THE FERMENTS OF THE PANCREAS. PART IV. STEAPSIN.

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Introduction.

In 1891 Rachford (1) investigated the properties of steapsin contained in pancreatic juice obtained from a rabbit by means of a fistula. He considered himself fortunate if he obtained 1 c.c. of juice from any one rabbit. But despite this fact he was able to show that bile increased the action of steapsin threefold, that the velocity of the reaction was doubled by raising the temperature from 18° C. to 37° C., and that the action of the ferment was retarded by hydrochloric acid. In 1910 an extensive series of researches on the steapsin of pancreatic juice, obtained by means of secretin, were published by Terroine (2). He found that the ferment was destroyed after heating the juice to 60° C. for ten minutes and that its activity was greatly diminished after heating to 50° C. for forty-five minutes; that pancreatic juice added to olive oil immediately after activation hydrolysed the fat to a considerable extent; that the same juice five hours after activation contained practically no steapsin; and that the addition of coagulated egg white to active juice preserved the steapsin to a considerable degree. The coenzyme of steapsin has been investigated by Rosenheim and Shaw Mackenzie (3). They have found that serum has a well-marked accelerating action on lipase contained in a glycerine extract of the

pancreas, a fact which has been confirmed by Miniami (4). Hamsik (5) has shown that in order to obtain an active lipase from fresh pancreas the glycerine extract must be turbid, but that if dried pancreatic tissue be used a clear glycerine extract possesses well-marked fat splitting activity.

The quantitative estimation of steapsin. The amount of ferment present in a solution was estimated by determining the rate at which emulsified olive oil was hydrolysed by it. An emulsion containing 50 % olive oil was made by means of tragacanth mucilage. To 2 c.c. of this 50 % olive oil emulsion the ferment solution and water were added to bring the volume to 3 c.c. The mixture was incubated at 40° C. for one hour. After this time 3 c.c. of alcohol and two drops of a 1 % solution of phenol phthalein were added to it. The addition of alcohol to this extent has been shown by Kanitz (6) to facilitate the titration of the fatty acids. The degree of acidity generated by the steapsin was determined by titration against NaOH (·1 N), a correction being made, by means of a control experiment, for the original acidity of the oil emulsion. The rate of hydrolysis of this oil emulsion by the steapsin contained in varying quantities of fresh pancreatic juice is shown in the following figures.

Olive oil (50 %)	H_2O	P. J.	NaOH (·1 N) required for neutralisation after one hour
2	·9	·1	1·4 c.c.
2	·8	·2	2·5
2	·7	·3	2·8
2	·6	·4	3·5
2	·5	·5	3·8

The above figures show that the amount of fat hydrolysed in an hour is not directly proportional to the amount of ferment added. From the curve expressing the relation between the amount of juice added and the amount of fatty acid formed the relative amounts of ferment in any of the following experiments may be calculated. In the majority of cases however the amount of steapsin present in a solution has been expressed in terms of the number of c.c. of NaOH (·1 N) required to neutralise the fat digestion mixture after one hour's incubation at 40° C.

The stability of steapsin.

The rate of destruction of steapsin varies with the conditions under which it may be placed. In order to analyse the effects observed a number of experiments were made on fresh pancreatic juice under varying conditions of reaction and temperature.

Alkali. Pancreatic juice of an alkalinity approximately Na_2CO_3 (·12 N) was left at 40° C. for five hours. At the end of each hour the lipolytic power of the juice was determined. The following numbers were obtained.

	At once	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
Steapsin in terms of NaOH (·1 N)	3·8	3·5	2·9	2·6	2·3	1·8 c.c.

The figures show a progressive destruction of steapsin—after heating the juice to 40° C. for five hours two-thirds of the ferment had disappeared. During this time no trypsin was produced in the juice. The trypsinogen was not diminished in any degree since on adding a small quantity of enterokinase to the portion of juice remaining after the five hours' heating a tryptic activity of one thousand units was developed. The significance of this fact will be evident later when we discuss the relation of trypsin to steapsin. At present it may be noted that pancreatic juice heated to 40° C. loses steapsin at the rate of 10 % per hour whilst trypsinogen is unaffected by this treatment.

