THE ACTIVATION OF PANCREATIC JUICE. By BARBARA AYRTON.
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SINCE the discovery by Pawlow and Chepowalnikow (1) that the trypsinogen of the pancreatic juice is activated under normal circumstances by a special ferment, enterokinase, secreted by the intestine, the mode of action of enterokinase has been the subject of a number of researches, and the attention of physiologists has been brought back to the question of the conditions which may determine the activation of trypsinogen apart from the action of enterokinase. Delezenne's (2) suggestion that the interaction of enterokinase and trypsinogen is comparable to the interaction of amboceptor and complement in the process of hæmolysis may be regarded as definitely negatived by the experiments of Bayliss and Starling (3). The results obtained by these observers can only be interpreted if we assume with Pawlow that enterokinase exercises a ferment-like action on the trypsinogen. The time relations of the interaction of these two substances, as well as the study of the influence of quantitative variations in the enterokinase on its action, cannot be interpreted by assuming an actual union between enterokinase and trypsinogen like that which occurs between amboceptor and complement. There are, however, certain divergences between the work of these two authors and that of Delezenne which still await satisfactory explanation. Thus, according to Delezenne, various additions, especially of leucocytes and lymph corpuscles, can effect the activation of trypsinogen, whereas Bayliss and Starling were unable to obtain activation by any means other than the addition of enterokinase, and therefore ascribed the so-called spontaneous activation of pancreatic juice to an accidental contamination with small traces of enterokinase.

Shortly after this Languier des Bancelles (4) found that pancreatic juice becomes active in the presence of a suitable electrolyte and a suitable colloid. The electrolytes employed by him were calcium, barium, and magnesium nitrates, and he ensured the presence of a suitable colloid by colouring with toluidine blue the albumen to be digested.

In 1905 a paper was published by Delezenne (5) in which this author stated that pancreatic juice could be activated by the addition to it of lime salts, e.g. calcium chloride, as well as of certain other salts such as those of magnesium or barium. These results were confirmed by Zuntz (6), who

carried out a comparative study of the relative activating effects of the salts of various metals without, however, adding materially to the results obtained by Delezenne.

According to the latter author, the activation takes place suddenly, as a rule after from twelve to sixteen hours in the incubator, but if the optimum amount of calcium salt be added it may occur much more rapidly. The activated juice can be freed from its calcium salt by dialysis or by the addition of sodium fluoride or ammonium oxalate without losing its activity. The phenomenon is influenced by the nature of the surface in contact with the juice. Thus it is delayed by a surface coated with paraffin. If the inactive juice be dialysed against sodium chloride, a mere trace of calcium salt is sufficient to activate it. Delezenne does not consider the action of calcium salts comparable to that of enterokinase, nor does he find these salts necessary to the action of enterokinase, for enterokinase activates pancreatic juice in the presence of excess of sodium fluoride or oxalate. He suggests that the calcium takes part in the formation of enterokinase from a precursor, prokinase, in a manner analogous to that in which calcium determines the formation of fibrin ferment from its precursors, thrombokinese and thrombogen.

At Professor Starling's suggestion, and with his assistance, I have undertaken a re-examination of this question, with especial reference to the relation between the action of lime salts and of enterokinase. A number of experiments had already been performed on this subject by Dr de Souza, and I shall have occasion to refer to his results in the course of the paper.

Experimental Methods.

Secretin juice was used for all the experiments: it was obtained in the following way. A cannula was tied in the lower duct of the pancreas, and the juice was allowed to flow through a glass tube into a flask; the flow of juice was produced by the intra-venous injection of secretin. The secretin was obtained by grinding up a superficial scraping of the mucous membrane of the duodenum with sand; the mixture was then boiled with dilute hydrochloric acid (12 c.c. per litre), and while boiling was just neutralised with dilute sodium hydrate; it was then filtered hot, and the filtrate, secretin, was injected into the jugular vein at the rate of about 2 c.c. every ten minutes.

Every care was taken to prevent the possibility of any contamination with enterokinase; all instruments, vessels, and collecting tubes used in the experiment were carefully sterilised in boiling water, and to ensure the juice being quite free from any enterokinase that might have adhered to the mouth of the pancreatic duct the first 6 or 7 c.c. were collected in a separate flask and thrown away.

As a rule, Fermi's modification of Mett's tubes, about 2 cms. long, were used for determining the rate of digestion. Five grammes of gelatin were

dissolved in 100 c.c. of water. A few drops of methyl violet were added to the mixture in order to make it easier to measure the quantity of gelatin digested in the tubes. The hot mixture was sucked up into sterilised capillary tubes about a foot long, and then allowed to cool. When the gelatin was quite cold the tubes were cut into lengths of two centimetres.

Sometimes cubes of egg-white were used instead of gelatine tubes; these were obtained in the following manner. The egg was hard boiled, and then with a razor cut up into small cubes, which were kept in toluol until required.

