THE ISOLATION OF SECRETIN—ITS CHEMICAL AND PHYSIOLOGICAL PROPERTIES.

By JOHN MELLANBY.

(*From the Sherrington School of Physiology, St Thomas's Hospital, London.*)

THE properties of secretin were described by Bayliss and Starling(1) in 1902. Since that time very little additional information has been published regarding the properties of this remarkable substance. In 1912 Dale and Laidlaw(2) published a preliminary note on the preparation of secretin picrate from the duodenal mucosa by precipitation with mercury salts and picric acid. In 1917 Dalmau(3) obtained a secretin preparation of high potency and low toxicity by precipitating secretin solutions with nine volumes of acetone. In 1926 Luckhardt, Barlow and Weaver(4) prepared a highly active solution by a modification of the original Bayliss and Starling method. About 100 c.c. of 0·4 p.c. HCl are introduced into the closed excised duodenum of a dog, and the loop with its contents is incubated for a variable time at either 37° C. or room temperature.

Bayliss and Starling stated that the physiological properties of secretin were (1) a powerful secretory action on the pancreas, and (2) a feeble secretory action on the bilary apparatus of the liver. Various observers have assigned other properties to secretin. Abelous and Soula(5) state that secretin increases nitrogenous metabolism, the secretion of urine, and respiratory exchange. Pitcairn(6) observed that secretin acts as a diuretic, but only when prepared from the same species as the injected animal. Downs and Eddy(7) state that secretin augments the red and white cells of blood by its action on bone marrow and lymphoid tissues. This hæmopoietic property of secretin was also described by King(8). Further, according to Downs and Eddy, secretin has a powerful action on striated muscle since purified secretin, perfused through frog's muscle, increases the work done and lengthens the fatigue time. In regard to carbohydrate metabolism Downs and Eddy state that secretin causes an increased output of sugar from some source in the organism. On the other hand, Santos(9) states that secretin exerts a

hypoglycæmic action in man, dog, and rabbit. Lambert and Hermann (10) observed a transient hyperglycæmia followed by hypoglycæmia.

The isolation of secretin.

The method adopted for the isolation of secretin depends upon three observations: (1) secretin may be extracted from the intestinal mucosa by absolute alcohol; (2) precipitated bile acids adsorb secretin from solution; and (3) dilute acid precipitates secretin from aqueous solution.

In a series of papers (11, 12, 13, 14) proofs have been given that secretin is present in a preformed condition in the intestinal mucosa, and that powerful secretin solutions may be prepared by extracting the duodenal mucosa with absolute alcohol. Further, it has been shown that in the living animal the secretion of pancreatic juice is determined by the entrance of bile into the duodenum. An analysis of these phenomena showed that bile salts, when absorbed from the intestine, carry secretin contained in the cells of the duodenal mucosa into the portal blood and so to the pancreas. This deduction indicated that secretin and bile salts come into intimate relation with one another owing to their chemical or physical properties. The original method of Bayliss and Starling for the preparation of secretin extracts indicated that secretin is soluble in dilute acid. In point of fact secretin in aqueous solution is precipitated by dilute acid. For convenience the description of the method is divided into a series of processes. The difficulties which may arise at each stage are considered in a following section.

(1) The mucosa is scraped off a number of duodena. In these experiments pigs duodena were used. After grinding with sand, 250 grams of mucosa are extracted with a litre of absolute alcohol for 30 minutes at room temperature. The mixture is filtered through a coarse filter paper (Chardin) giving a clear yellow filtrate.

(2) This filtrate is distilled *in vacuo* to about 40 p.c. of its volume, *i.e.* until the liquid becomes opalescent and starts to froth. The solution is made up to the original volume with distilled water. This causes partial aggregation of the colloidal soaps and fats contained in the extract.

(3) The removal of fats and soaps is effected by means of $CaCl_2$ (0·01 *N*) at a temperature of about 20° C. For this purpose 1 c.c. of $CaCl_2$ (*N*) is added to each 100 c.c. of the slightly warmed fluid from (2). Within a few minutes the whole of the fats and soaps aggregate and form a well-marked precipitate which may be quickly filtered off by a coarse filter paper.

(4) The clear filtrate from (3) is cooled in an ice chest. Two c.c. of

10 p.c. bile salt (commercial sodium tauroglycocholate) are added to each 100 c.c. of the filtrate. After complete mixing, acetic acid to the extent of 0·2 p.c. is added. A small precipitate is produced on adding bile salt, but this precipitate is increased by the addition of the acetic acid. The precipitated bile acids adsorb secretin and carry it out of solution. Flocculation and settling of the precipitate occur more rapidly at low temperatures. Therefore, after addition of the bile salt and acetic acid, the mixture is left for one or two hours in an ice chest.

