THE MECHANISM OF PANCREATIC SECRETION.

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(From the Physiological Laboratory of University College, London.)

CONTENTS.

I. HISTORICAL.

IT has long been known that the activity of the pancreas is normally called into play by events occurring in the alimentary canal. Bernard[1] found that the pancreatic secretion could be evoked by the introduction of ether into the stomach or duodenum, and Heidenhain[2] studied the relation of the time-course of the secretion to the processes of digestion going on in the stomach and intestines.

Our exact knowledge of many of the factors determining pancreatic secretion we owe to the work of Pawlow and his pupils[3], who have shown that the flow of pancreatic juice begins with the entry of the chyme into the duodenum and is not excited directly by the presence of

[1] *Physiologie expérimentale*, II. p. 226. Paris, 1856.

[2] Hermann's *Handbuch d. Physiologie*, V. p. 183. 1883.

[3] *Die Arbeit der Verdauungsdrüsen.* Trans. from Russian, Wiesbaden. 1898. Also *Le travail des glandes digestives.* Paris, 1901.

food in the stomach itself. The exciting influence of the chyme is due chiefly to its acidity, and a large secretion can be brought about by the introduction of 0·4% hydrochloric acid into the stomach, whence it is rapidly transferred to the duodenum. Pawlow found, however, that other substances, *e.g.* water, oil, introduced into the stomach had a similar, though less pronounced, effect. In each case the effect was produced only when the substances had passed into the duodenum. Pawlow has, moreover, drawn attention to a remarkable power of adaptation presented by the pancreas, the juice which is secreted varying in composition according to the nature of the food which has passed into the duodenum. Thus, with a diet of meat the tryptic ferment is present in relatively largest amount, while a diet of bread causes the preponderance of the amylolytic ferment, and a diet of milk or fat that of the fat-splitting ferment.

Pawlow regards the secretion evoked by the presence of acid in the duodenum as reflex in origin, and ascribes the varying composition of the juice in different diets to a marvellous sensibility of the duodenal mucous membrane, so that different constituents of the chyme excite different nerve-endings, or produce correspondingly different kinds of nerve-impulses, which travel to the gland, or its nerve-centres, and determine the varying activity of the gland-cells.

In searching for the channels of this reflex, Pawlow has shown that, if proper precautions be taken, it is possible to excite a secretion of pancreatic juice by excitation of the divided vagus or splanchnic nerves. The vagus nerves, also, according to him, contain inhibitory fibres.

The question as to the mechanism by which a pancreatic secretion is evoked by the introduction of acid into the duodenum has been narrowed still further by the independent researches of Popielski[1] and of Wertheimer and Lepage[2]. These observers have shown that the introduction of acid into the duodenum still excites pancreatic secretion after section of both vagi and splanchnic nerves, or destruction of the spinal cord, or even after complete extirpation of the solar plexus. Popielski concludes, therefore, that the secretion is due to a peripheral reflex action, the centres of which are situated in the scattered ganglia found throughout the pancreas, and ascribes special importance to a large collection of ganglion cells in the head of the pancreas close to the pylorus. Wertheimer and Lepage, while accepting Popielski's

[1] *Gazette clinique de Botkin* (Russ.) 1900.

[2] *Journal de Physiologie*, III. p. 335. 1901.

explanation of the secretion excited from the duodenum, found that secretion could also be induced by injection of acid into the lower portion of the small intestine, the effect, however, gradually diminishing as the injection was made nearer the lower end of the small intestine, so that no effect at all was produced from the lower two feet or so of the ileum. Secretion could be excited from a loop of jejunum entirely isolated from the duodenum. They conclude that, in this latter case, the reflex centres are situated in the ganglia of the solar plexus, but they did not perform the obvious control experiment of injecting acid into an isolated loop of jejunum after extirpation of these ganglia. They showed that the effect was not abolished by injection of large doses of atropin, but compared with this the well-known insusceptibility to this drug of the sympathetic fibres of the salivary glands.

The apparent local character of this reaction interested us to make further experiments on the subject, in the idea that we might have here to do with an extension of the local reflexes whose action on the movements of the intestines we have already investigated[1]. We soon found, however, that we were dealing with an entirely different order of phenomena, and that the secretion of the pancreas is normally called into play not by nervous channels at all, but by a chemical substance which is formed in the mucous membrane of the upper parts of the small intestine under the influence of acid, and is carried thence by the blood-stream to the gland-cells of the pancreas[2].

II. Experimental methods.

All our experiments were made on dogs which had received a previous injection of morphia, and were anæsthetized with A.C.E. mixture during the course of the experiment. In order to keep the animals' condition constant, artificial respiration was usually employed, a procedure which is especially necessary when both vagi are divided, the anæsthetic bottle being introduced in the course of the blast of air from the pump. The animals had received no food for a period of 18 to 24 hours previously. In the earlier experiments, where a considerable degree of preliminary operative manipulation was re-

[1] This *Journal*, XXIV. p. 99. 1899.

[2] A preliminary abstract of the main results of this work was published in the *Proc. Roy. Soc.* LXIX. p. 352. 1902. The experiments, of which an account is given in the present paper, were completed in March, 1902, their publication being delayed by extraneous circumstances.

quired in the abdominal cavity, the animals were placed during the remainder of the experiment in a bath of warm physiological saline, the level of the fluid being above that of the abdominal wound. This method was found to keep them in such good condition throughout a long experiment that it was adopted as a routine practice in all cases. The arterial pressure was always recorded by means of a mercurial manometer connected with the carotid artery in the usual way. The pancreatic juice was obtained by placing a cannula in the larger duct which enters the duodenum on a level with the lower border of the pancreas. To the cannula was connected a long glass tube filled at first with physiological saline; the end of this tube projected over the edge of the bath so that the drops of the fluid as they were secreted fell upon a mica disc cemented to the lever of a Marey's tambour, which was in connection, by means of rubber tubing, with another tambour which marked each drop upon the smoked paper of the kymograph.

