THE RELATION OF SECRETIN FORMATION TO THE ENTRANCE OF ACID CHYME INTO THE SMALL INTESTINE—THE PROPERTIES OF SECRETIN.

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In a previous paper(1) the hypothesis was put forward that the metabolism of the pancreatic enzymes is under the control of the vagus nerves, whilst secretin causes the cells of the pancreas to produce a copious flow of sodium bicarbonate (·14 *N*) which carries the pancreatic enzymes with it. On this hypothesis, secretin ensures the presence in the intestine of an adequate supply of sodium bicarbonate to preserve the neutrality of the intestine during the action of the pancreatic and intestinal enzymes. According to Bayliss and Starling(2) secretin is derived from prosecretin by the action of acid, and prosecretin exists only in that situation where it is in a position to be acted upon by the acid chyme and to discharge into the blood the substance which acts as a timely stimulus to the pancreatic cells. As a corollary to these statements the secretion of pancreatic juice, produced by secretin, has been causally connected with the secretion of hydrochloric acid by the gastric mucosa. In so far that apparently normal intestinal digestion may be associated with the complete absence of hydrochloric acid from gastric juice, it follows from this hypothesis that the vagus plays the dominant rôle, whilst the secretin mechanism may be adjuvant, but is not essential, to pancreatic secretion. This conclusion, however, is difficult to reconcile with the demonstrable facts of pancreatic secretion. Vagal pancreatic juice though rich in enzymes is extremely scanty in quantity and after prolonged vagal stimulation the cells of the pancreas show marked signs of exhaustion. Secretin juice, on the other hand, although relatively poor in enzymes compared to vagal juice, is secreted in copious quantities, and after a long period of secretion the cells of the pancreas show no sign of exhaustion under the secretin stimulus. Therefore, in order to elucidate these difficulties, and more particularly to reconcile the facts of normal pancreatic digestion with achlorrhydria, the distribution of prosecretin in the alimentary canal, the assumed existence of prosecretin and the properties of secretin were investigated.

The distribution of prosecretin in the alimentary canal. Bayliss and Starling(3) found that secretin extracts of the duodenum were more effective in causing a flow of pancreatic juice than those of the jejunum, whilst those of the ileum were inactive. Similarly, Lalou(4) made secretin extracts from the mucous membrane of various parts of the alimentary canal and compared their secretin contents on the same dog. He found that the extract of the duodenum contained approximately ten times as much secretin as the corresponding extract from the ileum and eighty times as much as the stomach extract. In the investigation of the distribution of secretin in the small intestine, the anatomical division of that portion of the gut into duodenum, jejunum and ileum was not followed. It appeared more reasonable to estimate the relative quantities of secretin in those portions of the gut in which digestion and absorption varies to the greatest extent. For this reason the secretin contents of the mucous membrane of the stomach (fundus and pylorus), small intestine (upper, middle and lowest third) and ascending colon were estimated. These experiments were carried out at the beginning of the investigation when it was assumed that secretin exists in the inactive form (prosecretin) in the mucous membrane. The extracts were therefore made by ·2 p.c. HCl in the method of Bayliss and Starling. The results are, however, comparable to those obtained with other extractives of secretin.

The mucous membrane was scraped from the alimentary canal of a goat within one hour of its death: 20 grm. of the mucous membrane from various portions, after being well ground up with sand, were boiled with 40 c.c. of HCl ·2 p.c., neutralised and filtered according to the accepted method for making secretin; ·4 c.c. of each of these filtrates was injected in turn into the femoral vein of an anæsthetised cat (urethane 1·5 grm. per kilo) and the quantity of resulting secretion from the pancreatic duct was measured.

Part of gut taken	Pancreatic juice secreted in c.c.
Fundus of stomach	Nil
Pylorus of stomach	Nil
Upper third of small intestine	2·75
Middle third of small intestine	2·6
Lowest third of small intestine	0·5
Ascending colon	0·2

These figures confirm to some extent the observations of Bayliss and Starling and of Lalou, on the distribution of prosecretin. The fact, however, which we desire to emphasise is that prosecretin exists in practically undiminished quantities in two-thirds of the small intestine

of the goat. It is evident, therefore, on the acid hypothesis of secretin formation, that although hydrochloric acid of the gastric juice may be an important factor in the formation of secretin from prosecretin, yet other substances produced in the intestine may enter into the mechanism. In this connection, amino acids produced from the digestion of protein in the duodenum and jejunum were considered as a possible source of acid. This hypothesis appeared to be verified by certain experimental results. Active secretin extracts were obtained by boiling the intestinal mucosa with water containing glutamic acid, leucine, tyrosine, etc. It appeared, therefore, that the occurrence of prosecretin in considerable quantities in the mucous membrane of the intestine well below the action of acid chyme might be appreciable on the hypothesis that amino acids could actively participate in the reaction. As an extension of the hypothesis, it is evident that fatty acids obtained from the digestion of fat by the first secreted pancreatic lipase might also participate in this mechanism since, as Moore and Rockwood(16) have shown, a meal of fat induces a weak acidic reaction throughout the greater part of the small intestine in the dog. In order to test the accuracy of the hypothesis that amino acids and organic acids might convert prosecretin into secretin and thus supplement the initial excitatory action of the acid chyme, the capacities of a variety of solvents to extract secretin from the intestinal mucosa were determined.