When the pancreatic juice was dialysed, small parchment dishes were employed; these were first sterilised by being placed in a beaker which was put in the steriliser and steamed in the usual way.

The rate of digestion was determined by placing a couple of gelatin tubes with the juice into a small bottle plugged with cotton-wool; at regular intervals the gelatin digested from either end of each of the tubes was measured, and an average taken of the four measurements thus obtained.

All solutions employed with the juice were first raised to boiling-point in order to do away with any possibility of contamination with enterokinase.

The Action of Lime Salts on the Pancreatic Juice.

It is easy to confirm the statement of Delezenne. This is shown by the following experiments carried out by Dr de Souza.

In order to test whether calcium salts activate pancreatic juice, the following series was arranged. A 10 per cent. solution of calcium chloride was used for these experiments:—

TABLE I.¹
ACTIVATING EFFECT OF CaCl_2 ON FRESH PANCREATIC JUICE.

Flask.	Solutions used.	Amounts digested.				
		24 hrs.	44 hrs.	48 hrs.	72 hrs.	92 hrs.
1.	P.J. 5 c.c. + CaCl_2 0.1 c.c.	0	0	0	0	0
2.	P.J. 5 c.c. + CaCl_2 0.2 c.c.	0	0	0	0	1.1
3.	P.J. 5 c.c. + CaCl_2 0.4 c.c.	0	0	0	0	4
4.	P.J. 5 c.c. + CaCl_2 0.8 c.c.	0	all	...	... + tube	all
5.	P.J. 5 c.c. + CaCl_2 2 c.c.	0	0	0	all + tube	all
6.	P.J. 5 c.c. + CaCl_2 5 c.c.	0	0	0	all + tube	all

¹ In the tables, P.J.=pancreatic juice; Ek.=solution of enterokinase; all means "more than 10"; "+ tube" signifies that a fresh tube was added. Gelatin tubes were added in every case (unless otherwise mentioned), and the digestion is expressed in millimetres.

Complete digestion took place in flask 4 in 44 hours, and in flasks 5 and 6 in 72 hours, and digestion had started in flasks 2 and 3 in 92 hours when the juice was still sweet. 5 c.c. of the juice were activated by 0.1 c.c. of an enterokinase solution in less than 24 hours.

The result with flask 4 is very striking, and a like result was obtained in other experiments. I find, with Delezenne, that a definite amount of calcium chloride must be added to provide the optimum effect. In the case of 5 c.c. of pancreatic juice, this amount lies between 0.5 c.c. and 1 c.c.

The disappearance of the gelatin from the tubes does not necessarily imply true activation of the juice, as the gelatin might possibly have been dissolved out. Here, however, it was a true activation, for, while it took 44 or 72 hours to get rid of the gelatin from the first tubes, that from fresh tubes added at the end of this time disappeared in less than 20 hours. Moreover, I set out another series similar to the above, but with the pancreatic juice replaced by distilled water, and found that at the end of 72 hours the gelatin was still projecting beyond the ends of the tubes.

With regard to the time taken to activate the juice when the optimum amount of calcium salt is added to it, my experiments show that this is usually more than 24 hours and less than 48 hours. As a rule, activation has occurred by the 40th hour, and this is so whether digestion is allowed to proceed at room temperature with gelatin tubes, or in the incubator at 36° C. with albumin tubes. In one experiment in which the mixture had been kept in the incubator at 36° C. for 24 hours it was found, on cooling, to be activated for gelatin at room temperature, while the juice from which the mixture was made was still inactive at the end of 72 hours. In every other case when activation occurred in less than 24 hours it was found that the juice itself was active, i.e. that it had probably been contaminated with enterokinase.

Delezenne states that the activation takes place suddenly, and lays great stress upon this point. The following observation is of particular interest in this connection :—

TABLE II.

COMPARISON OF THE EFFECTS OF CALCIUM SALTS WITH THAT OF ENTEROKINASE.

Flask.	Solutions used.	Amounts digested.		
		24 hrs.	32 hrs.	44 hrs.
1.	P.J. 3 c.c.	0	0	0
2.	P.J. 3 c.c. neutralised with CaCl ₂	0	6.4	14
3.	P.J. 3 c.c. + Ek. 0.001 c.c. . . .	1	5	12

The juice with calcium chloride was inactive for the first 24 hours, then

it digested 6.4 mm. of gelatin in the next 8 hours, and 7.6 mm. in the following 12 hours. The rate of digestion was less in this last interval than in the preceding one, so that full activation must have occurred between the 24th and 32nd hours. As a contrast, the enterokinase mixture had digested 1 mm. of gelatin in the first 24 hours, then it digested 4 mm. in the next 8 hours, and 7 in the following 12 hours. The rate of digestion, therefore, gradually increased. Evidently, then, the activation by calcium salts is more sudden than that by enterokinase, though it will be seen that there is a gradual action of calcium, the curve of activation being, however, steeper in the case of this salt than in the case of enterokinase.