III. The effect of the injection of acid into the duodenum and jejunum.

It is unnecessary to describe at length the results obtained under this heading. We are able to confirm the statements made by our predecessors. The result of injecting from 30 to 50 c.c. of 0·4 % hydrochloric acid into the lumen of the duodenum or jejunum is to produce,

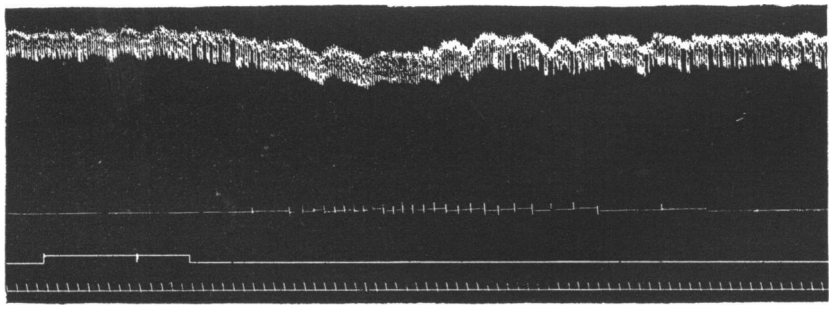

Fig. 1. *Effect of injection of acid into duodenum after destruction of cord.* Upper curve—blood-pressure. Uppermost of three lines—drops of pancreatic secretion. Middle line—signal marking injection of 50 c.c., 0·4 % HCl. Bottom line—time in 10″. Blood-pressure zero = level of time marker.

after a latent period of about two minutes, a marked flow of pancreatic juice. Further, this effect is still produced after section of both vagi,

section of the spinal cord at the level of the foramen magnum, destruction of the spinal cord, section of the splanchnic nerves, or extirpation of the solar plexus, or any combination of these operations.

Fig. 1 will serve as an illustration of the fact. In this case the spinal cord was destroyed from the 6th thoracic vertebra downwards, and both vagi and splanchnic nerves were cut. At the period of time marked by the signal, 50 c.c. of acid were injected into the duodenum, about 2 minutes from the beginning of the injection the first drop of secretion is recorded, and a rapid series of drops commences at 4 minutes, to last for some 3 or 4 minutes and gradually cease after 11 or 12 minutes.

In two or three of these experiments we noted an effect which is perhaps worth recording. During the injection of repeated doses of acid, the effect of each dose was less than that of the preceding one, and sooner or later a point was reached at which no effect was produced, even by 1·6 % HCl. On now injecting into a vein about 50 c.c. of 3 % sodium carbonate solution a considerable flow of juice was obtained without any further injection of acid into the duodenum.

Intravenous injection of sodium carbonate solution, without previous introduction of acid into the gut, had no effect on the pancreatic secretion.

Our experiments, therefore, confirm those of previous observers in so far as we find that after exclusion of all nerve-centres, except those in the pancreas itself, a secretion of pancreatic juice is obtained by the introduction of acid into the duodenum. But, as pointed out above, the *experimentum crucis* of taking an isolated loop of intestine, dividing the mesenteric nerves supplying it, and then injecting acid into it, had not been performed.

It is plain that this experiment cannot be performed on the duodenum for anatomical reasons. Fortunately, however, as Wertheimer and Lepage have shown, the jejunum, separated by section from the duodenum, is also capable of exciting the pancreas to activity, when acid is introduced, and in this case the centre for the "reflex" must be in the cœliac or mesenteric ganglia. The possibility of our crucial experiment is given here, and the results are contained in the next section.

IV. The crucial experiment.

On January 16th, 1902, a bitch of about 6 kilos weight, which had been fed about 18 hours previously, was given a hypodermic injection of morphia some 3 hours before the experiment, and during the experiment itself received A.C.E. in addition. The nervous masses around the superior mesenteric artery and cœliac axis were completely removed and both vagi cut. A loop of jejunum was tied at both ends and the mesenteric nerves supplying it were carefully dissected out and divided, so that the piece of intestine was connected to the body of the animal merely by its arteries and veins. A cannula was inserted in the large pancreatic duct and the drops of secretion recorded. The blood-pressure in the carotid was also recorded in the usual way. The animal was in the warm saline bath and under artificial respiration.

The introduction of 20 c.c. of 0·4 % HCl into the duodenum produced a well-marked secretion of 1 drop every 20 secs. lasting for some 6 minutes; this result merely confirms previous work.

But, and this is the important point of the experiment, and the turning-point of the whole research, the introduction of 10 c.c. of the same acid into the enervated loop of jejunum produced a similar and equally well-marked effect.

Now, since this part of the intestine was completely cut off from nervous connection with the pancreas, the conclusion was inevitable that the effect was produced by some chemical substance finding its way into the veins of the loop of jejunum in question and being carried in the blood-stream to the pancreatic cells. Wertheimer and Lepage have shown[1], however, that acid introduced into the circulation has no effect on the pancreatic secretion, so that the body of which we were in search could not be the acid itself. But there is, between the lumen of the gut and the absorbent vessels, a layer of epithelium, whose cells are as we know endowed with numerous important functions. It seemed therefore possible that the action of acid on these cells would produce a body capable of exciting the pancreas to activity. The next step in our experiment was plain, viz. to cut out the loop of jejunum, scrape off the mucous membrane, rub it up with sand and 0·4 % HCl in a mortar, filter through cotton-wool to get rid of lumps and sand, and inject the extract into a vein. The result is shown in Fig. 2. The first effect is a considerable fall of blood-pressure, due, as we shall show later, to a

[1] *Journal de Physiologie*, III. p. 695. 1901.

body distinct from that acting on the pancreas, and, after a latent period of about 70 secs. a flow of pancreatic juice at more than twice