(5) The precipitate from (4) is obtained by centrifugalisation, and is dissolved in a small volume of absolute alcohol. Some of the precipitate does not dissolve. This may be removed by spinning and rejected.

(6) The alcoholic solution of secretin and bile acid is added to five volumes of acetone. After a short time a flocculent precipitate of secretin separates out and may be obtained as a compact mass by spinning in a high speed centrifuge. The precipitate is washed with acetone (twice) and dried with ether.

(7) Further purification is obtained by dissolving the precipitate from (6) in a small volume of water in which this secretin is freely soluble. Acetic acid is added to the solution to the extent of 0·1 p.c. The secretin is precipitated and may be obtained by spinning, and drying whilst in the centrifuge tube with acetone and ether. The final product is very active and has all the characteristic properties which are described in the following pages.

Notes on the various stages in the preparation of secretin. (1) Secretin is rapidly destroyed by autolytic and digestive enzymes. Therefore the duodena should be obtained from recently killed animals. (2) The optimum temperature for the extraction of secretin from the mucosa by alcohol is between 15° C. and 30° C. At low temperatures (5° C.) only a small quantity of secretin is extracted; at high temperatures (70° C.) a great part of the secretin is destroyed. (3) Secretin in alcoholic solution is rapidly destroyed at temperatures above 60° C. Therefore distillation of the alcoholic extract should be carried out, *in vacuo*, at a low temperature. Distillation may be taken below a 40 p.c. volume without injury to the secretin. (4) The precipitation of the suspensoid colloids of fats and soaps by $CaCl_2$ occurs more completely and rapidly at 20° C. than at 10° C. Therefore this process is facilitated by warming the solution. At this stage there is always some loss of secretin since $CaCl_2$ precipitates the bile salts which are always present to some extent in the alcoholic extract of the duodenal mucosa. These precipitated bile salts carry some secretin out of solution. The loss however is small—usually about 10 p.c. of the

total secretin contained in the original alcoholic extract. (5) The adsorption of secretin by precipitated bile acids requires care. Commercial sodium tauroglycocholate contains varying quantities of sodium glycocholate and sodium taurocholate depending upon the source of the original bile. These two salts possess very different properties as regards precipitation by acid. A solution of sodium glycocholate becomes opalescent and slowly precipitates on the addition of small quantities of acetic acid; a similar solution of sodium taurocholate is not precipitated by large quantities of acetic acid. Further, according to Hammarsten (15) taurocholic acid keeps glycocholic acid in solution and so prevents precipitation on acidifying a mixture of both salts. The precipitation properties of these two bile acids are too obscure to be discussed in detail in this paper. As a matter of routine a commercial preparation of sodium tauroglycocholate was used which readily precipitated under the conditions described in the method. This stage must be controlled by animal experiments until the optimum conditions for the precipitation of the bile salt and the consequent adsorption of the secretin from solution have been determined. The method described is adequate for all commercial preparations of bile which, when dissolved in water, become opalescent on adding acetic acid to the extent of 0·1 p.c. The aggregation of the bile acid-secretin adsorption compound is assisted by carrying out this process at a low temperature. (6) Secretin is readily adsorbed from solution by a large variety of substances. Hardened filter paper is a particularly active adsorbant of secretin. Therefore after precipitating secretin from the crude solution by means of bile acid it is essential to centrifuge and not to filter all subsequent solutions.

Methods of animal experiment. It is advisable when carrying out this process for the isolation of secretin to control all stages by animal experiment. In experiments on cats the following procedures have been used:

A nonvolatile anæsthetic (usually veronal 0·45 grm. per kg. of body weight) is administered subcutaneously three hours before starting the experiment. After this time the animal, if kept warm, is usually well under the influence of the anæsthetic. In some cases however it is essential to give chloroform during the operative procedures. Cannulæ are tied into the right femoral vein and gall bladder, in the latter case the cannula being of large size to permit the free flow of bile. A large dose of secretin (0·1 mgm.) is injected into the femoral vein. This causes the pancreas to secrete copiously and the viscid pancreatic juice present in the ducts is excreted into the duodenum. After five minutes a ligature is

placed under the common bile duct and pancreatic duct near their entrance into the duodenum. The pancreatic duct is opened by a fine incision—a correct incision being evidenced by the free flow of pancreatic juice from the cut surface. This flow of juice greatly facilitates the insertion of the cannula into the pancreatic duct, and the rapid flow of juice into the cannula indicates that the procedure has been correctly carried out. The ligature by which the cannula is tied into the pancreatic duct includes the common bile duct to prevent any bile entering the duodenum during the course of the experiment. The necessity for preventing the entrance of bile into the duodenum when standardising secretin preparations is evident from previous experiments in which it has been shown that the absorption of bile from the duodenum leads to a copious flow of pancreatic juice. The cannula inserted into the gall bladder allows the free exit of bile from the liver during the course of the experiment and is essential since bile is continuously secreted by the liver. Finally, it must be emphasised that cats vary considerably in their capacity to secrete pancreatic juice. Some cats have a small pancreas and secrete about 1 c.c. of pancreatic juice in 30 minutes; others possess a well-developed pancreas and may secrete 5 c.c. of pancreatic juice in that time. Therefore comparative experiments should be carried out on the same animal.