Effect of Neutralisation.

Is the effect of calcium due to neutralisation? When calcium chloride is added to pancreatic juice a precipitate occurs which consists chiefly of calcium carbonate. At the same time the alkalinity of the juice is diminished. It was found that the optimum amount of calcium chloride for activation was about the amount necessary to neutralise the juice, supposing the alkalinity of the juice to be equivalent to that of a decinormal solution of sodium carbonate. It was therefore necessary to compare the behaviour of juice treated with calcium chloride with that of juice neutralised in other ways. Accordingly the following series of experiments was arranged:—

TABLE III.
COMPARISON OF EFFECTS OF CALCIUM SALTS WITH THAT PRODUCED
BY NEUTRALISATION.

Flask.	Solutions used.	Amounts digested.			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
1.	P.J. 5 c.c.	0	0	0	0
2.	P.J. 5 c.c. + CaCl ₂ 0.2 c.c.	0	0	0	1
3.	P.J. 5 c.c. + CaCl ₂ 0.5 c.c.	0	all + tube	all	
4.	P.J. 5 c.c. + CaCl ₂ 1 c.c.	0	0	0	all
5.	P.J. 5 c.c. + CaCl ₂ 2 c.c.	0	0	0	0
6.	P.J. 5 c.c. + HCl till just alkaline	0	0	0	3
7.	P.J. 5 c.c. + HCl till neutral	0	0	0	4
8.	P.J. 5 c.c. + HCl till just acid	0	0	0	0

The juice rendered neutral with HCl did become active, but only to a slight extent, after 96 hours. The specimens were still quite sweet at the time. The activation was considerably slower than that of the optimum calcium mixture. A slightly alkaline mixture also became active, but a very small amount of acidity was sufficient to prevent activation.

In another series, activation had begun in 66 hours, but was again slower than that of the calcium mixture.

TABLE IV.
ACTIVATING EFFECT OF CaCl_2 ON FRESH PANCREATIC JUICE COMPARED WITH
THAT OF NEUTRALISATION.

Flask.	Solutions used.	Amounts digested.			
		24 hrs.	42 hrs.	48 hrs.	66 hrs.
1.	P.J. 3 c.c. + water 1.1 c.c.	0	0	0	0
2.	P.J. 3 c.c. + CaCl_2 0.3 c.c. + water 1 c.c.	0	0	0	0
3.	P.J. 3 c.c. + CaCl_2 0.5 c.c. + water 1 c.c.	0	all (11) + tube	4	all
4.	P.J. 3 c.c. + CaCl_2 0.8 c.c. + water 1 c.c.	0	11 + tube	4	all
5.	P.J. 3 c.c. neutralised with HCl + water 1 c.c.	0	0	0	1.5

Water was added for comparison with another series to be dealt with later.

One had to determine next whether such a neutral mixture became active if all traces of calcium had been removed from it. This was tried in various ways. The juice was neutralised with hydrochloric acid or acetic acid, then sodium fluoride or ammonium oxalate was added; or the fluoride or oxalate was added first and the mixture was then neutralised; or the juice was neutralised with calcium chloride and filtered, and the filtrate treated with ammonium oxalate. In none of these cases did activation take place, either at room temperature with gelatin tubes for 114 hours, or in the incubator at 36°C . with albumin tubes for 72 hours, although such mixtures were capable of activation by enterokinase.

It would seem, then, that if all traces of calcium salt be absent, pancreatic juice is not activated by neutralising it. This would account for the fact that Bayliss and Starling got no activation when they neutralised pancreatic juice, for they used sodium fluoride as a preservative, and thus got rid of all traces of calcium salt.

The Effect of Dialysis.

Delezenne has found that if juice be neutralised by dialysis against sodium chloride it remains inactive, but that a very small amount of calcium chloride added to it will activate it in 12 hours in the incubator. I have investigated the effects produced by dialysing the juice against distilled water as well as against $\frac{\text{N}}{10}$ sodium chloride solution. We considered the juice completely dialysed when it no longer gave an alkaline reaction with litmus; this occurred after about 48 hours in the case of

distilled water, and 72 hours when sodium chloride solution was used as the outer fluid. The following experiment was then performed with the dialysed juice, another experiment being carried on at the same time with normal undialysed juice:—

Juice A was dialysed against water.

Juice B was dialysed against $\frac{N}{10}$ NaCl.

Juice C was simply normal untouched juice.

TABLE V.

THE INFLUENCE OF DIALYSIS ON FRESH JUICE, WITH AND WITHOUT THE ADDITION OF NEUTRAL SALTS.

Experiments, October 1907: Average Results.