Neutral solution. The destruction of steapsin in neutral solution at 40° C. occurs at practically the same rate as in alkaline solution. Pancreatic juice was neutralised by the addition to it of an equal quantity of HCl (·1 N). The following figures indicate the amounts of steapsin contained in ·1 c.c. of the neutralised and diluted juice after varying intervals of time.

	At once	1 hr.	2 hrs.	3 hrs.
Steapsin in terms of NaOH (·1 N)	2·5	2·2	2·0	1·7 c.c.

The rate of destruction is only a little slower than in pancreatic juice which has not been neutralised.

Acid. Steapsin is fairly stable at room temperature (16° C.) in alkaline or neutral solution but the presence of a minute quantity of free mineral acid rapidly destroys it. The following experiment illustrates this statement.

(A) ·5 c.c. P. J. + ·8 c.c. HCl (·1 N) (slightly acidic).
(B) 5 c.c. P. J. + ·8 c.c. H_2O.

After fifteen minutes at 40° C. 2 c.c. of a 50 % olive oil emulsion were added to each of these solutions. After one hour's incubation at 40° C. the acid developed in each tube required for neutralisation (A) 1 c.c. NaOH (·1 N) and (B) 5 c.c. NaOH (·1 N).

The presence of a small quantity of free HCl in the juice (approximately HCl ·02 N) diminished the lipolytic activity to less than one-fifth of its former value. Experiments, detailed later, show that electrolytes do not affect the action of steapsin on fat. We must therefore conclude that HCl ·02 N actively destroys this ferment.

The presence of organic acids to the extent of about ·2 N in the above fat digestion mixture (B) indicates the stability of steapsin in the presence of organic acids. Apparently the stability of the ferment in acidic solution is related to the hydrogen ion concentration of the solution. We propose to deal with this problem in a subsequent paper.

Temperature. It has been shown that steapsin is gradually destroyed when pancreatic juice is heated to 40° C., the rate of destruction being approximately 10 % per hour. The following experimental results show that this rate of destruction is considerably augmented at 50° C.

Period of heating to 50° C.	Activity of juice in terms of NaOH (·1 N)
0 mins.	3·5 c.c.
15	1·7
30	1·0
45	·5
60	·1
75	0

From these figures it may be seen that within fifteen minutes more than 50 % of the steapsin disappeared from pancreatic juice heated to 50° C. This rapid destruction of steapsin in pancreatic juice heated to 50° C., confirms the results obtained by Terroine (2) and is comparable to that described by us in the case of trypsin. In the case of this latter ferment the whole of it contained in activated pancreatic juice is destroyed by heating to 50° C. for forty-five minutes. Further experiments showed that steapsin is completely destroyed by heating pancreatic juice to 60° C. for five minutes. A similar temperature of destruction has been shown to hold good for trypsin also.

The relation of steapsin to trypsin.

From the experiments of Terroine (2) it appeared that steapsin is unstable in the presence of trypsin. Our method of estimating tryptic activity has enabled us to show in detail that as the tryptic power of pancreatic juice develops the lipolytic activity diminishes. This disappearance of steapsin with the development of trypsin is illustrated in the following experimental results.

Pancreatic juice was neutralised by the addition to it of $CaCl_2$ (·1 N), and allowed to become active. After varying times the lipolytic and tryptic activities of the juice were determined.

	Activity after					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
Steapsin in terms of NaOH (·1 N)	4·0	3·7	3·5	·2	0	0
Units of trypsin	0	0	0	660	1250	1000

From these figures it may be seen that in the first three hours no trypsin was developed and the steapsin remained practically intact. In the fourth hour 600 units of trypsin were produced and the steapsin disappeared.