A protocol of an experiment is given to illustrate the method: 200 grm. of duodenal mucosa (pig) were ground up with sand and extracted with 800 c.c. of absolute alcohol for 30 mins. at room temperature (16° C.), 800 c.c. of filtrate were distilled *in vacuo* to 310 c.c. at a temperature not exceeding 50° C. This was diluted to 800 c.c. with distilled water and warmed to 20° C. Eight c.c. of $CaCl_2$ (*N*) were added, the fluid was well shaken and allowed to stand for five minutes. The precipitated fats and soaps were removed by filtration through a coarse paper. The clear filtrate was cooled to 5° C. and 16 c.c. of 10 p.c. sodium tauroglycocholate and 8 c.c. of 20 p.c. HA were added. The mixture was well shaken and allowed to stand in an ice chest for two hours. At the end of that time the precipitate was centrifuged off and dissolved in 20 c.c. of absolute alcohol. The portion of the precipitate which did not dissolve in the alcohol was separated by centrifuging and rejected. The alcoholic solution was added to 100 c.c. of acetone. A fine precipitate was formed and this was allowed to settle for one hour. At the end of this time the precipitate was obtained as a compact mass by spinning, and after twice washing with acetone was dried with ether. The weight of the precipitate was 15 mg. This precipitate was now dissolved in 15 c.c. of water in which it was completely soluble. Acetic acid was added to this solution to the extent of 0·1 p.c.

A precipitate was produced which, after 15 min., was obtained as a compact mass by spinning the fluid. This precipitate, after drying with acetone and ether, weighed 6·0 mg. The pancreatic activities of the various fractions were as follows:

1 c.c. of the original alcoholic extract gave 1·2 c.c P.J.
1 c.c. of the filtrate after removal of the fats and soaps by $CaCl_2$ gave 1·0 c.c. P.J.
0·05 mg. of the first dried precipitate gave 1·7 c.c. P.J.
0·05 mg. of the second dried precipitate gave 5·5 c.c. P.J.
0·017 mg. of the second dried precipitate gave 2·5 c.c. P.J.

On the assumption that the first and last solutions may be compared, it follows that 1 c.c. of the original extract contained $\frac{1}{120}$ mg. of secretin and that a gram of the original mucosa yielded $\frac{1}{30}$ mg. of secretin.

THE CHEMICAL PROPERTIES OF SECRETIN.

Secretin is an amorphous powder of a pale brown colour.

Solubility. Secretin slowly dissolves in water. Solution occurs more readily on adding a little sodium bicarbonate. This solution is a pale yellow colour even in concentrations as low as 0·05 p.c. The solution in water gives a stable froth on shaking. Secretin is insoluble in acetone, ether or absolute alcohol, but alcohol containing 5 p.c. of water dissolves a recognisable quantity of it.

Colour reactions. Secretin contains carbon, hydrogen, nitrogen, oxygen, sulphur and phosphorus. The colour reactions of secretin tested on a 0·5 p.c. solution gave the following results. *Biuret*: the colour developed was faint but positive. *Xanthoproteic*: this reaction was definitely positive. *Millon's* reaction was well marked as was also *Pauly's* reaction. *Glyoxylic*: this reaction was very indefinite but probably positive. The *Ninhydrin* and the *Molisch* reactions were both negative. After the oxidation of secretin by HNO_3 and H_2SO_4 the solution gave a well-marked phosphate, but only a faint sulphur reaction. These qualitative tests indicate that secretin is a polypeptide containing tyrosine and probably histidine together with a large amount of organic phosphorus and a trace of sulphur. The negative ninhydrin reaction suggests the absence of a free amino group in the molecule.

The precipitation of secretin. Secretin is precipitated from solution in water by saturation with ammonium sulphate. On this account it may be classed as a secondary albumose. Secretin dissolved in water is readily precipitated by small quantities of acetic acid (0·1 p.c.). It is also precipitated by picric acid and tannic acid. These precipitations are probably due to the reaction of the fluids and not to the formation of secretin

picrate or tannate. It is not practicable to precipitate secretin dissolved in water by alcohol or acetone, but secretin dissolved in 80 p.c. alcohol is precipitated by five volumes of acetone.

