THE MECHANISM OF PANCREATIC DIGESTION—THE FUNCTION OF SECRETIN. By J. MELLANBY.

(*From the Physiological Laboratory, St Thomas's Hospital, London.*)

A SECRETION of pancreatic juice may be evoked by appropriate stimulation of the vagus (Pavlov(1)) or by the action of secretin (Bayliss and Starling(2)). Generally speaking vagal juice is scanty in quantity but rich in proteins and enzymes; secretin juice, on the other hand, is copious in quantity but relatively poor in protein and enzymes. There appears to be nothing common to the mechanisms involved in these two secretory processes since atropine paralyses the secretory fibres of the vagus but has no apparent action on secretin.

The hypothesis of the chemical control of the digestive functions of the pancreas has been accepted by many observers since the discovery of secretin by Bayliss and Starling in 1902. Evidence however has been shown by various members of the Russian school (Kudrewezki, Babkin, Savitsch) that pancreatic secretion is due, in part, to reflex action by way of the vagus, and that this nervous mechanism affects especially the protein and enzyme content of pancreatic juice. A detailed account of this aspect of pancreatic secretion is given by Babkin(3). There are also certain inherent difficulties in accepting the doctrine of Bayliss and Starling that the digestive functions of the pancreas depend primarily upon the action of acid chyme on prosecretin, and that prosecretin exists only in that situation in which it may be easily acted upon by acid chyme. Among such difficulties may be mentioned the facts of apparently normal nutrition associated with gastrectomy, achlorhydria, or jejunal alimentation. More particularly the experiments of Dodds and Bennett(4) on duodenal feeding in the normal man directly negative the assumption of the chemical control of pancreatic digestion by gastric acidity. Dodds and Bennett deduced from numerous experiments that the alveolar carbon dioxide pressure gives a measure of pancreatic secretion, and by this method found that the direct introduction of a neutral or even alkaline fluid into the duodenum causes an immediate secretion of pancreatic juice.

In order to determine the relative functions of secretin and the vagus nerves in regulating the digestive functions of the pancreas, the quanti-

tative composition of pancreatic juice was determined under a variety of conditions. The results indicate that the vagus controls the enzyme content of pancreatic juice whilst the volume of bicarbonate solution in which these enzymes are contained is determined by the action of secretin.

Methods. The animals used in all the experiments were cats anæsthetised by urethane (1·5 grm. per kilo of body weight). A cannula was inserted into the pancreatic duct, and, in experiments involving continuous secretion, the juice was collected in successive quantities of 3 c.c. These portions were analysed for total alkali, trypsinogen, amylase and lipase.

Alkali. 1 c.c. of juice was diluted to 25 c.c. with distilled water and titrated against H_2SO_4 ·04 *N* using methyl orange as an indicator. The comparative absence of protein from diluted pancreatic juice enables a fairly sharp endpoint to be observed, especially if indicator controls are used.

Amylase. 0·2 c.c. of each specimen of juice was added to 2·3 c.c. of 1 p.c. starch containing 0·1 p.c. NaCl and the achromic time observed. The addition of sodium chloride to the starch paste is necessary since cat's pancreatic juice contains less than 0·2 p.c. NaCl, a quantity less than the optimum required for the action of amylase on starch. The amount of amylase in each sample of juice was determined from a curve showing the achromic times of a similar quantity of starch with known quantities of amylase. It is noteworthy that cat's pancreatic juice contains very little amylase.

Trypsinogen. The juice was activated by the addition of an optimal quantity of enterokinase and after one hour the capacity of 0·1 c.c. of the activated juice to clot 2 c.c. of calcified milk (milk to which an equal volume of $CaCl_2$ 0·1 *N* had been added) was determined. The details of this estimation and the reasons for regarding pancreatic rennin as identical with trypsin have been described in a previous paper (5).

Lipase. An emulsion of olive oil was made by adding dilute sodium hydroxide to commercial olive oil until the mixture was just alkaline to phenolphthalein. The soap formed during the neutralisation of the fatty acid in the oil facilitated the formation of a permanent emulsion on shaking the oil with water. To 2 c.c. of oil emulsion 0·5 c.c. of fresh pancreatic juice was added and the mixture was incubated at 40° C., the tube being shaken every ten minutes to preserve complete mixing of the oil and juice. After one hour 2·5 c.c. of absolute alcohol was added to the mixture and the amount of alkali required to bring solution back

to a reaction just alkaline to phenolphthalein determined. This quantity of alkali gave a direct measure of the lipase content of the juice. It is important to estimate the lipase when the juice is freshly secreted since lipase is very rapidly destroyed by trypsin, which may spontaneously develop in the juice *in vitro* even without the addition of enterokinase (Mellanby and Woolley(6)).