Many experiments have conclusively shown that in fresh pancreatic juice steapsin is present in large quantities. On the addition of enterokinase to the juice steapsin persists only so long as minute quantities of trypsin are present. When full proteolytic activity is developed the steapsin entirely disappears.

The rapid destruction of steapsin by trypsin is illustrated in the following experiment.

To 1 c.c. of fresh pancreatic juice ·2 c.c. of enterokinase was added and the lipolytic and tryptic activities determined at once and after twenty minutes' incubation at 40° C.

	At once	After 20 mins.
Steapsin in terms of NaOH (·1 N)	3·8	·2 c.c.
Units of trypsin	0	1000

Within twenty minutes the juice lost the whole of its steapsin and developed full proteolytic activity.

The following experiment differentiates between the gradual destruction of steapsin in pancreatic juice heated to 40° C. and that caused by trypsin. For a period of five hours juice was heated to 40° C. and its fat splitting and proteolytic activities determined every hour. At the end of this time enterokinase was added to the remaining juice and the activities of the mixture determined after a lapse of an additional thirty minutes.

		Activity after					
	At once	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	5½ hrs.
Steapsin in terms of NaOH (·1 N)	3·8	3·5	2·9	2·6	2·3	1·8	·2 c.c.
Units of trypsin	0	0	0	0	0	0	1000

Up to five hours the steapsin disappeared at the rate of 10 % per hour; after the addition of enterokinase full proteolytic activity was developed and the remaining steapsin was destroyed within thirty minutes.

These experimental results show conclusively that steapsin cannot exist in the presence of trypsin. They afford an adequate explanation of the observation that pancreatic juice which contains trypsin is devoid of fat splitting activity. The importance of the fact in relation to fat digestion will be discussed in the concluding section of this paper.

The influence of serum and egg albumen. The following mixtures of pancreatic juice, ox serum, and enterokinase were made up:

P. J.	E.	Ox serum	H_2O	
·5	·0	·5	·0 c.c.	(A)
·5	·05	·45	·0	(B)
·5	·05	·0	·45	(C)

After an hour's incubation at 40° C. (C) contained a large quantity of trypsin but no steapsin, whilst (A) and (B) contained no trypsin but an undiminished quantity of steapsin. The hypothesis which we put forward to account for the preservation of steapsin is that the antitrypsin present in the serum neutralised the trypsin as it was produced and so prevented the destruction of steapsin. This hypothesis was confirmed by determining the preservative action of egg albumen on steapsin.

The following mixtures of fresh pancreatic juice, enterokinase and albumen were made up:

P. J.	E_{10}	H_2O	Alb.	
·5	·1	·35	·05 c.c.	(A)
·5	·1	·4	0	(B)

After two hours (A) was devoid of proteolytic activity whilst (B) contained a large quantity of trypsin. At the same time the lipolytic activities of (A) and (B) were compared with that of a similar quantity of fresh pancreatic juice.

	Oil emulsion (50 %)	H_2O	P. J.		NaOH (·1 N) required after one hour
(Control)	2	·9	·1		3·0 c.c.
	2	·8	·2	(A)	2·6
	2	·8	·2	(B)	·1

Comparing the fat splitting powers of (A) and (B) with that of the control it may be seen that the presence of egg albumen (A) had preserved the steapsin almost intact, whilst in experiment (B), in which enterokinase only had been added to the juice, the steapsin had disappeared. In the one tube, (A), the added egg albumen neutralised the trypsin and thereby preserved the steapsin. In the other tube, (B), to which no albumen had been added, a large quantity of trypsin was produced and the steapsin was destroyed.

We may conclude therefore that the preservative actions exerted by serum and egg albumen on the steapsin of pancreatic juice are due to the antitrypsins contained in them. Steapsin cannot exist in the presence of free trypsin. If the development of trypsin in pancreatic juice be prevented, or if the trypsin when first formed be neutralised by antitrypsin, then the steapsin remains intact. This observation, also, is of fundamental importance in considering the digestion of fat in the small intestine.