Hydrolysis by acid and alkali. Secretin dissolved in water may be boiled without any loss of activity. It is rapidly hydrolysed by dilute acid or dilute alkali. Seventy-five p.c. is destroyed when a solution of it in 0·1 p.c. HCl is heated to 100° C. for five minutes; similar heating in 0·1 p.c. NaOH results in its complete destruction.

Hydrolysis by trypsin and pepsin. Secretin is rapidly destroyed by both trypsin and pepsin. An equal volume of cat's pancreatic juice activated by enterokinase (trypsin) destroys secretin within five minutes at 37° C. Pepsin in 0·2 p.c. HCl also destroys it within a few minutes.

Destruction by autolytic enzymes. Secretin rapidly disappears when washed duodenal mucosa is ground up with sand and is incubated at 38° C. Probably this destruction is due to the autolytic enzymes contained in the cells of the mucosa. In conformity with this action of proteolytic enzymes it is found that secretin injected into the lumen of the duodenum rapidly disappears but causes no secretion of pancreatic juice. Probably these effects are due to the non-absorption of secretin from the lumen of the intestine by the cells of the villi and its digestion by the intestinal enzymes. The facts emphasise the impossibility of administrating secretin by the mouth.

Destruction by bacteria. Secretin, dissolved in water and sterilised by heat, preserves its activity for a considerable time. If the solution is left exposed to the air its activity usually disappears in the course of two days. This destruction is probably due to the growth of bacteria in the solution. Secretin dissolved in 85 p.c. alcohol preserves its activity for many days.

Relation of secretin to acids and bases. Certain indications suggest that secretin forms salts with sodium, magnesium and calcium. In the isolation of secretin the first precipitation with acetone takes place much more readily if magnesium and not calcium has been used to precipitate the fats and soaps. Also, secretin obtained after the use of calcium is more readily precipitated by acetone if a few milligrams of potassium oxalate are added to the solution. On the other hand, secretin in the presence of strong acids (HCl 0·2 *N*) appears to act as a base. Hydrochloric acid of this strength dissolves considerable quantities of secretin which cannot be precipitated from solution by means of acetone. This apparent capacity of secretin to act as an acid or base corresponds with the assumption of its polypeptide nature. Probably the absence of free amino groups and

the large content of organic phosphorus determine the acidic character of the compound.

Adsorption of secretin. Secretin, dissolved in water, is readily removed from solution by adsorption. In the preparation of secretin many attempts were made to obtain a non-pigmented product by means of charcoal. Charcoal, added to a neutral solution of secretin in water, adsorbs the whole of the secretin; in alkaline solution the quantity of secretin adsorbed by charcoal depends on the degree of alkalinity, the more alkaline the solution the less the amount adsorbed. The adsorption of secretin in acid solution by charcoal cannot be tested because secretin is precipitated from solution by small concentrations of acid. In those solutions in which partial adsorption by charcoal was produced the change in activity was proportional to the change in pigmentation. This result indicates that the active principle is a pigmented substance. Alumina adsorbs considerable quantities of secretin from solution in water. On extraction of the alumina with alcohol the secretin is recovered in its original pigmented form. In alkaline solution alumina does not adsorb secretin; in acid solution the acid alone precipitates secretin and thereby removes it from solution in water. Secretin is not adsorbed by precipitated cholesterol when an alcoholic solution of cholesterol is added to a solution of secretin in water. Hence secretin cannot be purified in a way similar to that described for insulin. Similarly attempts were made to purify secretin by adsorption with benzoic acid. Sodium benzoate was added to a secretin solution and acid was added to the mixture to precipitate the benzoic acid. Secretin however is precipitated from solution by much smaller quantities of acid than are required to decompose sodium benzoate and effect precipitation of the benzoic acid. Finally, it must be emphasised that hardened filter paper adsorbs secretin to a marked degree. Filtration through hardened filter paper often diminishes the activity of a solution by 50 p.c. This fact indicates the necessity of separating precipitates from secretin solution by centrifugilisation and not by filtration.

Crystallisation of secretin. Attempts were made to crystallise secretin. These experiments were carried out on two general lines in order to produce very slow precipitation of secretin: (*a*) adding minimal quantities of acetic acid to a solution of secretin in water, and (*b*) adding small quantities of acetone to a solution of secretin in 90 p.c. alcohol. No success was obtained by either method, the resulting precipitates being amorphous and pigmented.

THE PHYSIOLOGICAL PROPERTIES OF SECRETIN.

Experiments have been made to determine the physiological actions of secretin. The results are briefly summarised.

Administration.

(*a*) *Intravenous injection.* In the following experiments secretin was dissolved in water and injected into the right femoral vein. The resulting secretion of pancreatic juice usually lasts about 30 minutes, but secretion may continue for an hour.