The composition of successive portions of pancreatic juice. Pancreatic juice continuously secreted by a cat under the stimulus of secretin injected into a femoral vein was collected in seven portions. The following figures show the relative quantities of alkali, trypsinogen and amylase in the successive portions. The enzymes are given in terms of arbitrary units obtained from the standard curves. All the figures in this and the following experiments are comparable with one another:

$NaHCO_3N$	·128	·124	·134	·128	·132	·128	·124
Trypsinogen	665	640	360	400	230	280	150
Amylase	340	290	160	160	70	70	60

Two facts are evident from the above figures: (1) throughout the experiment the quantity of $NaHCO_3$ contained in the successive portions of juice remained approximately constant about a mean value of 0·128 *N*, and (2) the quantities of trypsinogen and amylase continually decreased, in the case of trypsinogen the content of the final fraction being less than one quarter, and of amylase approximately one-sixth of the quantity of enzyme in the first fraction.

The influence of pilocarpin on the composition of pancreatic juice. It is evident that the diminution in the enzyme content of successive fractions of pancreatic juice may be due to the exhaustion of the gland under the constant secretin stimulus. This hypothesis, however, does not accord with the histological appearance of the gland at the end of a long period of secretion. Thus a gland obtained from a dog which had secreted 150 c.c. of pancreatic juice under the stimulus of secretin had a typical resting structure. To test the exhaustion hypothesis, five successive fractions (3 c.c.) of pancreatic juice were obtained by means of secretin from a cat, during a period of three hours. At the end of this time secretin containing 2 mgrm. of pilocarpin was used as the pancreatic stimulant and an additional quantity (4 c.c.) of juice collected. The following figures give the analyses of the various fractions of juice:

	Secretin only					Secretin containing 2 mgrm. pilocarpin
$NaHCO_3N$	·150	·158	·156	·148	·146	·146
Trypsinogen	1350	1300	1150	1000	800	1300
Amylase	270	210	160	160	60	200

The figures show the facts previously described—the constancy of the bicarbonate and the steady diminution in trypsinogen and amylase content of successive fractions of juice secreted under the stimulus of secretin only. After the injection of pilocarpin the quantity of alkali remained the same as that in the previous fractions of juice, but the trypsinogen and amylase contents were considerably raised. From general considerations it may be assumed that pilocarpin stimulates the secretory nerve endings of the vagus in the pancreas. Therefore the last sample of juice owed its composition to the simultaneous stimulation of the secreting cells of the pancreas by secretin and the vagus. It contained the same quantity of bicarbonate as the portions of juice produced under the influence of secretin only. Its enzyme content was, however, considerably greater than that of the juice secreted immediately before it and approximated to that of the first sample of juice secreted in the experiment. Therefore secretin caused the pancreas to produce a free flow of 0·15 *N*. $NaHCO_3$ which carried with it the enzymes contained in the cells of the pancreas. On the other hand, the enzyme content of the juice appeared to be determined by the secretory fibres contained in the vagus nerve. This hypothesis receives support from the observations of Babkin, Rubaschkin and Savitsch(7) on the histological appearances of the pancreas after the injection of secretin and after stimulation of the vagus nerve. They found that in the case of secretin the pancreatic cells after the production of a copious secretion show no sign of fatigue, being still full of fine granules; after vagus stimulation marked cellular changes are apparent. "Under the influence of secretin, water flows through the cells in quantity and one sees in the cells what look like channels of fluid. This current carries out the zymogen granules into the ducts where they can be seen as granules but soon become dissolved. After nerve stimulation, on the contrary, the granules inside the cells undergo a transformation sometimes forming large vacuoles before passing into the duct." This dual hypothesis of the relative functions of the vagus and secretin, brings into line the work of Pavlov on the nervous mechanism of pancreatic secretion with the work of Bayliss and Starling on the chemical control of pancreatic secretion.

The influence of atropine on the composition of pancreatic juice. Wertheimer and Lepage(8) observed that the secretion of pancreatic juice initiated by the introduction of HCl into the duodenum was not annulled by atropine. Similarly Bayliss and Starling observed that atropine had no effect on the amount of pancreatic juice produced under

the influence of secretin. During the course of these experiments, I have observed that atropine appears to augment the amount of juice secreted under the influence of secretin. Thus after the cessation of a flow of pancreatic juice initiated by the intravenous injection of secretin, the flow recommences after the injection of 5 mgrm. of atropine. Atropine therefore appears to stimulate the secretory activities of the pancreas. This anomalous action, however, cannot be produced in an animal which has not previously been treated with secretin. The effect is probably due to the paralysis by atropine of the vagal endings contained in the plain muscle fibres which lie along the pancreatic ducts, as described by Anrep(9). The relaxed muscle thereby allows the juice previously held up in the constricted ducts to issue from the gland. Although atropine has no influence on the volume of pancreatic juice secreted under the secretin stimulus other than that just described, yet it has a marked effect on the composition of pancreatic juice. Thus in one experiment pancreatic juice, obtained from a cat by the intravenous injection of secretin was collected in six portions of 3 c.c. After the second sample was collected 10 mgrm. of atropine sulphate was injected into the blood. The following figures show the quantities of $NaHCO_3$, trypsinogen, amylase and lipase contained in the six portions of juice:

	Before atropine		After atropine			
$NaHCO_3$ *N*	·15	·14	·146	·134	·132	·126
Trypsinogen	2800	2400	1100	900	650	600
Amylase	500	340	140	60	70	50
Lipase	51	32	5	2	1	1

After the atropine injection the rate of secretion and the quantity of bicarbonate in the juice was practically unaltered, but there was a considerable diminution in trypsinogen, amylase and lipase. This fact becomes more evident if the average composition of 6 c.c. of juice secreted before and after the intravenous injection of atropine is considered thus:

	6 c.c. juice before atropine	6 c.c. juice after atropine
$NaHCO_3$ *N*	·145	·140
Trypsinogen	2600	1000
Amylase	420	100
Lipase	42	4

The figures afford confirmatory evidence in favour of the hypothesis that secretin causes a flow of bicarbonate solution through the cells of the pancreas whilst the vagus nerve determines the elaboration of enzymes in the cells of the gland.