The influence of electrolytes and bile salts on the digestion of fat by steapsin.

Briefly stated, bile salts augment to a marked degree the digestion of fat by steapsin, but electrolytes have practically no effect on this hydrolysis. These general statements are illustrated in the following experiments:

Sodium glycocholate in varying amounts, and ·1 c.c. of fresh pancreatic juice, were added to 2 c.c. of a 50 % olive oil emulsion and the degree of acidity developed after an hour's incubation at 40° C. was determined by titration against NaOH (·1 N).

Olive oil emulsion (50 %)	H_2O	P. J.	Sodium glycocholate (5 %)	NaOH (·1 N) required after one hour
(A) 2	·9	·1	·0	3·6 c.c.
(B) 2	·0	·1	·9	6·8

The presence of 1·5 % sodium glycocholate more than doubled the amount of oil hydrolysed in one hour by the steapsin contained in ·1 c.c. of pancreatic juice. Exp. (B) illustrates the remarkable lipolytic activity of fresh pancreatic juice in the presence of bile salts. A mixture containing only 3 % of pancreatic juice hydrolysed a 33 % olive oil emulsion at such a rate that organic acid to the extent of ·2 N was developed in an hour.

Similar results were produced by the addition of bile to the digestion mixture.

Olive oil	P. J.	H_2O	Bile	NaOH (·1 N) required after one hour
2	·1	·7	·2	5·0 c.c.
2	·1	·9	·0	3·8
2	·0	·8	·2	·0

From these figures it may be seen that whereas bile itself had no action on the oil yet a similar quantity of bile (6 % approximately) acting in conjunction with steapsin doubled the amount of oil hydrolysed in one hour.

The marked action of bile salts on steapsin is in striking contrast with the absence of effect of electrolytes on this ferment. The following figures show the rate of digestion of olive oil by fresh pancreatic juice, and by similar quantities of juice in the presence of various electrolytes.

Olive oil emulsion (50 %)	H_2O	P. J.	Electrolyte	NaOH (·1 N) after 1 hr.
2	·9	·1	0	3·5 c.c.
2	·8	·1	·1 HCl (·01 N)	3·3
2	·8	·1	·1 NaOH (·01 N)	3·3
2	·8	·1	·1 NaCl (N)	3·8
2	·8	·1	·1 $CaCl_2$ (N)	3·3
2	·8	·1	·1 $MgCl_2$ (N)	3·1

These results are opposed to those of Terroine (2) who finds that electrolytes augment the action of steapsin on fat. The small effects which we have noted may appear to support Terroine's work but we think that they must be ascribed to experimental error and possibly the effect of the electrolyte on the permanence and degree of the oil emulsion rather than to any adjuvant action on the lipase. Possibly the effect of the electrolytes on the permanence of the fat emulsion is important since Terroine working with unemulsified olive oil found that cane sugar augmented fat digestion tenfold. We have been unable to confirm this remarkable result working with emulsified oil. The addition of cane sugar to a mixture of 50% olive oil emulsion and pancreatic juice neither augments nor diminishes the degree of fat digestion. Since the only factor in which our experiments differed from those of Terroine consisted in the emulsification of the oil it is clear that the extraordinary adjuvant action exerted by cane sugar in the digestion of olive oil by steapsin must be ascribed to the emulsifying property of the added syrup. But apart from the question of the effect of cane sugar on fat digestion it is evident that the influence exerted by electrolytes on steapsin is by no means comparable to the effects produced by these substances on amylopsin. In this latter case the achromic time for a mixture of pancreatic juice and starch may be extended tenfold or diminished to a corresponding degree by the addition of certain electrolytes in suitable concentrations.

The question of a coenzyme to steapsin.