(*b*) *Subcutaneous injection.* In the anæsthetised animal a subcutaneous injection of secretin may produce a small secretion of pancreatic juice, but in many instances no result is observed. If, however, secretin is given subcutaneously to a cat 15 minutes before the administration of a volatile anæsthetic, then the pancreas yields a small secretion of juice when the duct is opened. It appears that secretin is absorbed slowly into the blood from the subcutaneous tissues. In the anæsthetised animal, which has been submitted to operative procedures, the rate of absorption may be too slow to stimulate the pancreas.

(*c*) *Alimentary administration.* The destruction of secretin by pepsin and trypsin is so rapid that it is useless to give secretin by the mouth. It was of interest to determine, however, whether secretin, admixed with bile, is adsorbed from the small intestine. To determine this point it was necessary to inject the mixture of bile and secretin into a portion of the small intestine in which the mucous membrane contains no secretin, since the absorption of bile only through the duodenum leads to a secretion of pancreatic juice. The ileum of the cat contains no secretin. Therefore a solution consisting of 2 c.c. ox bile, 7 c.c. Ringer and 1 c.c. $NaHCO_3$, 1·5 p.c. containing 1 mg. of secretin was injected into the ileum of a fasting cat in which cannulæ had been tied into the pancreatic duct and common bile duct. Within ten minutes the secretion of bile by the liver increased five-fold (due to the absorption of bile salts from the ileum). This increased secretion of bile continued for two hours. During that period there was no secretion of pancreatic juice and therefore no absorption of secretin, although the bile-secretin mixture contained as much secretin as about fifty maximal intravenously injected doses. The result indicates that the association of bile salt with secretin is very labile, since the bile salt was rapidly absorbed into the portal blood whereas the secretin remained in the ileum.

Pancreas.

(*a*) *External secretion.* The action of secretin on the external secretion of the pancreas has been discussed in a previous paper (16). Experimental results were given which indicated that the metabolism of the enzymes of the pancreas is controlled by the vagus nerves. Under the stimulus of secretin the cells of the pancreas secrete a dilute solution of sodium bicarbonate (approximately 0·15 p.c. $NaHCO_3$) by which the enzymes of the gland are carried into the intestine. This bicarbonate solution also provides the optimum medium for the enzymes to exert their digestive activities.

(*b*) *Internal secretion.* It has been stated by a number of observers that secretin acts on the cells producing the internal secretion of the pancreas; the evidence adduced being that secretin produces hypoglycæmia when injected into the intact unanæsthetised animal. These statements have been investigated in a number of experiments on rabbits. In the case of secretin preparations of a high degree of activity, large quantities (1 mg.), injected subcutaneously, have no influence on the glucose content of the boood. In some early experiments relatively impure secretin preparations, injected subcutaneously, produced a small fall in the blood sugar. Further purification resulted in the disappearance of this hypoglycæmic reaction. Probably a small quantity of insulin derived from the intestinal mucosa was associated with the secretin in the impure products. It may be definitely stated that the action of secretin is limited to the external secretion of the pancreas.

Liver.

The action of secretin as a cholagogue has been discussed in a previous paper (17). A series of experiments proved that this action of secretin is secondary to its action on the pancreas. As a result of the secretin stimulus products of pancreatic activity pass into the portal blood and stimulate the liver cells to secrete bile.

Red blood corpuscles.

A detailed investigation into the secretion of bile shows that many cholagogues have active hæmolytic qualities. In the early stages of this work various preparations of secretin were found to act as hæmolysins when added to suspensions of red blood corpuscles. With further purification however it was found that the hæmolytic property was lost whilst the cholagogue activity of the preparations remained.

Smooth muscle.

The results of many early experiments in which the secretion of pancreatic juice followed the absorption of bile from the duodenum indicated that secretin facilitated digestion by increasing intestinal peristalsis and by expelling bile from the contracted gall bladder (18). Therefore the action of secretin on the smooth muscle of the small intestine and uterus was investigated.

Intestinal muscle.

One inch of the jejunum of a young rabbit was suspended in oxygenated Tyrode solution and the contractions were recorded by a lever. This portion of the rabbit's intestine shows marked rhythmic contractions as recorded by the tracing (Fig. 1 *A–B*). The contractions occur about every eight seconds, the rate of contraction being greater the less the amplitude of the contractions. At the point (*B*) 0·1 mg. of secretin was added to the Tyrode solution (25 c.c.), the concentration of secretin being 1 : 250,000. A large increase in the general tonus of the muscle was produced, this being followed by a return of the rhythmic contractions at the higher tonic level (Fig. 1). After the solution was removed and replaced by Tyrode solution the intestine returned within a few minutes to its original condition. Fig 2 shows the effect of adding 0·03 mg. of secretin to the Tyrode solution, the concentration of secretin in this case being 1 : 750,000. The resulting increase in tone was small, indicating that a concentration of secretin less than this quantity does not affect intestinal muscle.