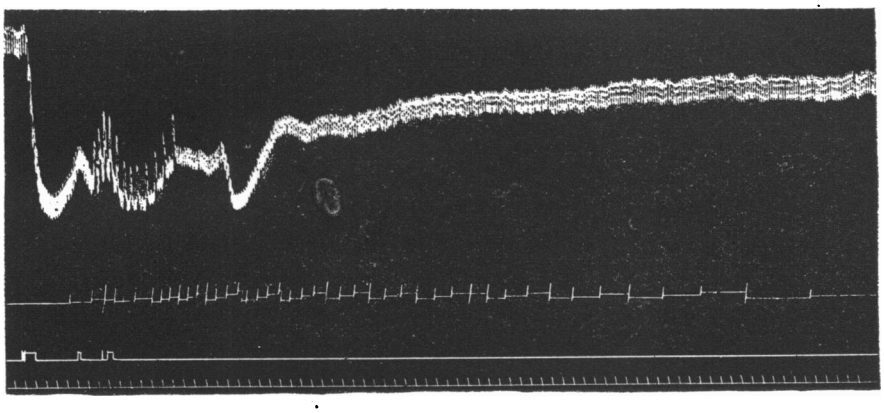

Fig. 2. *Effect of injecting acid extract of jejunal mucous membrane into vein.* Explanation as Fig. 1. The steps on the drop-tracing are due to a gradual accumulation of secretion on the lever of the drop-recorder, which fluid falls off at intervals. Blood-pressure zero = level of drop recorder.

the rate produced at the beginning of the experiment by introduction of acid into the duodenum. We have already suggested the name "secretin" for this body, and as it has been accepted and made use of by subsequent workers it is as well to adhere to it.

In the same experiment we were able to make two further steps in the elucidation of the subject. In the first place the acid extract was boiled and found undiminished in activity, secretin is therefore not of the nature of an enzyme. In the second place, since Wertheimer and Lepage have shown that the effect of acid in the small intestine diminishes in proportion as the place where it is introduced approaches the lower end, so that from the last 6 inches or so of the ileum no secretion of the pancreas is excited, it was of interest to see whether the distribution of the substance from which secretin is split by acids is similar in extent. Fig. 3 shows the result of injecting an extract from the lower 6 inches of the ileum made in the same way as the jejunum extract. The fall of blood-pressure is present, but there is no effect on the pancreas. Another preparation from the ileum just above this one also had no effect on the pancreas. A preparation from the jejunum below the previous one had a marked effect, but less than that of the loop above. The distribution of "prosecretin," as we have proposed to

call the mother-substance, corresponds therefore precisely with the region from which acid introduced into the lumen excites secretion from the pancreas.

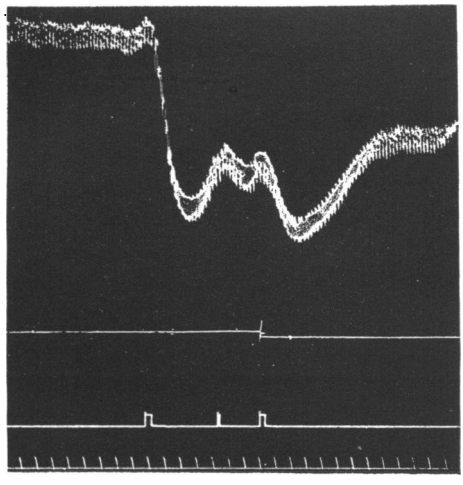

Fig. 3. *Effect of acid extract of lower end of ileum.* Explanation as before.

In reply to the objection of Pflüger to this experiment[1], we admit that it is difficult to be certain that all nerve-channels were absolutely excluded, since the walls of the blood vessels were intact, but we submit that since the result of the experiment was such as has been described it does not in the least matter whether the nerves were all cut or not; the only fact of importance is that it was the belief that all the nerves were cut that caused us to try the experiment of making an acid extract of the mucous membrane and that led to the discovery of secretin.

As to the further objection of Pflüger that it is in no way extraordinary that a body should be extracted from intestinal mucous membrane capable of acting as a stimulant to gland activity since there are many such bodies known, our reply is that secretin, as will be shown more fully later on, is of an entirely specific nature; the experiment described above shows that even from the ileum no such substance can be obtained, and subsequent experiments showed that from no other part of the body could any body be extracted which caused secretion in the pancreas. And further no other substance known to us, even pilocarpin, which acts so powerfully on most glands, has any effect on

[1] *Pfluger's Archiv*, xc. p. 32. 1902.

the pancreas at all comparable with that of secretin; nor has secretin any action on glands other than the pancreas, except perhaps to a small degree on the secretion of bile.

V. Properties and action of secretin.

Some physical and chemical characters. Having shown that boiling an acid extract of the mucous membrane does not destroy its activity, we proceeded to test whether the reaction had any effect in this way and found that a short boiling in either acid, neutral or alkaline solution was harmless. Since a preparation containing less proteid and more easily filterable can be made by neutralizing while boiling, our final routine method of preparing an active solution for ordinary purposes is the following. The duodenum and jejunum are cut out, slit up with scissors, washed under the tap, the mucous membrane scraped off and well rubbed with sand and a little 0·4% HCl in a mortar, allowed to stand under about 2 or 3 times its volume of 0·4% HCl for some minutes, the whole boiled over free flame in a porcelain dish, and while boiling brought to the alkaline side of neutrality by strong caustic soda, then made slightly acid by acetic acid, strained and pressed through muslin and filtered through paper. The solution thus obtained contains very little proteid, but becomes slightly turbid on cooling, partly owing to a trace of albumoses, but chiefly to gelatin.