Ten years ago Magnus (7) described experiments by which he separated the lipase of liver into two components, neither of which alone was capable of hydrolysing fat, but which, when added to one another, produced the activity of the original extract. The one component possessed the properties of the original ferment solution as regards heat destruction, etc., the other component was dialysable, heat stable at 100° C., and soluble in alcohol. To this latter component the name coenzyme was given. Loewenhart (8) found that bile salts possessed all the properties of this coenzyme of liver lipase. Rosenheim and Shaw Mackenzie (3) found that steapsin contained in a glycerine extract of the pancreas could be separated into two inactive components by precipitation with a large volume of water, the one component being found in the precipitate, the other component (the coenzyme) being present in the filtrate. Further Rosenheim and Shaw Mackenzie

found that serum had a well-marked accelerating action on pancreatic lipase similar to that shown by the above-mentioned coenzyme.

We may say at the outset that we have been unable to confirm these results as regards the steapsin contained in fresh pancreatic juice. Owing to the fact that dialysis occupies a considerable time, and that dialysed pancreatic juice develops a proteolytic activity with consequent disappearance of the steapsin, we have been unable to adopt the method originally employed by Magnus with liver lipase. We have, however, employed a method which is completely successful in separating amylopsin of pancreatic juice into enzyme and coenzyme, namely, by precipitating fresh pancreatic juice with 95 % of absolute alcohol. By this method we find that the enzyme component of amylopsin is contained in the precipitate and the coenzyme in the filtrate. If pancreatic lipase be similar to liver lipase this method ought to separate it into two components since a striking property of the coenzyme of the liver lipase is its solubility in alcohol (cf. Magnus). The following gives the results of an experiment:

2 c.c. of fresh pancreatic juice were added to 30 c.c. of absolute alcohol cooled to −10° C. The resulting solution was centrifuged at a temperature below zero and the supernatant fluid was removed from the precipitate with a pipette. The precipitate, when dissolved in water, gave a clear solution. The activities of the original juice and the alcohol precipitate dissolved in a volume of water equal to that of the original juice were as follows:

Olive oil	H_2O	P. J.	NaOH (·1 N) required for neutralisation after one hour
2	·8	·2	3·5 c.c.
2	·8	·2 (X)	2·8

There was a small diminution in activity of the precipitated juice but this loss was due to the destruction of the steapsin by alcohol. The activity of the precipitate was not restored to its original value by adding the filtrate, freed from alcohol, to it. We have found no evidence that steapsin contained in pancreatic juice can be separated into enzyme and coenzyme from a number of similar experiments made with variations in the amounts of alcohol added, the time allowed for precipitation, and the temperature of the solutions.

We are the more inclined to assume that steapsin contained in pancreatic juice cannot be divided into two components corresponding to enzyme and coenzyme since we find that electrolytes neither augment nor diminish its activity to any degree. All the effects produced by the coenzyme of amylolytic ferments can be simulated by inorganic salts, such as sodium chloride or calcium chloride, and these salts have an

extraordinary accelerating influence on the activities of ptyalin or amylopsin contained in solutions which have not been treated so as to separate them into their components. In fact it appears probable that fluids containing these enzymes as saliva and pancreatic juice do not contain an optimal quantity of coenzyme. By analogy it may be assumed that if steapsin has a similar constitution similar facts should hold good for it. In the absence of these facts we may deduce that the two types of ferments are not analogous and that steapsin contained in pancreatic juice is not formed of two components which can be separated from one another.