Solutions used.		Amounts digested.		
Oct. 11th, 4.50 P.M., 16° C.		12th, 11.40 A.M. 17° C.	13th, 11.45 A.M. 16.5° C.	14th, 11.45 A.M. 16° C.
Juice A. Dialysed against Distilled Water.	1. 2 c.c. juice + 1 c.c. $\text{CaCl}_2 \frac{N}{100}$. .	5.9	all	
	2. " " " $\text{BaCl}_2 \frac{N}{100}$. .	4	7.3	all
	3. " " " $\text{MgCl}_2 \frac{N}{100}$. .	5.6	8.1	all
	4. " " " H_2O	3.1	5.9	all
	5. " " " $\text{H}_2\text{O} + 0.001$ c.c. of enterokinase	all		
Juice B. Dialysed against Sodium Chloride.	1. 2 c.c. juice + 1 c.c. $\text{CaCl}_2 \frac{N}{100}$. .	5.3	8.3	all
	2. " " " $\text{BaCl}_2 \frac{N}{100}$. .	0	3.7	5.5
	3. " " " $\text{MgCl}_2 \frac{N}{100}$. .	3.9	6.8	all
	4. " " " H_2O	1.9	3.7	7.9
	5. " " " $\text{H}_2\text{O} + 0.001$ c.c. of enterokinase	all		
Juice C. Pure Juice.	1. 2 c.c. pure juice + 1 c.c. $\text{CaCl}_2 \frac{N}{100}$. .	4.7	7.5	all
	2. " " " $\text{BaCl}_2 \frac{N}{100}$. .	2.1	3.2	4.8
	3. " " " $\text{MgCl}_2 \frac{N}{100}$. .	3.3	5.4	8.7
	4. " " " Alone	0	2	4.1
	5. " " " $\text{H}_2\text{O} + 0.001$ c.c. of enterokinase	all		

These experiments were repeated many times, and the results shown in Table V. are quite typical of all those obtained; indeed, the results of the different experiments varied very little.

The facts that are chiefly brought under our notice are:—

(1) That dialysation very much helps the activation of the juice, especially when distilled water is used in the dialyser. Possibly this may be connected with the fact that after the juice has been dialysed against sodium chloride it has lost more calcium than when distilled water is used; this is easily proved by testing the two dialysates with ammonium oxalate.

(2) Calcium is of great assistance in the activation of both dialysed and undialysed juice.

(3) Other salts besides the calcium do accelerate the activation of the juice, but not to the same extent.

(4) Enterokinase is very much more powerful than anything else in activating the juice.

(5) The juice always becomes spontaneously active if given time.

It might at first sight be thought that the activation of the juice, which is brought about by simple dialysis, shows that calcium salts cannot be concerned in the process. It must be remembered, however, that the sodium salts which determine the alkalinity of the juice will dialyse much more rapidly than the calcium salts, so that after dialysing a certain number of hours we shall have a neutral solution still containing a fair proportion of lime salts, i.e. conditions which according to Delezenne are especially favourable for activation to take place. The fact, however, that simple dialysis against distilled water is much more potent than mere neutralisation in bringing about activation shows that some other factor must be concerned: possibly a removal of certain salts or electrolytes which are unfavourable to the activating effect of calcium; or the alteration of the balance of electrolytes, producing a corresponding change in the condition of the calcium salts, brings them into a form more adapted to acting on trypsinogen.

There must then be something more in dialysation than merely neutralising the juice, because the dialysed juice is more active than merely neutralised juice. If these results are compared with those on page 205, it will be seen that even the juice dialysed against sodium chloride is more active than merely neutralised juice. That neutralisation is helpful in activating the juice is evident, because the neutralised juice digests the tubes with considerably greater rapidity than pure juice. Dialysation, however, must in some way place the juice under much more favourable conditions for activation than neutralisation; for not only does it activate more powerfully, but it does so under what would appear to be very adverse circumstances, since it removes some of the calcium which we know to be so helpful in the activation of the juice. Indeed, when sodium chloride is the dialyser used a comparatively large quantity of calcium is removed. If it were not for this fact, one would conclude that the

quickened action was caused by the fact that calcium activation could take place more easily in a neutral medium than in an alkaline one, but that would not account for the fact that the activation is still further quickened when it is neutralised by a process that removes some of the calcium. It may be, however, that the reaction is still a calcium one, but that dialysation in some way places the calcium under such very favourable conditions for activation, that in spite of the fact that there is less calcium present, all that is there is so much affected that it enables the juice to activate more easily than when all the calcium is present and only helped as far as neutralisation is able to assist it.