In order to obtain a purer preparation we attempted to precipitate the solution by alcohol, and were somewhat surprised to find that the

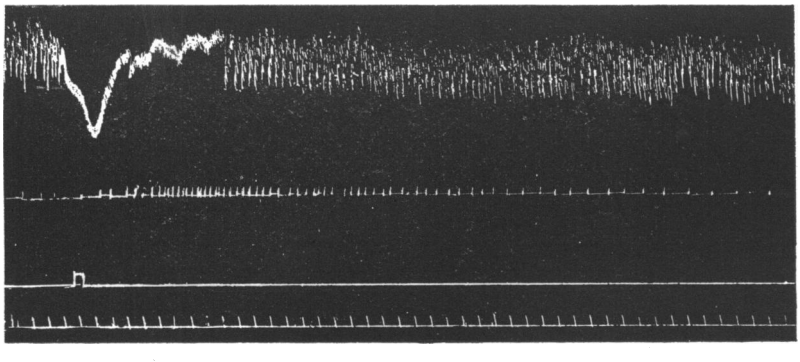

Fig. 4. *Effect of saline extract of dried residue from alcoholic solution of secretin.* Explanation as before. There was a certain amount of secretory activity already present owing to a previous injection the effect of which had not passed off. Blood-pressure zero 20 mm. below time-marker.

active substance is soluble in alcohol, at all events in alcohol of a strength up to 90 %. Dr W. A. Osborne, who is engaged in investigating the chemical nature of secretin, found later that it is insoluble in absolute alcohol, as also in ether. Fig. 4 will serve to show the fact of the solubility in alcohol. A solution of secretin made as above was mixed with 5 times its volume of absolute alcohol, a little ether added to cause agglomeration of the precipitate, filtered, the filtrate evaporated in dryness under diminished pressure and various extracts made of the residue. The effect of the saline extract is shown in the figure. It will be noticed that there is very little fall of blood-pressure. The alcoholic and ethereal extracts of the above residue were evaporated to dryness, taken up again in saline, and the facts as to solubility already given were made out.

Although short boiling does not destroy it, on concentrating a weak solution by prolonged evaporation at atmospheric pressure the activity was found to disappear, this we think was due to slow oxidation, and in fact the activity of a strong solution is very readily abolished by weak potassium permanganate. Dr Osborne finds also that any attempt to precipitate a solution of secretin by the addition of metallic salts such as those of mercury, lead, or iron, or by phosphotungstic acid leads to destruction of the active body, since it has disappeared from both filtrate and precipitate. Tannin may be used, however, and a very

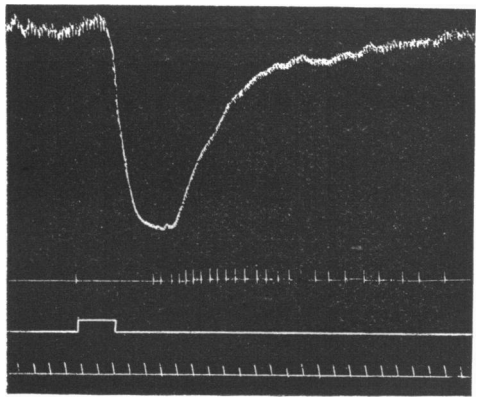

Fig. 5. *Effect of filtrate from precipitating secretin solution by tannin.* Explanation as before. Blood-pressure zero 15 mm. below time-marker.

pure preparation may be obtained by precipitating the excess of tannin in the filtrate by gelatin, and the gelatin afterwards by alcohol. The

effect of such a solution is shown in Fig. 5. It will be seen however that by this treatment alone the body causing fall of blood-pressure is not removed.

Secretin is non-volatile, it does not appear in the distillate obtained by passing steam through its solution.

It dialyses through parchment paper, but not readily.

The action of its solutions does not depend on their inorganic constituents, since the ash, prepared for us by Dr Osborne, has no effect on the pancreas.

It is destroyed by digestion with active tryptic solutions for one hour; the effect of peptic solutions is somewhat uncertain, digestion with gastric juice from the dog for one hour did not destroy the activity of a solution of secretin, but considerably diminished it.

Taking these facts together it will be seen that we cannot as yet give any definite suggestion as to the chemical nature of secretin, its solubility in alcohol and diffusibility point to its being a body of low molecular weight; since it is not destroyed by boiling it is not a ferment; and that it is not of the nature of an alkaloid or diamino-acid is shown by the fact of its not being precipitated by tannin.

The mode of action of secretin. In attempting to form some idea as to the mode of action of this body, the first necessity is to decide whether the substance causing fall of blood-pressure, which we shall in future, for the sake of brevity, call the depressor substance, is the same as that exciting the pancreas to secretion; the importance of this being because the vascular dilatation producing the fall of blood-pressure might be thought to be the cause of the increased activity of the pancreas, which organ no doubt would share in the general splanchnic dilatation. That this is not so, however, can be shown in several ways. Albumoses (Witte's pepton) cause considerable vaso-dilatation in the abdominal viscera, but no secretion of the pancreas, at least not in the doses by which a large fall of blood-pressure is produced (5 c.c. of 5 % solution). But we have been able to obtain secretin solutions acting powerfully on the pancreas, with minimal or zero effects on the blood-pressure. The mucous membrane of duodenum and jejunum is rubbed up with sand in a mortar without the addition of acids, this mass is then folded in filter-paper and extracted with absolute alcohol in a Soxhlet apparatus for 24 hours, the mass removed, and boiled with 0·4 % HCl, neutralized and filtered in the usual way. The effect of injecting this preparation is shown in Fig. 6. There is no fall of blood-pressure, but a powerful effect on the pancreas.