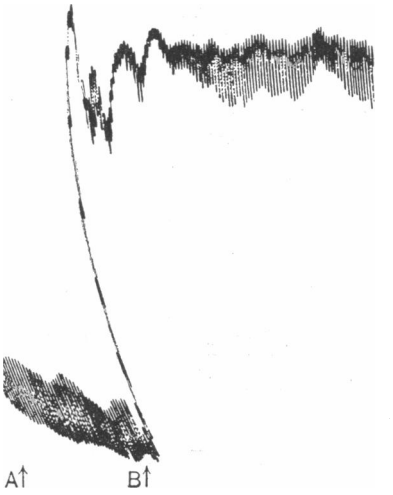

Fig. 1. 1 in. of jejunum of rabbit suspended in Tyrode solution (25 c.c.). *A–B*, normal rhythmic contractions. At the point *B*, 0·1 mg. secretin was added to the solution giving a concentration of secretin of 1 : 250,000.

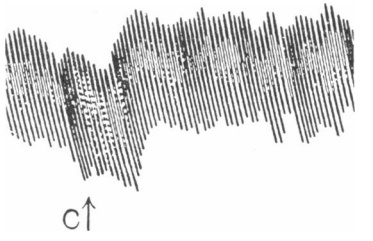

Fig. 2. Same as Fig. 1. At the point *C*, 0·03 mg. of secretin was added to the Tyrode solution giving a concentration of secretin of 1 : 750,000.

Uterine muscle.

The uterus of a guinea-pig was suspended in Tyrode solution. At the point (*A*), Fig. 3, 0·1 mg. of secretin was added to the solution. A large contraction of the uterine muscle resulted, followed by a return of the rhythmic contractions of the uterus which had been absent for the previous five minutes. The effect of secretin on uterine muscle in this experiment was great, but in many experiments it was observed that uterine muscle is less sensitive than the muscle of the intestine.

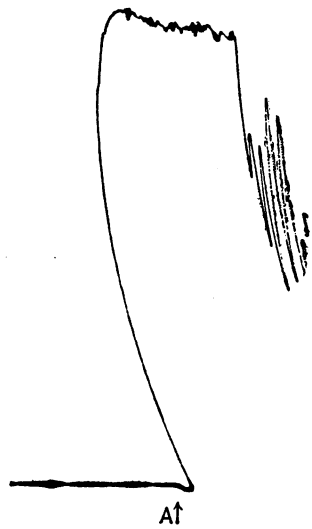

Fig. 3. Guinea-pig's uterus suspended in Tyrode solution (25 c.c.). At the point *A*, 0·1 mg. of secretin was added to the solution, giving a concentration of secretin of 1 : 250,000.

The problem arose whether quantities of secretin in the blood which produce a secretion of pancreatic juice also stimulate smooth muscle—in fact, whether secretin shares its normal activities between the pancreas and the plain muscle of the body. The effects produced by the intravenous injection of secretin into a cat showed that the pancreas is much more sensitive to the action of secretin than the muscle of the intestine. The pancreatic activity of the secretin preparation used in the plain muscle experiments recorded was determined. It was found that the intravenous injection of 0·03 mg. of this secretin into a cat resulted in a maximum pancreatic secretion, 3·6 c.c. of pancreatic juice being secreted in 30 minutes. The volume of the cat's blood was 150 c.c., so that a concentration of one part of secretin in 4,500,000 parts of blood caused the pancreas to secrete at a maximal rate. The least concentration of secretin in Tyrode solution which influences the tone of intestinal muscle is approximately 1 : 500,000. Therefore the pancreas is about ten times as sensitive to the action of secretin as the plain muscle of the intestine. A concentration of secretin in the blood which produces a maximal stimulation of the pancreas should have no action on the plain muscle of the body.

This experiment was confirmed by an experiment on the urinary bladder of an anæsthetised cat. A cannula, connected with a reservoir of warmed salt solution, was tied into the bladder. The rhythmic contrac-

tions of the smooth muscle of the bladder wall produced corresponding variations in the volume of the salt solution reservoir and these were recorded by a tambour. The injection of secretin (0·03 mg.) into the blood caused a copious secretion of pancreatic juice (3·2 c.c. in 30 min.) but did not contract or relax the bladder wall.

The question therefore arises whether secretin influences intestinal movements. Probably it produces increased tone locally when it is absorbed into the blood in the normal manner, that is, when bile salts are absorbed from the intestine and carry secretin from the intestinal mucosa into the blood. In this way secretin may be absorbed in a sufficiently concentrated form to act on the smooth muscle in its immediate vicinity and so produce increased intestinal movement.