Since the addition of calcium makes so much difference to the rate of activation of the juice, probably removing the calcium that is present in normal juice will have an equally marked effect. To determine whether this was the case, we treated the juice with ammonium oxalate, thus removing all the calcium. In order that the action of the juice should in no way be interfered with by the presence of superfluous ammonium oxalate, we titrated a 1 per cent. ammonium oxalate solution against 100 c.c. of pancreatic juice, and found that 29 c.c. of ammonium oxalate solution precipitated all the calcium present; that is, 0.29 per cent. ammonium oxalate is required to decalcify normal pancreatic juice. Of course the quantity of calcium present in the juice is not absolutely constant, but it varies so little as to make the difference negligible. For instance, the above figures are an average taken from titrating three samples of juice, from which the figures obtained were 0.29 per cent., 0.29 per cent. and 0.28 per cent., giving an average of 0.29 per cent. We therefore used 29 c.c. of 1 per cent. ammonium oxalate per 100 c.c. of juice. We left the mixture overnight and filtered off the calcium oxalate in the morning, after which we tested a small quantity of the juice with ammonium oxalate, but no precipitate formed; the juice was therefore calcium free.

We then tried by various methods to activate the decalcified juice; dialysing it against distilled water and against sodium chloride solution, adding gelatin tubes to the pure and dialysed juices, and leaving them for a week or ten days, but without producing the slightest change in the juice. We also tried adding various salts, e.g. magnesium chloride, barium chloride, sodium carbonate, etc., to both dialysed and undialysed juice, but all without success. At length we thought that perhaps it might be possible to replace the calcium in the juice and thus produce the desired effect; this we attempted to do in sufficient quantity not only to give back what had been removed from the juice but also to have an excess present, and thus turn it into the comparatively quickly activating juice already described. Since calcium chloride was the most successful calcium salt in activating ordinary juice, we supposed that in this case too it would probably answer best; we therefore added an equal quantity of $\frac{N}{10}$ calcium chloride solution to both the dialysed and undialysed juices. After a week,

in each case the result was nil; different strengths and quantities of the salt solution were added, but still without producing any result whatever.

It occurred to us that possibly in removing the calcium we had broken up an organic molecule which could only be remade by the combination of organic atoms or molecules of the juice and calcium; we therefore supplied the calcium in an organic form as calcium citrate; this attempt, however, was as fruitless as the others. The result was that we were reluctantly compelled to accept the theory, that in losing calcium, pancreatic juice undergoes some radical change which can in no way be reversed to bring the juice back to its former state.

Is calcium concerned in the activation of juice by enterokinase? Since calcium evidently plays so large a part in the activation of juice in the absence of enterokinase, one is naturally led to wonder what part it plays in enterokinasic activation. With the idea of discovering this, we experimented with every possible combination of enterokinase, juice, and calcium. In one case we used extra calcium, in another juice from which the calcium had been removed, then we tried enterokinase under the same conditions, and finally juice and enterokinase with no calcium present at all—in all cases the activation was equally rapid and complete.

The conclusion we therefore came to was that there are two kinds of activation—one caused by enterokinase entirely, in which the calcium plays no part; and one, if not actually caused by calcium, caused by something in which calcium is an important factor; and, in fact, for whose action the presence of calcium is an absolute necessity.

Is enterokinase concerned in the activation by calcium salts? As already mentioned, a suggestion was put forward by Delezenne to the effect that calcium might produce enterokinase from a prokinase already present in the juice as secreted. If this were the case, one would expect to find enterokinase in the juice after activation by calcium, just as one finds fibrin ferment in the serum of oxalate plasma which has been made to clot by the addition of lime salts. Such an activated juice would therefore bring about activation in another specimen of fresh juice even in the presence of lime salts. To determine whether this is the case, we have compared the activity of fresh pancreatic juice to which some calcium-activated juice had been added some time previously, with that of a mixture of the same quantity of calcium-activated juice with sodium carbonate solution, and also with that of some of the fresh juice treated with a minute trace of enterokinase. The results are given in the following table:—

[TABLE.]

TABLE VI.

THE ACTION OF CALCIUM-ACTIVATED JUICE UPON PURE JUICE.

Experiments, November 1907: Average of Results.

	Solutions used.	Amounts digested.				
Temp. Date Time	17° C. November 26th 10.15 A.M.	17° C. 26th 5.15 P.M.	18° C. 27th 10 A.M.	18° C. 27th 6 P.M.	17° 5° C. 28th 10 A.M.	18° C. 28th 5 P.M.
	No. 1 { 2 c.c. calcium-activated juice + 1 c.c. pure juice	1	3.1	4.3	5.9	7.3
	2 c.c. calcium-activated juice + 1 c.c. $\text{Na}_2\text{CO}_3 \frac{\text{N}}{100}$	0.7	2.8	3.7	4.7	6.1
Temp. Date Time	18° C. November 27th 10 A.M.	18° C. 27th 5.30 P.M.	19° C. 28th 10.15 A.M.	19° C. 28th 6.15 P.M.	18° C. 29th 10 A.M.	18° C. 29th 6 P.M.
	No. 2 { 2 c.c. calcium-activated juice + 1 c.c. pure juice	0	0.8	1.4	1.9	2.5
	2 c.c. calcium-activated juice + 1 c.c. $\text{Na}_2\text{CO}_3 \frac{\text{N}}{100}$	0.5	1.6	2.3	3.1	3.8
Temp. Date Time	17° C. November 29th 10 A.M.	17° C. 29th 6 P.M.	17° C. 30th 10 A.M.	17° 5° C. 30th 5.30 P.M.	18° C. Dec. 1st 10.15 A.M.	18° C. 1st 6.15 P.M.
	No. 3 { 2 c.c. calcium-activated juice + 1 c.c. pure juice	1.3	2.6	3.3	4.9	5.6
	2 c.c. calcium-activated juice + 1 c.c. $\text{Na}_2\text{CO}_3 \frac{\text{N}}{100}$	1.7	2.8	3.5	5	5.7