The question then arises as to the possible explanation of the differences between our experimental results obtained with steapsin contained in fresh pancreatic juice, and those described by other experimenters working with a glycerine extract of the pancreas. A most important point is the condition of the steapsin in the two preparations. In pancreatic juice the steapsin is held in complete solution and no amount of filtration diminishes the lipolytic activity of the juice. On the other hand the lipase contained in a glycerine extract of the pancreas is not held in solution since the fluid obtained after filtration through ordinary filter paper is practically devoid of any lipolytic action. In the preparation of such an extract of the pancreas it is essential that the resulting fluid should be turbid and not water clear, an end which is achieved by pressing the extracting mixture through muslin. We regard this suspensoid condition of the steapsin contained in glycerine extracts of the pancreas as responsible for the fact that the precipitate obtained from such an extract by dilution with water is devoid of lipolytic activity. The steapsin contained in the precipitate is insoluble in water (Hamsik) and it is essential that the ferment should at least approximate to a condition of solution before it can exert its specific action. This condition may be produced by the substances present in the filtrate after precipitation of the glycerine extract with water, or by various electrolytes such as alkaline carbonates, or by fluids containing these electrolytes in solution as serum.

The capacity of serum to simulate the action of a coenzyme is also evident from the experiments of Rosenheim and Shaw Mackenzie in which they showed that the addition of serum to a glycerine extract of the pancreas greatly augments the lipolytic activity. In this action two factors are probably involved: (*a*) the action discussed above, *i.e.* the more adequate solution of the lipase contained in the glycerine extract by certain components of the serum, and (*b*) the preservation of

the steapsin from the action of trypsin by the antitrypsin contained in the added serum.

We have previously discussed the effect of serum on the destruction of steapsin by trypsin, the only question which now arises being whether a glycerine extract of the pancreas contains trypsin or enterokinase and trypsinogen, in addition to steapsin.

At the outset it may be noted that the addition of serum to fresh inactive pancreatic juice has no effect whatever upon its lipolytic activity. Fresh pancreatic juice diluted with water hydrolyses as much olive oil emulsion in an hour as a similar quantity of fresh pancreatic juice diluted with serum. Such an experiment effectively dismisses the possibility of the existence of a coenzyme in serum for steapsin contained in fresh pancreatic juice. (It is essential that this juice should not contain more than a trace of enterokinase.) A glycerine extract of the pancreas however contains not only steapsin but also trypsinogen and enterokinase. We(11) have previously shown that the slow development of trypsin and the continued existence of steapsin in such a solution is due to the inhibitory action of strong glycerine on the activation of trypsinogen by enterokinase, and on the proteolytic activity of trypsin. Dilution of such an extract removes the inhibitory effects produced by the glycerine and there results activation of the trypsinogen by the enterokinase, and the subsequent disappearance of the steapsin at a rate depending upon the rate of trypsin production. The addition of the antitrypsin contained in serum to such a diluted glycerine extract neutralises the trypsin as it is produced and so permits the steapsin to act for the whole of the digestive period instead of for a time depending upon the rate of conversion of trypsinogen into trypsin. In this way serum simulates the action of a coenzyme merely by virtue of the antitrypsin contained in it.

Finally we have added serum and bile salts to pancreatic juice which has lost its lipolytic property owing to the development of trypsin within it. In no case has the added serum or bile salt generated steapsin, showing that the loss of lipolytic activity from pancreatic juice, produced by trypsin, is not due to the disappearance of a coenzyme but to an absolute destruction of the ferment itself.

Discussion of results.

From the experimental results described in the previous pages two main questions arise for discussion: (*a*) the properties of steapsin, and (*b*) the mechanism of fat digestion in the small intestine.

(*a*) *The properties of steapsin.* The problem as to the ultimate nature of ferments has given rise to much speculation and experimental work. The greater part of the work has been directed towards the purification of ferment solutions and analysing the final products. The majority of workers have concluded that ferments consist essentially of protein. The main criticism against this conclusion is that the protein tests are given by substances associated with the ferment, rather than by the ferment itself.

The relation of trypsin to steapsin appears to settle this problem conclusively so far as steapsin is concerned. It has been fully proved that so soon as trypsin develops in pancreatic juice steapsin disappears. The fact admits of only one explanation—that steapsin is digested by trypsin, and since trypsin is a proteolytic ferment then steapsin consists essentially of protein.