The composition of pancreatic juice before and after cutting the vagus nerves. As a corollary to the above experiment the effect of vagal section on the volume and composition of pancreatic juice secreted under the influence of secretin was determined. After the secretion of two portions of pancreatic juice, each containing 3 c.c., both vagi were cut in the neck. After this procedure three additional quantities of 3 c.c. were collected. The analysis of these successive portions of juice gave the following figures:

	Before vagal section		After vagal section		
$NaHCO_3N$	·134	·138	·138	·132	·128
Trypsinogen	1250	800	330	280	100
Amylase	520	200	75	75	50
Lipase	30	19	9	2	5

The results are comparable to those observed before and after the intravenous injection of atropine. The rate of secretion and the quantity of alkali (0·136 *N*. $NaHCO_3$) contained in the juice was unaltered by section of the vagi. The enzyme content, however, was considerably diminished, trypsinogen falling from 1000 to 300 units, amylase from 360 to 75 units, and lipase from 25 to 5 units. This result indicates that the enzyme content of the juice is determined by impulses along the vagus nerves probably reflexly through the vagus nuclei in the bulb.

Discussion of Results.

The question arises as to the importance of secretin in the mechanism for pancreatic digestion. The experiments recorded indicate that secretin stimulates the cells of the pancreas to produce a copious flow of a dilute solution of sodium bicarbonate which carries the pancreatic enzymes with it. The metabolism of the enzymes of the pancreas, however, appears to be under the control of the vagus nerves, and in this respect the results confirm the previous conclusions of Babkin and Savitsch. Secretin therefore ensures the presence in the intestine of an adequate supply of sodium bicarbonate to preserve the neutrality of the intestinal contents during the process of digestion. The importance of the secretion of this alkaline fluid is evident from the fact that its reaction is such as to secure an optimal medium for the activity of lipase, amylase and trypsin.

There are a large number of well established observations that normal intestinal digestion may be associated with the complete absence of hydrochloric acid from gastric juice. In these cases, on the hypothesis of Bayliss and Starling that secretin formation depends on the action

of hydrochloric acid on prosecretin contained in the duodenal mucosa, no secretin action is possible. Therefore the vagal control of pancreatic secretion may play the dominant role in pancreatic digestion as stated by the Russian School of Physiologists, whereas the production by secretin of a copious flow of a dilute bicarbonate solution in which the pancreatic enzymes are contained may not be essential to normal digestion.

SUMMARY.

1. Pancreatic juice secreted after the intravenous injection of secretin contains a constant quantity of $NaHCO_3$ (approximately 0·14 *N*) but diminishing quantities of trypsinogen, amylase and lipase as secretion proceeds.

2. The diminution in the quantities of enzymes secreted is not due to the exhaustion of the gland, since after long continued secretion the quantities of enzymes may be increased to their original values by vagal stimulation.

3. Removal of vagal control from the cells of the pancreas either by atropine or by section of the vagi in the neck diminishes the quantities of enzymes contained in pancreatic juice, but does not diminish the quantity of bicarbonate contained in this juice, nor the rate of secretion.

4. The hypothesis is put forward that the enzyme content of pancreatic juice is determined by the vagus nerves, whereas the quantity of bicarbonate solution in which these enzymes are contained is determined by secretin. On this hypothesis, secretin may play a subsidiary part in pancreatic digestion, since it only ensures the presence in the intestine of an optimal reaction for the activity of the pancreatic enzymes.

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

REFERENCES.

(1) Pavlov. The work of the digestive glands. Trans. by W. H. Thompson, 2nd edition. 1910.
(2) Bayliss and Starling. This Journ. 28, p. 325, 1902; 29, p. 174. 1903.
(3) Babkin. Die äussere Sekretion d. Verdauungsdrüsen (Berlin), 1914.
(4) Dodds and Bennett. This Journ. 55, p. 382. 1921.
(5) Mellanby. Ibid. 45, p. 345. 1912.
(6) Mellanby and Woolley. Ibid. 48, p. 287. 1914.
(7) Babkin, Rubaschkin and Savitsch. Arch. f. mik. Anat. 74, p. 68. 1909. My quotation is from Bayliss. General Physiology, 4th edition, p. 346.
(8) Wertheimer and Lepage. Journ. de Physiol. 3, p. 335. 1901.
(9) Anrep. This Journ. 50, p. 421. 1915.