This result is to be explained by the fact that the depressor substance exists preformed in the mucous membrane, and being soluble

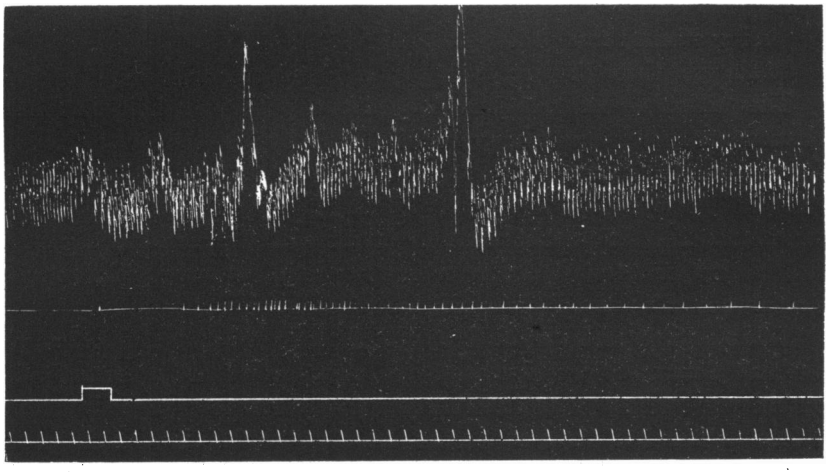

Fig. 6. *Effect of secretin prepared from mucous membrane extracted with absolute alcohol (Soxhlet).* Explanation as before. Blood-pressure zero 21 mm. below time-marker.

in alcohol is extracted by that body. On the other hand prosecretin is insoluble in alcohol and is not converted into the soluble secretin by alcohol even when hot, but is left in the mucous membrane in a state capable of being converted into secretin by the action of acid subsequently.

There is also another way of obtaining a secretin solution free from depressor substance. It was found by one of us (Starling), in the course of a research on lymph formation, that if the aorta is occluded above the cœliac axis for an hour to an hour and a-half, and then the blood allowed free course again and the animal killed after a further half-hour, the epithelial coat of the small intestine is for the most part shed and forms a pasty mass in the lumen of the intestine. This fact gives us an opportunity of testing whether secretin comes from the epithelium or from more deeply-lying substances. The experiment was performed by the introduction of an obturator (consisting of a metal tube with an india-rubber balloon at the end) into the femoral artery. This was pushed up until the balloon was above the cœliac axis, the balloon was then distended by forcing water into the tube until pulsation ceased in the other femoral artery. It was allowed to remain

in this condition for 1½ hrs., the water then let out and the obturator withdrawn sufficiently far to allow the circulation in the intestines to be reestablished. After half-an-hour the animal was killed and a secretin preparation made in the usual way from the desquamated epithelium found in the lumen of the upper part of the small intestine. The result of injecting this is shown in Fig. 7. The fall of blood-pressure is negligible, while the secretory effect is large. The slow rise of blood-pressure is due to a diminution in the amount of A.C.E. given, which had been accidentally given in too great an amount previously.

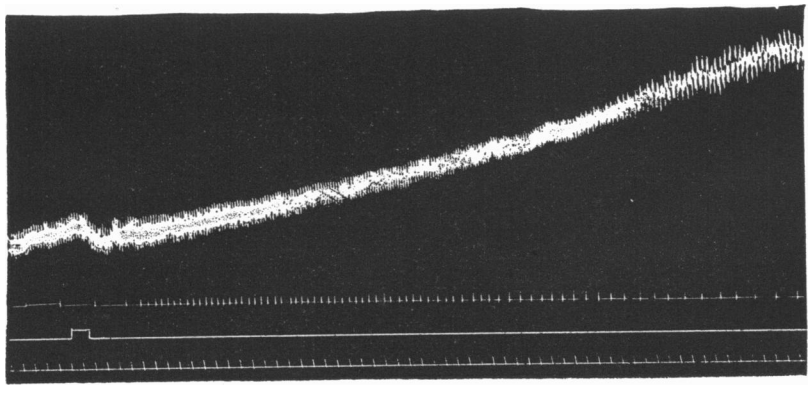

Fig. 7. *Effect of acid extract of desquamated epithelial cells from duodenum.* Explanation as before. Blood-pressure zero = level of time-marker.

A secretin preparation was also made from the mucous membrane of the same animal from which the epithelium had for the most part been shed, the effect being shown in Fig. 8. A very considerable fall of blood-pressure is produced and also a powerful secretory effect, the latter most probably being due to the fact that much of the epithelial layer had been left *in situ*, or, although desquamated, was held between the villi, and so escaped removal by the short washing under the tap to which the intestine was subjected before the preparation was made. We think, however, that the conclusion is justified that the secretin comes from the epithelial cells, and the depressor substance from more deeply lying structures, possibly the muscularis mucosæ.

We had also an opportunity of investigating the effect of the desquamated epithelial cells found in the intestine of a dog which had received repeated doses of leech extract, for the purpose of another research which was being carried on in the laboratory. Fig. 9 shows

the action of this preparation. There is here no fall of blood-pressure if anything a slight rise.

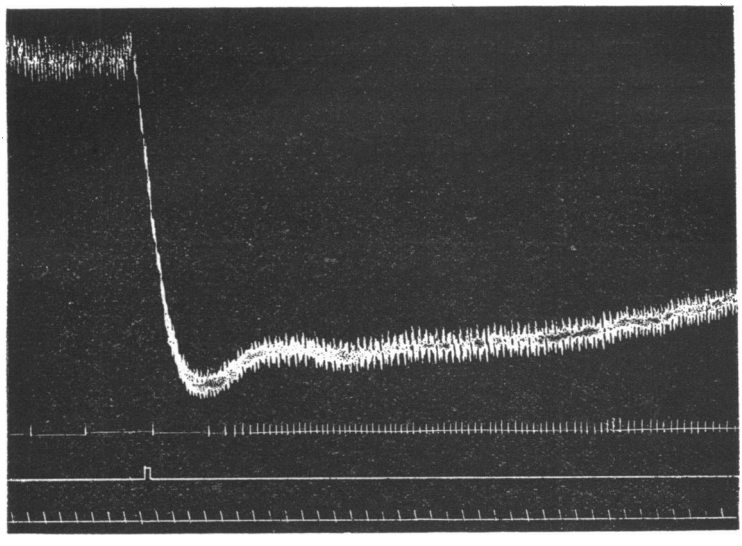

Fig. 8. *Effect of acid extract of mucous membrane from which most of the epithelial cells had been desquamated.* Explanation as before. Blood-pressure zero 6 mm. above time-marker.