The existence of prosecretin. According to Bayliss and Starling(5) secretin is formed from prosecretin by a process of hydrolysis. They found that mineral acids were more effective than organic acids in producing this reaction and that a weak acid like carbonic acid was ineffective. These results were confirmed and extended by Camus(6) who, in addition to carbonic acid, found that boric acid was incapable of producing secretin from its precursor in the intestinal mucous membrane. In marked contrast to these results, a large number of observers have produced active extracts of secretin from the intestinal mucous membrane by solutions containing substances other than acids. Among such substances may be mentioned sodium oleate (Fleig(7)), chloral hydrate (Falloise(8)), ethyl alcohol (Fleig(9)), sodium chloride (Delezenne and Pozerski(10)), Witte's peptone (Gley(11)), cane sugar, glycine, urea and soaps (Frouin and Lalou(12)). In order to test the hypothesis that the precursor of secretin (prosecretin) exists in the mucous membrane of the small intestine and that this precursor is effectively hydrolysed by acids only, and thereby converted into secretin, the following experiment was carried out:

The mucous membrane of the upper two-thirds of the small intestine of a pig was ground up with sand and divided into ten equal portions. Each portion was boiled with twice the quantity by volume of (1) 75 p.c. alcohol, (2) 75 p.c. acetone, (3) 5 p.c. NaCl, (4) ·7 p.c. NaCl, (5) H_2O, (6) ·05 p.c. NaOH, (7) ·1 p.c. NaOH, (8) ·2 p.c. NaOH, (9) phosphate *p*H 6·5 and (10) phosphate *p*H 7·5. After neutralisation (if necessary) and filtering, the filtrates were tested for secretin on a cat anæsthetised with urethane (1·5 grm. per kilo). The number of drops of pancreatic juice and the rate of secretion were determined in each case after the intravenous injection of 2 c.c. of the filtrates into the femoral vein.

	75 p.c. alcohol		75 p.c. acetone		5 p.c. NaCl		·7 p.c. NaCl		H_2O	
Drops	m.	s.	m.	s.	m.	s.	m.	s.	m.	s.
2		47	1	30		47	1	40	1	55
4	1	16	2	9	1	20	2	25	2	45
6	1	57	3	7	1	53	3	6	4	5
8	2	43	4	2	2	24	3	58	5	35
10	3	15	4	47	2	58	4	46	7	3
12	3	46	5	33	3	36	5	36	8	56
16	4	59	7	20	5	23	8	6	16	0
20	6	16	9	49	7	45	12	10	—	
24	7	47	14	57	11	9	—		—	
28	9	45	—		—		—		—	

	·05 p.c. NaOH		·1 p.c. NaOH		·2 p.c. NaOH		Phosphate *p*H 6·5		Phosphate *p*H 7·5	
Drops	m.	s.	m.	s.	m.	s.	m.	s.	m.	s.
2	1	36	2	5	1	25	1	50	1	55
4	2	25	3	31	2	20	2	40	2	53
6	3	9	4	52	3	35	3	55	4	5
8	3	51	6	54	4	53	5	30	5	38
10	4	48	10	52	6	35	7	50	8	10
12	5	49	17	30	9	40	13	20	13	0
16	8	18	—		—		—		—	
20	13	12	—		—		—		—	

It may be observed that the filtrates from the 75 p.c. alcohol extract contain only about 50 p.c. alcohol owing to the quantity of water contained in the intestinal mucosa which is subjected to extraction. Similar remarks apply to the acetone extract. The results indicate the apparent marked diversity of solutions which can be used as effective extractives of secretin. The efficiency of dilute alcohol for the preparation of secretin is well illustrated. Within 10 minutes of the injection of 2 c.c. of alcoholic secretin into the blood stream, the pancreas secreted 2 c.c. of juice. In order to emphasise the efficiency of dilute alcohol as an extractive for secretin, an experiment was carried out in which dilute alcohol and ·2 p.c. HCl were compared directly. The results obtained are given below (p. 126).

The result emphasises the marked superiority of dilute alcohol over acid for the preparation of secretin extracts from the intestinal mucous membrane. The comparative records of the two experiments show that acid is a less effective extract for secretin than alcohol (50 p.c.), acetone (50 p.c.), 5 p.c. NaCl and ·7 p.c. NaCl.