A second fact observed in the properties of steapsin is that the stability of this ferment in alkaline or neutral solution at any temperature is approximately the same as trypsin (always assuming that no trypsin is present in the solution). The rapid disappearance of trypsin from solution has been assumed to be due to autodigestion. We have previously put forward arguments against this assumption and have indicated that its disappearance is due to inherent instability. These arguments receive considerable support from the observations stated above: that the stability of steapsin in alkaline or neutral solution is approximately the same as trypsin. It is in the highest degree improbable that steapsin which consists essentially of protein should undergo autodigestion since its function is to hydrolyse fat. And arguing from these facts we may legitimately conclude that the disappearance of trypsin from solution is not due to autodigestion but to its inherent instability.

Another point which arises from the relation of steapsin to trypsin is the question of the identity of the milk clotting ferment of pancreatic juice with trypsin. This problem has been discussed by one (9) of us in considerable detail, and we concluded from a critical survey of the subject that pancreatic rennin and pancreatic trypsin are identical with one another—in fact that the coagulation of milk by trypsin is an

expression of a general law that all proteolytic ferments coagulate milk provided an adequate amount of calcium be contained in it. Exception has been taken to this hypothesis by Edie (10) who finds that trypsin, as estimated by its capacity to hydrolyse protein, is preserved to a somewhat greater extent after boiling in acidic solution than is denoted by the figures which we have given in this connection. The unique fact that the ferment or ferments in pancreatic juice which digest protein and coagulate milk should withstand boiling in acidic solution is practically conclusive proof that the two actions are produced by one and the same substance. But as additional evidence in favour of the identity of the milk clotting ferment with trypsin we offer this relation of the milk clotting ferment to steapsin. If pancreatic rennin exists as a separate ferment then the disappearance of steapsin from pancreatic juice when rennin develops is unintelligible. If however pancreatic rennin and trypsin are identical then the disappearance of steapsin from activated pancreatic juice receives a ready explanation.

(*b*) *The mechanism of fat digestion in the small intestine.* The relation of steapsin to trypsin throws some light on the mechanism of fat digestion in the small intestine. Since steapsin is quickly destroyed by trypsin it is essential that the digestion of fat should take place either in the absence of trypsin, or, if present, under such conditions that its destructive action on steapsin is prevented. The rate of activation of trypsinogen by enterokinase is admirably adapted to secure an adequate fat digestion in the upper part of the small intestine. We (11) have previously investigated this phenomenon in detail: suffice it to say that at the beginning of the reaction the rate of activation of trypsinogen is very slow, but as the change proceeds this rate is continually accelerated until finally the velocity is relatively large. From this it follows that steapsin may remain intact and fat digestion may take place in the upper part of the small intestine during the time occupied by the initial stages of trypsinogen activation.

The question next arises as to the conditions which augment or diminish the rate of trypsinogen activation. We have previously shown that the rate of trypsinogen activation is directly proportional to the amount of enterokinase present, and that the reaction proceeds most rapidly in neutral solution, is inhibited by alkalies, and stopped by acids. A consideration of these facts enables a deduction to be made as to the optimal conditions for fat digestion in the small intestine.

Enterokinase is contained in the succus entericus, and the upper two-fifths of the small intestine contain much more enterokinase than the

remaining portion of the gut. The active factor involved in the secretion of succus entericus is stated to be the mechanical excitation of the intestine by the food contained in it. Consequently the secretion of enterokinase will be small and fat digestion facilitated on a diet which contains a minimum of material capable of mechanically exciting the mucous membrane of the upper part of the small intestine.