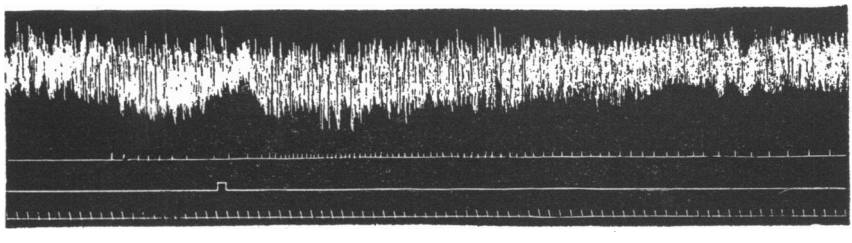

Fig. 9. *Effect of acid extract of epithelium desquamated after injection of leech extract.* At *A* a small dose, at *B* another dose of twice the amount. Explanation as before. Blood-pressure zero 30 mm. below time-marker.

The independence of the two bodies, secretin and depressor substance, is also proved by the fact already referred to, that acid extracts of the ileum have either no secretory effect at all, or a very feeble one, while the fall of blood-pressure produced by them is as great as that of similar preparations from the duodenum, having a powerful action on the pancreas.

It may be well to mention here that we have made special experiments to test whether the action of these extracts on the pancreas is due to *bile salts;* this is not so, bile salts have no effect on the pancreas when injected intravenously, and the very active preparations made from alcohol-extracted mucous membrane contained no bile-salts.

General conditions of action of secretin. So far as we have as yet made out this action is wonderfully independent of the state of the animal. It is shown equally well in a dog in the stage of digestion or 20 hours after a meal. The height of the blood-pressure has very little effect, as shown by Fig. 10. Anæsthetics in all usual doses have no

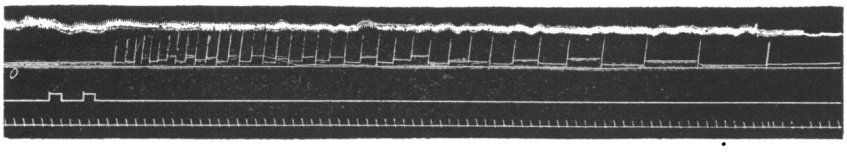

Fig. 10. *Effect of ordinary secretin preparation in case of very low blood-pressure.* Blood-pressure zero is the line immediately below that of the drop-marker. Other explanations as previous figures.

appreciable influence; in this connection we may refer to a statement made by Camus[1], who, having found the effect of secretin diminished by a previous dose of chloroform and abolished by a strong dose, concludes that the nervous system plays a considerable part in the excitation of the pancreas by this substance. So far as the central nervous system is concerned its influence is excluded by the first experiments in which we injected secretin solutions, since in these the pancreas was cut off from the central nervous system, as well as from the peripheral ganglia, except those in its own substance. With regard to these latter it is plain that we cannot absolutely deny the possibility of secretin acting indirectly through nerve-cells, but we submit that the well-known effect of chloroform as a general protoplasmic poison is quite sufficient to explain the results of Camus.

The effect of secretin is unaltered by previous injection of atropin. In fact it is impossible to paralyse the pancreatic secretion by any dose of this drug. The same insusceptibility to atropin was found by Wertheimer to hold good for the secretion evoked by introduction of acid into the duodenum.

The relations in some other animals. All the experiments described

[1] *C. R. Soc. de Biologie*, 25 Avril, 1902, p. 443.

so far were performed on dogs, and it is of obvious importance to discover whether there is a similar mechanism in other animals.

We find that secretin preparations made from the duodenum of the cat, rabbit, ox, monkey, man and frog are all active as regards the pancreatic secretion of the dog. On the pancreas of the rabbit and monkey we also tested secretin of the dog, rabbit, monkey and man and obtained positive results. We conclude, therefore, that the secretin of all these animals is one and the same body; we hope to extend the list of animals tested at a subsequent date.

Fig. 11 is an example of the effect of secretin from the cat on the pancreatic secretion of the dog.

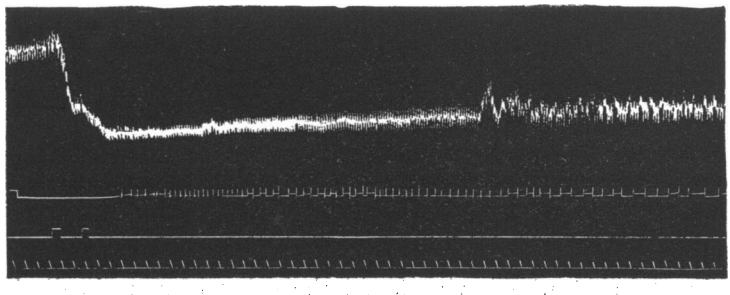

Fig. 11. *Effect of acid extract of cat's duodenum on dog's pancreas.* Explanation as previous figures. Blood-pressure zero 5 mm. below time-marker.

VI. Prosecretin.

Secretin is formed from its mother-substance apparently by a process of hydrolysis, mineral acids being more powerful than organic acids, although even boiling water has some action in this respect. The acids we used were hydrochloric, sulphuric, lactic, oxalic and acetic, and found all effective. Carbonic acid had apparently no effect, and this is perhaps to be expected since it is always present in the mucous membrane and it is undesirable that the pancreas should be constantly excited. The action of stearic acid in solution in bile was doubtful, but its solubility even in bile is very low. Since we made the above experiments Camus has described similar ones[1], in which he finds nitric, phosphoric, and citric acids, in addition to those we tested, to be active, while carbonic and boracic were ineffective.