In this experiment we see that the activity of the mixture of fresh pancreatic juice and of calcium-activated juice remained approximately the same throughout the experiment. There has been no evidence that the mixture acquired further activity by the activation of the trypsinogen present in the fresh juice. De Souza attempted to decide this question in another way. By the injection of enterokinase into an animal such as the rabbit, the serum of this animal acquires after a time an antibody to enterokinase. It is possible by the use of such an antikinasic serum to test for the presence of kinase in any mixture. The following experiment shows that the serum used had really antikinasic properties:—

TABLE VII.

ANTIKINASIC EFFECT OF SERUM.

- A. P.J. 3 c.c. at 36° C. for 1 hour, then cooled and added water 2 c.c. and gelatin tube.
- B. P.J. 3 c.c. + Ek. 1 c.c. at 36° C. for 1 hour, then cooled and added water 1 c.c. and gelatin tube.
- C. P.J. 3 c.c. + Ek. 1 c.c. at 36° C. for 1 hour, then cooled and added serum 1 c.c. and gelatin tube.
- D. Serum 1 c.c. + Ek. 1 c.c. at 36° C. for 1 hour, then cooled and added P.J. 3 c.c. and gelatin tube.

Result :—

	After 24 hours.	After 44 hours.
A.	0	0
B.	4	7
C.	2	5
D.	0	0

The result with D showed that the serum was antikinasic. A comparison of B and C showed that it possessed some antitryptic properties as well.

This serum was used to prevent the action of enterokinase in the following experiment :—

TABLE VIII.
ATTEMPT TO PREVENT ACTIVATION OF JUICE BY CaCl_2 BY ADDITION OF
ANTI-KINASIC SERUM.

Flask.	Solutions used.	Amounts digested.				
		24 hrs.	42 hrs.	48 hrs.	66 hrs.	114 hrs.
A1.	P.J. 3 c.c. + CaCl_2 0.3 c.c. + water 1 c.c.	0	0	0	0	all
A2.	P.J. 3 c.c. + CaCl_2 0.5 c.c. + water 1 c.c.	0	all + tube	0	all	
A3.	P.J. 3 c.c. + CaCl_2 0.8 c.c. + water 1 c.c.	0	11 + tube	0	all	
B1.	P.J. 3 c.c. + CaCl_2 0.3 c.c. + serum 1 c.c.	0	0	4	0	all
B2.	P.J. 3 c.c. + CaCl_2 0.5 c.c. + serum 1 c.c.	0	0	0	0	0
B3.	P.J. 3 c.c. + CaCl_2 0.8 c.c. + serum 1 c.c.	0	4.2	10 + tube	all	

All in table means "more than 11."

The series A shows the ordinary calcium activation result. If activation was due to enterokinase, it should have been prevented by the serum in series B. This was the case in B2, but activation was only delayed in B3 as compared with A3, and not affected in B1 as compared with A1.

The mixture of antitryptic with antikinasic effects renders, however, these results difficult to interpret, and I have therefore not carried out any further experiments on the same lines.

The Spontaneous Activation of Normal Juice.

Having decided how pancreatic juice behaves under artificial conditions, that is, in the absence of all calcium or in the presence of an unnaturally large quantity of it, the next question that presents itself is, How does it behave under normal conditions?

Bayliss and Starling could get no spontaneous activation of juice at all; this, however, is explained by the fact that they only worked with fluoride juice, in which, of course, calcium can play no part, so that the juice was not really in a natural condition.

In all our experiments we used pure juice straight from the pancreas; the results obtained in this way invariably were that the juice became active within forty-eight hours, after which digestion, though slow, was always complete if given time. It sometimes, however, required four or five days to completely digest the Mett's tubes.

Throughout all these experiments, whenever an unexpected result of a positive kind was obtained we were immediately confronted with the unanswerable question, Is the result a true or a false one? Is the activation of the juice really spontaneous, or has it been caused by the juice in some way becoming contaminated with enterokinase? We know from Bayliss and Starling's results that the presence of the slightest trace of enterokinase will ultimately cause activation of even a large quantity of juice. In spite of all possible precautions having been taken, one could never be absolutely certain that the juice was entirely free from the ferment; for instance, there might even have been some slight trace present in the pancreas itself, or rather at the mouth of the duct, in which case the juice would inevitably become contaminated. It was obvious, therefore, that no really satisfactory results could be obtained until it could be proved with absolute certainty that the so-called pure juice was really pure, and uncontaminated by the faintest trace of enterokinase.