The reaction of the contents of the small intestine appears to play a less important part in the digestion of fat than appeared probable from the early determinations of the alkalinity of pancreatic juice. According to many observers the alkalinity of pancreatic juice varies between Na_2CO_3 (·1 N) and Na_2CO_3 (·2 N). Auerbach and Pick (12), however, have recently shown that pancreatic juice, secreted from a fistula, is practically neutral in reaction, since the juice is saturated with CO_2, the alkali thus existing as $NaHCO_3$ and not as Na_2CO_3. The well-marked alkalinity ascribed by earlier workers may be explained on the assumption that their determinations were made on old samples of juice which had lost their CO_2. Our experiments fully confirm those of Auerbach and Pick. Fresh pancreatic juice obtained by means of secretin has a hydrogen ion concentration which corresponds to NaHCO (·2 N) and which is only slightly alkaline to phenol-phthalein. Succus entericus also is only slightly alkaline in reaction. It may be assumed therefore that the contents of the small intestine are approximately neutral in reaction, and that the conditions as regards reaction are such as to determine a maximum rate of trypsinogen activation by enterokinase.

The destruction of enterokinase by hydrochloric acid may be dismissed as outside the range of physiological action since the acid control of the pylorus prevents the contents of the small intestine from becoming acidic. In any case, free acid, to the extent of HCl (·02N) in the small intestine, would not only prevent the activation of trypsinogen but would also destroy any steapsin and amylopsin with which it came in contact.

Finally, a factor which may exert a marked effect on fat digestion is the amount of protein contained in the diet. The protective action of egg albumen on the steapsin of pancreatic juice has been considered in detail. This protective action depends mainly upon the adsorption of the trypsin by the egg albumen and the degree of protection depends upon the rate at which the albumen is digested by the attached trypsin. From this it follows that fat in a dietary containing protein is more favourably placed for digestion by steapsin than fat taken alone or in conjunction with carbohydrate.

However, apart from the factors which determine the optimal conditions for the digestion of fat, one thing is clear—in the upper part of the small intestine fat is digested by steapsin and trypsinogen is converted into trypsin by enterokinase. When free trypsin exists in the intestinal contents, the steapsin disappears and fat digestion ceases.

Finally the relation of trypsin to steapsin offers an adequate explanation for the presence of trypsinogen rather than trypsin in fresh pancreatic juice.

SUMMARY.

(1) The stability of steapsin in alkaline solution is similar to that of trypsin. Fresh pancreatic juice at 40° C. loses steapsin at the rate of 10 % per hour; at 50° C. this rate is augmented to 50 % per hour; and at 60° C. the whole of it is destroyed within five minutes.

(2) The stability of steapsin in acidic solution is related to the hydrogen ion concentration of the fluid in which it is contained. It is stable in the presence of large quantities of the higher fatty acids (·2N) but minute quantities of free mineral acids (HCl ·02 N) quickly destroy it.

(3) Steapsin cannot exist in the presence of free trypsin. Therefore, when pancreatic juice is activated by enterokinase, as trypsin develops steapsin disappears. This fact affords an explanation for the presence of trypsinogen rather than trypsin in fresh pancreatic juice.

(4) Serum, or egg albumen, added to activating pancreatic juice, protects steapsin from destruction owing to the presence of antitrypsin in them.

(5) The action of steapsin on fat is greatly augmented by bile and bile salts. Electrolytes, such as neutral salts, have no influence on the reaction.

(6) There is no evidence that steapsin in pancreatic juice can be separated into enzyme and coenzyme. Serum, when added to pancreatic extracts, may simulate the action of a coenzyme owing to the antitrypsin contained in it protecting the steapsin from any tryptic action.

(7) Steapsin cannot be restored to activated pancreatic juice by the addition of serum or bile. The destruction of steapsin by trypsin is absolute.

(8) From a consideration of the properties of steapsin and its relation to trypsin it appears:

(*a*) that steapsin consists essentially of protein; (*b*) that the destruction of trypsin in alkaline solution is not due to autodigestion

but to its inherent instability; (*c*) that although the conditions in the small intestine which favour trypsin production are inimical to the continued existence of steapsin, yet the presence of protein in a dietary may facilitate fat digestion by virtue of the capacity of the protein to adsorb the first formed trypsin.

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