[1] *C. R. Soc. de Biologie*, 25 Avril, 1902, p. 443.

We have been unable as yet to obtain prosecretin in solution, by which we mean a solution in itself inactive as regards the pancreas but capable of becoming so when treated with acid. Extracts of mucous membrane made with cold water, saline solutions or alkalis of various strength contained no substance of this nature.

The idea might perhaps be entertained that the production of secretin is a "vital" reaction on the part of the cell-protoplasm to the contact with acid. That this is not so and that there is a definite substance, apparently of a complex nature, and insoluble in all ordinary solvents, present in the cell is shown by the facts already mentioned in dealing with the preparation of secretin. It was then shown that boiling alcohol does not cause production of secretin, but that from mucous membrane treated by it, a powerful preparation of secretin can be made by extracting with acid. This is a useful fact, since it enables us to preserve mucous membrane dried after treatment with alcohol, and from it we can at any time prepare an active secretin solution. Fig. 12 is an example of the injection of a solution so prepared.

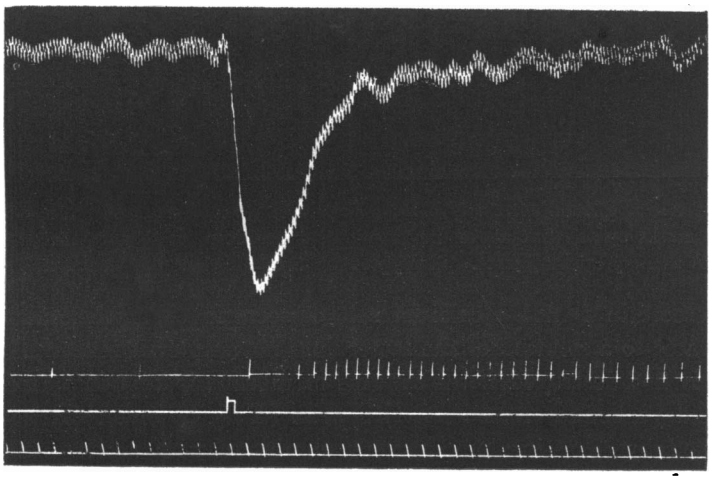

Fig. 12. *Action of acid extract of mucous membrane which had been dried after dehydration by alcohol.* Explanation as previous figures. Blood-pressure zero 4 mm. above time-marker.

The fact of the existence of prosecretin is also shown by boiling the mucous membrane in water, and subsequent washing in cold water, to remove the secretin formed by the boiling water. There is a large amount of prosecretin left unchanged since acid extracts of the coagu-

lated material are very active, while watery or saline extracts are inactive. Fig. 13 shows this fact. The extraction with water has, it will be noticed, removed nearly all the depressor substance.

Some attempts have been made to obtain cell-juice from the mucous membrane by grinding in Dreefs' mill and pressing through Kieselguhr in a hydraulic press in Buchner's method. The prosecretin, however, was destroyed in the process, probably by the iron contained in Kieselguhr. Experiments are now in progress in which the cell-juice is obtained by Rowland's disintegrating method and the juice centrifuged to get rid of cell *débris:* if necessary to use the press with Kieselguhr, this latter will need thorough extraction with acid before use.

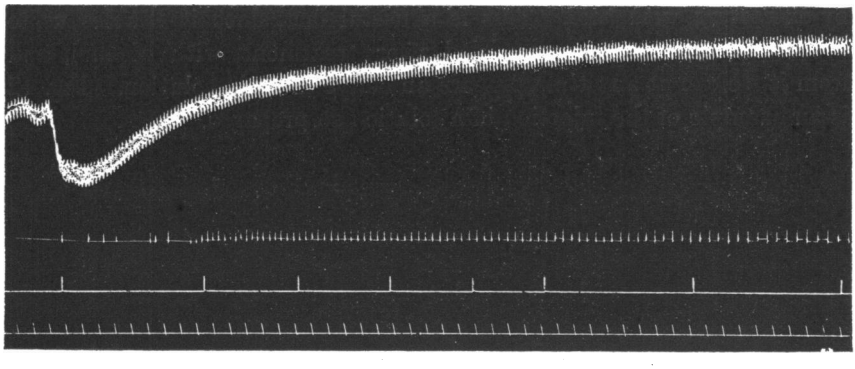

Fig. 13. *Action of acid extract of boiled and washed mucous membrane.* In this figure the marks on the line above the time-marker are drops of bile secreted, the other lines as in previous figures. Blood-pressure zero = level of time-marker.

Prosecretin is not a calcium salt of secretin, since the addition of lime-water to a solution of the latter does not render it inactive.

It is not destroyed if the mucous membrane is kept in an ice-chest for 2½ days before treatment with acid. It is, however, like secretin, destroyed by digesting the mucous membrane with active pancreatic juice for one hour.