Since enterokinase is a ferment and its presence can only be discovered by the activation of the juice, how is it possible to prove conclusively that active juice contains no trace of it? It occurred to us that it might be possible to get juice to work with in which any enterokinase that had been present was destroyed.

One of the simplest and most effective methods of destroying enterokinase is by heat in alkaline reaction. At 40° C. it is completely and rapidly destroyed. At a lower temperature the process is more gradual; in fact, from 20° C. upwards, as the temperature is raised the action of the ferment becomes proportionately inhibited. Accordingly the following experiments were made:—

About 60 c.c. of juice were taken from a dog, all care being taken to prevent, as far as possible, any contamination with enterokinase. This was divided into two parts, A and B; A was left pure, while to B 5 c.c. 0·001¹ per cent. enterokinase was added; A and B were then each divided into three portions, which were placed in tightly stoppered bottles; one bottle of each was put in a freezing mixture at 0° C., one was kept at room temperature, and the third was put in an incubator at 35° C. After twenty-four hours, all the bottles were changed to room temperature, namely 19° C., and gelatin tubes added. These were left for twenty-four hours. During this time they all began to activate. At the end of this time the amount of gelatin digested from the tubes was measured and an average taken for each bottle. Subsequently a reading was taken every four hours during the day for the next two days. The results shown in Table IX. are typical of those obtained in all the experiments.

¹ The numbers given to represent the amounts of enterokinase are, of course, purely arbitrary. An extract or superficial scraping of the intestinal mucous membrane was made in chloroform water. After standing 24 hours this was filtered, first through paper and then through a Berkefeld filter. The fluid thus obtained was called "enterokinase." One c.c. of this fluid in 100 c.c. water would be called a 0·01 per cent. solution of enterokinase, etc.

TABLE IX.

INFLUENCE OF TEMPERATURE ON THE SPONTANEOUS ACTIVITY OF JUICE, AND
ON ENTEROKINASE.

Experiments, February 1908: Average of Results.
Temperature 19° C.

		Solutions used.		Amounts digested.							
Date	Time			19th	19th	19th	20th	20th	20th	20th	21st
		February 18th		10 A.M.	2 P.M.	6 P.M.	10 A.M.	2 P.M.	6 P.M.	10 A.M.	
		A	Pure juice raised from 0° C. to 19° C.	0.3	0.3	0.5	0.9	0.1	1.2	1.8	
			Pure juice kept at 19° C.	0.7	0.9	1	1.5	1.5	1.8	2.1	
			Pure juice reduced from 35° C to 19° C.	1.9	2.1	2.4	3.5	3.7	4.1	5.2	
		B	Juice and enterokinase raised from 0° C. to 19° C.	all							
			Juice and enterokinase kept at 19° C.	all							
			Juice and enterokinase reduced from 35° C. to 19° C.	2	2.3	2.7	4.1	4.4	4.6	6.1	

Other experiments were made in which the juice was kept at 0° C., 19° C., and 35° C. respectively for a longer period than twenty-four hours; but apparently whatever changes take place in the juice when it is heated or cooled are quite finished within twenty-four hours, for the results did not in any way differ from those obtained in the former experiments.

One sees from this table what an enormous difference there is in the two juices A and B at 19° C., and how the rate of digestion is almost equal in the two juices when they are reduced from 35° C., showing how very much the activation of the pure juice is accelerated, while that of the juice with enterokinase is so much inhibited as to make the digestion results almost the same. If one inquires in detail into what really happens one sees that in the case of the juice which has been kept at 19° C. all the time, at the end of seventy-two hours, e.g. three days, only 0.21 cm. are digested from the tubes, while the tubes in the mixture of juice and enterokinase are completely digested in twenty-four hours under the same circumstances. In the case of both sets of juice A and B being reduced from 35° C., the results are 0.52 cm. and 0.61 cm. respectively, only differing by 0.09 cm., that is 14.7 per cent. as compared with something over 95 per cent. in the former case. Obviously, then, there would not be very much difficulty in telling whether the activation was due to enterokinase or not.

Probably in the case of the juice with enterokinase activating after being reduced from 35° C. there is practically no enterokinasic activation at all, for we have no reason to suppose that because enterokinase is mixed with the juice spontaneous activation is stopped; so it is very possible that

raising it to 35° C. does almost completely destroy the enterokinase, and that nearly all the subsequent activation which takes place is spontaneous; in fact, it would be quite reasonable to conclude that it is only the difference between the digestion of the two sets of tubes, e.g. 14·7 per cent., that is carried on by the enterokinase.