No definite evidence has been brought forward to show that the secretin isolated by this method and used in these experiments is chemically pure. These two physiological actions therefore may indicate that the product contains two principles, the first (secretin) which excites the pancreas to activity, and the second which stimulates plain muscle. In the previous pages it has been shown that proteolytic enzymes rapidly destroy the pancreatic activity of secretin solution. This property was therefore used to investigate the above hypothesis. An equal volume of cat's activated pancreatic juice was added to a strong solution of secretin and the mixture incubated at 38° C. for ten minutes. After this time the solution had no action on the pancreas or the intestinal muscle. Sometimes a secretin solution loses its pancreatic activity after 24 hours owing to the growth of bacteria in it. This change is accompanied by a corresponding loss of activity on intestinal muscle. In fact, changes in pancreatic activity always proceed *pari passu* with changes in smooth muscle activity, indicating that the two properties depend on one principle only. Incidentally it may be observed that the action of secretin on the intestinal muscle of the rabbit in dilutions of 1 : 250,000 is much more intense than that of a corresponding quantity of pure histamine, the substance most likely to be associated with secretin.

Vascular system.

An intravenous injection of secretin contained in an alcoholic extract of the duodenal mucous membrane causes a profound fall of arterial blood-pressure. There is no relation between the fall of blood-pressure and the pancreatic activity of the solution; and it has been established by the work of numerous observers that the depressor principle has no relation to secretin.

In view of the action of purified secretin on plain muscle (intestine and uterus) it was of interest to determine whether a similar action could be observed on the plain muscle of the vascular system. For this purpose a Symes cannula was placed in the aorta of a frog and the vascular system perfused with Ringer solution from a constant pressure bottle. The drops of perfused fluid were registered on a slowly revolving drum. After the flow was established 0·1 mg. of secretin dissolved in Ringer was added to the perfusing fluid in the open limb of the cannula. For a brief period, about one minute, the rate of the flow was increased. The number of drops then progressively decreased until only one drop per minute of fluid passed through the perfused vascular system. The following is an abstract of such an experiment:

Normal rate of flow: 30 drops per min.
Immediately after adding secretin (0·1 mg.) to the perfusing fluid: 45 drops per min.
Five min. later: 15 drops per min.
Fifteen min. later: 5 drops per min.

It is evident that secretin, in relatively large doses, produces a marked constriction of the perfused vascular system of a frog. Small quantities (0·01 mg.) of secretin added to the perfusing fluid produce no change in the rate of flow. The intense vaso-constriction produced by large doses of secretin is annulled by adding amyl nitrite to the perfusing fluid. Probably therefore secretin acts directly on the plain muscle of the arteries. The result of these experiments indicated that the intravenous injection into a cat of a large dose of secretin would produce a rise of arterial blood-pressure. To test this hypothesis the carotid blood-pressure of a cat anæsthetised with urethane was recorded in the usual way, cannulæ having been previously inserted into the pancreatic duct and the gall bladder. The intravenous injection of 1 mg. of secretin (approximately ten times the quantity of secretin required to produce a maximal secretion of pancreatic juice) produced only a small transient fall and rise of blood-pressure. When the flow of pancreatic juice started—about one minute after the injection—the arterial blood-pressure returned to its original value and remained at that level for the following 50 minutes, during which time pancreatic juice was secreted. The result indicated that secretin injected into the blood is quickly removed from the circulation by the pancreas and so reduced to a concentration below that at which it acts on the plain muscle of the arteries. Purified secretin therefore has no effect on the general arterial blood-pressure. This fact is in agreement with the observation that the secretion of pancreatic juice which follows the injection of dilute bile into the duodenum (previously shown to be due to

the absorption of secretin into the portal blood) is not accompanied by any change in the arterial blood-pressure. Direct inspection shows however that the secretion of pancreatic juice caused by the action of secretin is accompanied by vaso-dilatation of the pancreas, an effect which is probably secondary to the action of secretin on the cells of the pancreas.

Respiratory system.

The intravenous injection of a crude extract of secretin produces a more or less prolonged period of apnœa followed by compensatory dyspnoea. Large doses of crude alcoholic secretin, or repeated small doses, may kill an animal by paralysis of the respiratory centre. These toxic effects are due to the impurities contained in alcoholic extracts. Purified secretin has no action on the respiratory centre as evidenced by changes in the depth or frequency of the respiratory rhythm. The result is interesting, because the secretion of pancreatic juice involves the withdrawal of a corresponding volume of approximately 0·15 p.c. $NaHCO_3$ from the blood.

Kidney.

