with ·4 p.c. Na_2CO_3 and egg albumin (×2)
Pancreatic extract alone	100	56	100	56
+ ·05 p.c. of proteid	49	23	29	19
·2 p.c. ,,	39	5	13·6	10
·5 p.c. ,,	20	+17	8·4	8
1 p.c. ,,	10·9	+19	2·9	
2 p.c. ,,	5·5	+28		

[1] This *Journal*, XXVIII. p. 388. 1902.

normal value when in presence of ·05 % of egg white proteids, and to a tenth of its value in presence of 1 %. Pure egg albumin delayed the action of the ferment still more, ·05 and 1 % of it lowering the tryptic value to respectively 29 and 2·9 % of its normal amount. Even ·017 % of egg albumin, or 1 part in 6000, reduced the tryptic value to 45 % of the normal.

In the third and fifth columns of the table are given the percentages of trypsin destroyed when the ferment was kept for an hour at 38° with ·4 % Na_2CO_3 and *twice* the amounts of egg white or egg albumin given in the first column of the table. It will be seen that the apparent protective action upon the trypsin was very marked. In the presence of ·4 % of egg white only 5 % of the trypsin was apparently destroyed instead of 45 % as in the case of the other proteids, whilst in presence of from 1 to 4 % of egg white the tryptic value was actually enhanced. The explanation of this apparent anomaly is a simple one. Egg albumin, on exposure to the action of sodium carbonate and trypsin at 38°, is speedily converted into alkali albumin and is then to some extent digested and converted into albumoses and peptones. These digestion products of albumin retard the action of the trypsin on fibrin considerably less than undigested albumin, and hence the tryptic value is apparently increased. By keeping some of the ferment with ·4 % Na_2CO_3 and egg white for 24 hours at room temperature before allowing it to digest the fibrin, it was found that the tryptic value, as subsequently determined, might be as much as 70 % greater than the value obtained when similar quantities of ferment and egg white were added straight to the fibrin to be digested.

The antitryptic action of egg albumin persists to a considerable extent even after it has been coagulated by heat. Thus a sample of trypsin when acting upon fibrin in the presence of ·2 % egg albumin had its digestive value reduced to 12·6 % of the normal. When some of this albumin in 1·25 % solution had been previously heated to 60° C. for 15 minutes, it still slowed the action of the trypsin almost as much as before. When heated another 15 minutes to 70° it began to lose some of its antitryptic action, but this loss was not much increased on heating another 15 minutes to 80°, even though the albumin seemed to have undergone complete coagulation. In fact it was not until the albumin had been heated to 100° that it began to lose a considerable amount of its antitryptic action. From the table we see that after being heated for 3 hours to 100° C. (the vessel containing it being plunged into boiling water and the large lumps of coagulum formed being broken up

mechanically into small fragments) it still diminished the digestive power of the trypsin to 62 % of its normal value. Presumably still more prolonged heating would have further reduced the antitryptic action, but it does not look as if even indefinitely continued heating would have destroyed it altogether.

Trypsin acting in presence of						Tryptic value
·2 p.c. egg albumin ...	...	...	...	...		12·6
,, ,, previously kept for 15 mins. at					60°	12·8
,, ,, ,, ,, ,,					70°	15·6
,, ,, ,, ,, ,,					80°	16·0
,, ,, ,, ,, ,,					90°	20·0
,, ,, ,, ,, ,,					100°	27·9
,, ,, ,, ,, 1 hour at					100°	53·2
,, ,, ,, ,, 3 hours at					100°	62·0

This antitryptic action of egg albumin is presumably due to its combining with the trypsin molecules by means of "side-chains," and so delaying or preventing their digestive action. Not only is digestion delayed, but its course is to some extent altered. Under normal conditions the trypsin attacks the swollen fibrin at once, and causes its volume to diminish steadily from the very beginning of digestion. When egg albumin is present, however, the fibrin remains undiminished in volume during the first third or more of the total digestion time, and then rapidly passes into solution. However, if boiled fibrin be used instead of swollen unboiled fibrin, the course of digestion is just the same in each case, though the egg albumin still retards the action of the trypsin as much as before.

It should perhaps be mentioned that in all the above described experiments in which the effects of proteids and other substances upon the digestive activity of trypsin was determined, such a quantity of pancreatic extract was used as would take about 35 to 45 minutes to digest 80 % of the fibrin in the centrifugal tubes. That is to say the digestion time was kept as far as possible constant, whilst the quantity of ferment was varied. Supposing, for instance, that ·1 c.c. of pancreatic extract were used under normal conditions, then if some of the extract were previously exposed to the action of ·4 % Na_2CO_3 at 38°—whereby about 50 % of the ferment was destroyed—·2 c.c. of the extract would be used for digestion. Again, if the extract were to be allowed to act in the presence of egg albumin, as much as 1 c.c. of it might be employed, but under no conditions was this volume of 1 c.c. exceeded.

SUMMARY.

The protective value of various substances upon trypsin was estimated by keeping pancreatic extract with ·4 % sodium carbonate and a known percentage of the substance for 1 hour at 38° C. and determining the amount of trypsin thereby destroyed. As a rule the protective value depends almost entirely upon the power the substance possesses of neutralising the alkali, and so rendering it incapable of reacting upon the ferment.

Most proteids have practically the same protective value, about 45 % of the trypsin of an extract being destroyed per hour in presence of ·4 % of proteid, 27 % in presence of 1 %, 12 % in presence of 2 %, and 7 % in presence of 4 % of proteid. When no proteid was present 56 % of the ferment was destroyed. Hydrated proteids have a slightly greater protective value than native proteids, and the decomposition products of proteid hydrolysis a slightly greater one still. The protective value of individual substances depends solely upon their power of neutralising alkali, *e.g.* aspartic acid and glycocoll have a somewhat greater protective value than proteids, leucin and hippuric acid have about the same value, bile acids have considerably less, whilst urea, creatin and the sugars have none at all. Also if the acid radicles in the various substances be previously neutralised by the addition of alkali, they entirely lose their protective power over the ferment.

In certain cases there is a combination between ferment molecule and proteid. Thus egg albumin possesses a most marked antitryptic action, the digestive power of the ferment being reduced to 29 and 2·9 % of its normal value in the presence of ·05 and 1 % of the proteid respectively. Also a mixture of Witte's peptone with sodium carbonate sufficient for its complete neutralisation can exert a considerable protective action upon the ferment.

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