Drops	75 p.c. alcohol m.	s.	·2 p.c. HCl m.	s.
2		15	1	0
4		50	1	25
6	1	12	1	50
8	1	36	2	16
10	2	6	2	46
12	2	50	3	27
16	4	13	5	25
20	5	43	8	10
24	7	20	—	
28	9	14	—	
32	11	0	—	
Total juice	2·2 c.c.		1·4 c.c.	

It is evident that hydrochloric acid possesses no specific capacity for the preparation of secretin extracts from the intestinal mucosa. The fact that a comparatively strong solution of alkali (·2 p.c. NaOH) is able to extract secretin is a definite proof against the hypothesis that prosecretin is hydrolysed by acids with the formation of secretin. The experiments establish the fact that secretin is contained in a preformed condition in the mucous membrane of the upper two-thirds of the small intestine, and that this secretin is soluble in water, and is stable in dilute solutions of acid, acetone and alcohol.

Properties of secretin. According to the observations of Bayliss and Starling and W. A. Osborne(13), secretin may be regarded as a simple substance since it is stable when boiled in dilute acid or alkaline solutions and may be dialysed. Further, according to these observers, it is not precipitated from solution by tannic acid, a fact which differentiates it from a protein, alkaloid or diamino acid. On the other hand, it may be removed from solution by salts of the heavy metals. Dale and Laidlow(14) showed that the precipitation of secretin by mercury salts could be utilised to purify secretin preparations. It has been stated(15) that secretin appears to be an amine derived by decarboxylation of an amino acid, but the evidence in support of this statement is not available.

Solubility. From the foregoing experiments it is evident that secretin in the intestinal mucosa is freely soluble in water, and is comparatively stable when boiled in solutions of sodium chloride (5 p.c.), phosphate (*p*H 6·5 and 10·5), dilute acids (·2 p.c. HCl), dilute alkali (·1 p.c. NaOH), dilute alcohol (50 p.c.) and dilute acetone (50 p.c.).

Action of enzymes. Bayliss and Starling found that secretin is rapidly destroyed by trypsin. This fact would appear to indicate that secretin is a protein or polypeptide capable of being hydrolysed by this enzyme. Secretin, however, is readily destroyed by pepsin and since rapid peptic digestion proceeds only as far as proteoses it follows that

secretin must belong to that class of proteins. Alternatively, secretin may be a relatively simple substance which owes its solubility to its association with a proteose, and when this association is broken by digestive enzymes the labile secretin is destroyed. In this connection the action of intestinal intracellular enzymes is of practical importance. It is well known that secretin solutions made in a routine manner show considerable variations in activity. The circumstances underlying these variations were determined. An important factor was the duration of the interval between the death of the animal and the extraction of the intestinal mucosa. The following experiment illustrates this statement and indicates the cause of the variation:

The mucous membrane from the small intestine of a pig was divided into three equal portions and suspended in (*a*) H_2O, (*b*) ·2 p.c. HCl, and (*c*) alkaline phosphate (*p*H 10·5). The suspensions were left at 37° C. for 1 hour. After this time secretin solutions were prepared from them in the usual way by boiling, etc. The alkaline phosphate solution showed a slight amount of activity; the water and ·2 p.c. HCl extracts were completely inactive.

It is evident, therefore, that secretin is destroyed by the enzymes in the intestinal mucosa, rapidly in a neutral or acid medium and more slowly in an alkaline medium.

Precipitation. (*a*) *Ammonium sulphate.* The precipitation of secretin solutions by Am_2SO_4 gives characteristic results. The precipitate formed by half saturation with Am_2SO_4 contains no secretin; the precipitate obtained by saturation of the filtrate from this mixture with Am_2SO_4 contains all the secretin of the original solution.

(*b*) *Alcohol* added to a secretin solution to the extent of 85 p.c. at 0° C. produces a partial precipitation of secretin.

(*c*) *Tannic acid.* From the foregoing description it is evident that secretin is soluble in water, is not destroyed by heat (100° C.), is not precipitated by half saturation but is precipitated by full saturation with Am_2SO_4. Further, it is rapidly destroyed by trypsin, pepsin and the intracellular enzymes of the small enzymes of the small intestine. All these properties indicate that secretin is a secondary albumose. Bayliss and Starling, however, suggest that secretin belongs to a much simpler class of substances, since it is not precipitated by tannic acid. Detailed experiments were therefore carried out to analyse this precipitation. The complete precipitation of proteins by tannic acid is a matter of considerable difficulty. The reaction of the fluid must be made slightly acid and the degree of acidity and the required amount of tannic acid varies with every protein solution. These difficulties have been recognised by Almén, who has made a tannic acid reagent con-

taining acetic acid and alcohol which he states to be a more effective precipitate than ordinary tannic acid solutions. The activities of a secretin solution before and after precipitation by tannic acid and Almén's reagent are shown in the following figures which were all obtained from the same cat:

Drops	Original secretin solution m. s.	Filtrate after ppt. by tannic acid m. s.	Filtrate after ppt. by Almén's reagent m. s.
5	1 55	3 36	1 55
10	3 0	5 0	3 26
15	4 1	6 38	5 35
20	5 8	8 40	8 29
25	6 1	12 20	14 45
50	11 30	—	—
75	22 23	—	—
Total juice	4·3 c.c.	1·6 c.c.	1·4 c.c.