In view of the action of the kidney in maintaining the neutrality of the blood, experiments were made to determine whether secretin influenced the volume or reaction of the urine produced during the active secretion of pancreatic juice. The details of an experiment are given:

Pancreatic juice and bile were collected from the pancreatic duct and gall bladder, the common bile duct being ligatured. Urine was collected from a cannula with a bulbous end tied directly into the urinary bladder. The bulbous end of the cannula almost filled the bladder. In this way variations due to differences in the volume of the bladder were excluded. Successive quantities of secretin (0·05 mg.) were injected intravenously every hour for four hours. During the first 30 minutes after each injection of secretin pancreatic juice and bile were freely secreted; in the second half hour only small quantities of pancreatic juice and bile were obtained, the stimulating action of the secretin being exhausted in the first 30 minutes. During the whole time urine was secreted at varying rates.

	Time (min.)	Urine	P.J.	Bile
0·05 mg. secretin	30	2·4	4·0	0·8
	30	1·6	0·1	0·0
0·05 mg. secretin	30	1·1	4·2	0·9
	30	0·6 *A*	0·0	0·0
0·05 mg. secretin	30	1·1	4·1	1·1
	30	1·0	0·0	0·2
0·05 mg. secretin	30	1·1	4·5	1·1
	30	0·7 *B*	0·1	0·1
Total quantities in four hours		9·6	17·0	4·2

An analysis of these results shows that secretin does not stimulate the kidney to secrete urine. In three out of the four hourly periods there was less urine secreted in the second half hour. This effect was probably caused by the diminution in the body fluid owing to the excretion of approximately 5 c.c. of pancreatic juice and bile during the first 30 minutes. During four hours 17 c.c. of pancreatic juice and 4·2 c.c. of bile were excreted. These secretions contained about 0·15 p.c. $NaHCO_3$ and represented a bicarbonate content of a corresponding volume of blood (21 c.c.). It was observed that all the specimens of urine had approximately the same reaction except (*A*) and (*B*). In these two periods smaller quantities of urine were secreted of a slightly greater degree of acidity than those produced during the other six half hour periods. Therefore the acidity of the urine appeared to be determined by its rate of secretion and not by the quantity of pancreatic juice secreted. There was certainly no evidence that, in the anæsthetised experimental animal, the kidney attempts immediately to preserve the neutrality of the blood by secreting urine of a compensatory reaction to that of the alimentary secretion.

Salivary, gastric and intestinal secretions.

Secretin has no action on the salivary glands, the gastric mucosa, or the glands of the small intestine. Crude alcoholic extracts of secretin may produce a secretion of gastric juice, but this effect was never observed with purified preparations of secretin. Probably some of the crude alcoholic extracts contained histamine which possesses a definite stimulating action on gastric secretion.

Voluntary muscle.

In view of the results of Downs and Eddy(7) the action of secretin on voluntary muscle was determined. There was no evidence that purified secretin exerts any action on the contractile properties of frog's voluntary muscle either by altering the form of the contraction curve or by delaying the onset of fatigue.

Summary.

(1) The method described for the isolation of secretin is based on, (*a*) the extraction of secretin from the duodenal membrane by absolute alcohol, (*b*) the adsorption of secretin by precipitated bile acids, and (*c*) the precipitation of secretin from aqueous solution by dilute acid.

(2) Secretin is a polypeptide containing phosphorus. It is slightly

soluble in water, freely soluble in dilute alkali, but insoluble in alcohol, acetone and ether.

(3) Secretin, in aqueous solution, is rapidly destroyed by (*a*) dilute acid and alkali at 100° C.; (*b*) pepsin, trypsin and autolytic enzymes, at 38° C.

(4) Secretin is adsorbed from aqueous solution by bile acids, charcoal, alumina, and hardened filter paper.

(5) Secretin acts most effectually when intravenously injected; it produces a slow secretion of pancreatic juice when administered subcutaneously to the unanæsthetised animal; it is not absorbed from the alimentary canal even in the presence of bile.

(6) Secretin acts on the external secretions of the pancreas and the liver. It has no action on the glucose content of the blood.

(7) Secretin increases the tone of smooth muscle (intestine, uterus, blood vessels) in a concentration of 1 : 500,000 approximately; but secretin in concentrations in the blood which produce a maximal secretion of pancreatic juice (1 : 5,000,000 approx.) does not influence the general plain muscle of the body.

(8) Secretin has no action in the arterial blood-pressure, although it produces local vaso-dilatation in the pancreas.

(9) Secretin has no action on the respiratory system or the voluntary muscle of the body.

(10) Secretin has no action on the secretion of urine by the kidney, except that the flow of urine decreases slightly immediately after the secretion of a large quantity of pancreatic juice and bile produced by the intravenous injection of secretin.

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