The conclusions that one draws from these tables are, in short:—

1. That so-called pure juice is really pure, in so far that it is in no way contaminated by enterokinase.

2. Probably the spontaneous activation is a chemical process, since it follows the general rule for chemical processes, e.g. heat accelerates instead of inhibiting the action.

As calcium plays so large a part in the spontaneous activation of the juice, and even affects it to the extent of making the rate of activation practically proportional to the quantity of calcium present, up to a certain point, and since rise of temperature is also concerned in the rate of activation, the obvious thing to conclude would be that the most powerful activation of the juice in the absence of enterokinase would be in the presence of calcium chloride at a temperature of 35° C. But this is not the case. Directly the juice is mixed with calcium chloride, change of temperature within certain limits, namely reducing it to 0° C. or raising it to 35° C., ceases to have any effect upon the rate of activation. Table X. shows the rate of activation of juice with half its volume of $\frac{N}{100}$ calcium chloride solution added, as compared with pure juice. It will be seen that the juice and calcium together behave all the way through just as they did when it was simply a matter of adding calcium to juice where no temperature was concerned, as shown on p. 203. It will be noticed that the results are very much what would be expected.

TABLE X.

EFFECT OF TEMPERATURE ON SPONTANEOUS ACTIVATION AND ON ACTIVATION BY CaCl_2 .

Experiments, February 1908: Average of Results.
Temperature 19° C.

Date Time	Solutions used.	Amounts digested.						
		24th 10 A.M.	24th 2 P.M.	24th 6 P.M.	25th 10 A.M.	25th 2 P.M.	25th 6 P.M.	26th 10 A.M.
	February 23rd							
	10 A.M.							
	Juice and calcium :—							
	Juice and calcium raised from 0° C. to 19° C.	2·3	2·7	3·1	5·8	6·2	6·4	8·9
	Juice and calcium kept at 19° C. . .	2·1	2·7	0·3	5·3	5·7	6·2	8·6
	Juice and calcium reduced from 35° C. to 19° C.	2·4	2·7	3·2	5·7	6·0	6·3	8·7
	Pure juice :—							
	Pure juice raised from 0° C. to 19° C. .	0·5	0·7	0·1	1·3	1·8	0·2	2·6
	Pure juice kept at 19° C.	0·5	0·1	1·4	1·7	2·1	2·4	2·9
	Pure juice reduced from 35° C. to 19° C.	1·6	1·9	2·1	3·9	4·2	4·4	5·9

We thus see that the activating effect of calcium on pancreatic juice is independent of the temperature; on the other hand, that spontaneous activation no longer occurs after decalcification suggests that this process is concerned with the presence of calcium salts. So that we have the contradictory results that while one line of argument leads us to the conclusion that calcium salts are concerned in spontaneous activation, another line of argument, that derived from the study of the influence of heat on the process, would indicate that spontaneous activation, being quickened by rise of temperature, cannot be identical with activation by calcium. If, in spite of this divergence, we choose to assume that the spontaneous activation is due to lime salts, we have also to assume that (1) rise of temperature quickens some process which alters the calcium present so as to make it capable of activating the trypsinogen, but does not affect the rate of activation itself, and (2) that the activation by lime salts is due to the presence of calcium in a form or in combination with something which, when destroyed by the addition of oxalate or fluoride, cannot be reproduced by the addition of calcium.

It will be seen, therefore, that many interesting points still remain for investigation. The absolute identity of the final results of the action of an inorganic salt and of a ferment has, so far as I know, no analogies in the case of other ferments, and the curious relation between these phenomena and the temperature at which they are effected are at present difficult to understand. Further research along these lines should materially advance our knowledge of the intimate nature of the ferment action.

CONCLUSIONS.

I. There are two different kinds of activation of pancreatic juice: (*a*) the formation of trypsin by the action of the ferment enterokinase; (*b*) activation in the absence of enterokinase, in which calcium is intimately concerned, and which is, as far as one can tell, a chemical process.

II. It is conclusively proved by the temperature method that spontaneous activation may occur when no enterokinase or ferment of any kind is present; and that the activation of the juice is really spontaneous in so far that it is caused by the juice, or some of its constituents only, and not by any external agent.

III. The spontaneous activation of the juice is dependent on the presence of calcium salts in the juice, as well as on some other factor which is destroyed by decalcification. Hence it is not possible, after decalcification, to activate by further addition of calcium salts. Excess of calcium salts, on the other hand, may inhibit activation.

IV. (*a*) The spontaneous activation of juice is very much accelerated by heat.

(*b*) The calcium reaction of juice is in no way affected by change of temperature, within broad limits, e.g. under 40° C.

(c) Activation of the juice by enterokinase is very much retarded by raising the temperature to 35° C., on account of the destruction of this ferment by the heat in an alkaline medium. On the other hand, in a neutral medium the action of enterokinase, like that of all other ferments, is quickened by rise of temperature.

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