It is evident that tannic acid removes considerable quantities of secretin from solution, the amount removed being comparable to the proportion of protein precipitated. The results certainly offer no evidence in favour of the hypothesis that secretin is not a protein.

Colloidal iron and colloidal gold. Secretin is not precipitated from solution by either positively or negatively charged suspensoid colloids. The precipitation of colloidal iron in a secretin solution does not diminish the secretin activity of the resulting filtrate. A similar fact is true for colloidal gold precipitation. From this it may be inferred that secretin is electrically neutral when dissolved in water.

The action of the products of peptic digestion on pancreatic secretion. The foregoing facts suggested that secretin is a secondary albumose formed in gastric digestion which, when absorbed into the blood, stimulates the pancreas to secrete. This hypothesis, if correct, would correlate gastric and pancreatic functions. No experimental evidence was obtained in favour of it. The intravenous injection of proteose solutions and peptic digests in all stages of digestion into cats never produced any secretion from the pancreas, although control experiments with secretin evoked well-marked pancreatic secretion. The hypothesis was directly negatived by an experiment with a fœtal goat obtained from the uterus two weeks before full term. The small intestine of this fœtus, when extracted with ·2 p.c. HCl in the usual way for the preparation of secretin, produced a well-marked secretion of pancreatic juice when injected into a cat. It is evident, therefore, that secretin has no direct relation to gastric digestion unless the secretin in the fœtal mucous membrane is derived from the secretin in the maternal goat's blood and stored in it during development.

Secretin as a primary amine. Secretin is described by Robertson(15) as a primary amine derived by decarboxylation of an amino acid. This statement was tested by submitting a secretin solution to the action of nitrous acid. No destruction of secretin occurred. Since nitrous acid decomposes primary amines with the evolution of nitrogen, it is evident that the statement made by Robertson is inaccurate.

Discussion.

The experimental results show that considerable quantities of secretin exist in the mucous membrane of the upper two-thirds of the small intestine. There is no evidence that hydrochloric acid is necessary for the formation of secretin from an assumed precursor in the cells of the intestinal mucosa, nor is there any evidence that this assumed precursor exists only in that portion of the small intestine in which it is in a position to be acted upon by acid chyme. The experimental facts do not afford any basis for the assumption that pancreatic secretion and gastric hydrochloric acid are causally connected. Further, the results show that the secretin mechanism for the production of pancreatic juice may function in complete gastric achlorrhydria. An analysis of the properties of secretin shows that it is soluble in water, is not destroyed by heat (100° C.), is not precipitated by half saturation with Am_2SO_4, but by full saturation with this salt. Further, secretin is rapidly destroyed by pepsin, trypsin and the intracellular enzymes of the small intestine. All these properties indicate that secretin is a secondary albumose or is intimately associated with a secondary albumose. Experiments with various protein fractions obtained by peptic and tryptic digestion indicate that secretin is not derived from the digestion of protein foodstuffs. This conclusion was confirmed by the fact that the small intestine of an immature fœtal goat, taken directly from the uterus, contained large quantities of secretin.

Summary.

1. Secretin exists in a preformed state in considerable quantities in the mucous membrane of the upper two-thirds of the small intestine. A relatively small quantity of secretin may be extracted from the lowest third of the intestine.

2. Secretin contained in the mucous membrane of the intestine is soluble in water, and is relatively stable in dilute solutions of acid, alkali, alcohol and acetone. It may therefore be obtained by extracting the mucous membrane of the upper two-thirds of the small intestine with

water, ·2 p.c. NaOH, phosphate solutions (*p*H 7·5, 6·5), ·2 p.c. HCl, 75 p.c. alcohol and 75 p.c. acetone.

3. There is no evidence that gastric hydrochloric acid converts a precursor (prosecretin) in the duodenal mucous membrane into secretin and thereby excites a flow of pancreatic juice.

4. Secretin possesses all the properties of a secondary albumose, being soluble in water, precipitated on full saturation with Am_2SO_4 and destroyed by pepsin, trypsin and the intracellular enzymes of the small intestine.

5. Secretin is not derived from the alimentary digestion of protein, since it is present in considerable quantities in the small intestine of the fœtus.

6. There is no evidence that the chemical group to which secretin owes its activity is of the nature of a primary amine.

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