VII. The normal mechanism, chemical or nervous?

In our preliminary communication we expressed the opinion that the discovery of "secretin" rendered necessary the repetition of certain experiments of Pawlow in which excitation of the peripheral end of the vagus caused secretion of pancreatic juice, our reason being that passage

of acid from the stomach into the duodenum was not absolutely excluded in these experiments. Popielski[1] in answer to our article, and while admitting the justice of our criticism of the above-mentioned vagus experiments, states that he has been able to separate fibres from the vagus immediately above the diaphragm, excitation of which causes a secretion from the pancreas. This secretion is stated by him to come on as rapidly as is the case with the saliva obtained by stimulating the chorda tympani nerve, and not to be affected by previous ligature of the duodenum below the pylorus. If this fact is confirmed it undoubtedly proves the existence of nerves capable of causing secretion of the pancreas, and we may call to mind that we have in no way stated that our experiments disproved the existence of secretory nerves to the pancreas. So far, however, as our own experiments go we have been unable to obtain secretory effect from the vagus in the neck, either after administration of atropin, or without atropin but after section of the cardiac branches of the vagus. We hope to return to the question of the influence of nerves on the pancreas in a subsequent communication, and will content ourselves for the present with the statement that in our opinion the chemical mode of excitation, viz. by the production of secretin in the mucous membrane by the action of the acid chyme from the stomach upon it, is the normal one. At all events this mode of stimulation must take place, whether there is a concomitant nervous process or not, so that this latter is superfluous and therefore improbable.

It is of interest and importance that Wertheimer has recently[2] found that the blood coming from a loop of intestine into which essence of mustard had been introduced was capable of exciting the pancreas, and this fact seems to show that other substances besides acids, when acting on the mucous membrane *in situ*, are able to cause the production of secretin. It is well known that ether in the duodenum causes a copious secretion of pancreatic juice; oil is also stated by Pawlow to produce this effect. Fig. 14 illustrates the action of ether. Now there are two possible explanations of the action of ether, and the same will apply, *mutatis mutandis*, to the case of other bodies, such as oil or mustard. Secretin solution introduced into the stomach or intestine, if of neutral reaction, has no effect on the pancreas; evidently then the wall of the epithelial cells turned towards the lumen is impermeable to the substance, and it would seem not impossible that the action of

[1] *Centralblatt f. Physiol.* XVI. p. 44. April 26, 1902.

[2] *C. R. Société de Biologie*, 9 Mai, 1902, p. 475.

ether and certain other substances consists in making the cells perme able to secretin and so rendering possible its absorption from the cavity of the intestine. This explanation of course presupposes the

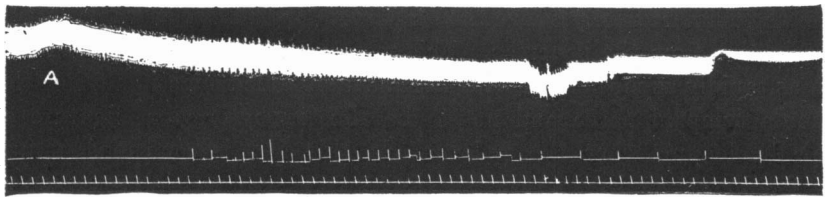

Fig. 14. *Effect of ether in duodenum on pancreas.* At *A* 5 c.c. ether into lumen of duodenum. Other explanation as previous figures. Blood-pressure zero 25 mm. below time-marker.

existence of secretin already in the intestine when the ether, etc. is introduced, and it is supported by the fact that the effect of ether is most marked after a number of previous injections of acid into the intestine. Frequently, indeed, ether has no effect at all, more especially when introduced at the beginning of an experiment. On the other hand, if secretin is introduced into the intestine, immediately after a previous ineffective injection of ether, there is no evidence on the part of the pancreas that it has been absorbed. The other possible explanation is, that all these bodies acting from the intestinal cavity are capable of producing hydrolytic change of prosecretin into secretin in the cells; ether, indeed, made to act on excised mucous membrane does not produce secretin, neither does oil; nor ether intravenously injected in the form of saline saturated with ether. It may, however, be necessary that the cells be intact and living in order that they may react to ether, etc. by the production of secretin.

Naturally, if we invoke the aid of nervous reflexes, there is no difficulty in explaining these effects, and Wertheimer[1] believes that he has shown the existence of a nervous mechanism in the case of mustard by the following experiments: Essential oil of mustard painted on the mucous membrane caused considerable flow of secretion, but the same result followed if the portion of intestine had been previously enervated; this fact comes to the support of the suggestion made above, that secretin can be produced by other means than acids. The complementary experiment was then made, viz. leaving the nerves, but

[1] *C. R. Soc. de Biol.* 9 Mai, 1902, p. 475.

putting a cannula in the vein and preventing the blood from reentering the circulation, the thoracic duct being also tied. In two experiments out of three a marked result was obtained. In 16 further experiments the vagi and splanchnic nerves were cut in order to show the peripheral nature of the reflex, and in 4 of these a positive result obtained, but not very pronounced. With regard to these experiments we think it a matter of considerable difficulty to be certain that there is no possible communication of the veins of the loop with the general circulation, and the flow of secretion was admittedly not great, so that a very small escape of secretin would account for it.

If we assume that the inflow of acid from the stomach into the duodenum is the normal exciting cause of the pancreatic secretion, some difficulty may be felt in endeavouring to account for the apparently normal condition of dogs from which the stomach has been removed. There are however in this case two circumstances to be taken into consideration. In the first place, Pawlow has already suggested that in such cases the lactic acid always produced by fermentation takes the place of the normal hydrochloric acid, and in the second place it has been pointed out by Carvallo and Pachon[1] that the extirpation of the stomach can never be complete in the dog for anatomical reasons, and that cats on which the complete operation can be performed do not remain long in health.

VIII. THE FATE OF SECRETIN IN THE ORGANISM.

The flow of pancreatic juice produced by an injection of secretin does not last more than about 10 minutes, and the rapid flow not more than 5 minutes, so that the secretin introduced must disappear from the blood.

Now if we make repeated injections of secretin it is possible to produce a continuous flow of juice, without apparent fatigue[2], for as long as we have made the attempt, that is for eight hours. We thought it possible, therefore, that secretin might be the source of the ferment or ferments of the juice, and that the pancreatic cell might not have very much work to do to convert it into these ferments. If this were so, however, it ought to be possible to obtain secretin back again, either from the pancreatic tissue or the juice. This we have been

[1] *Arch. de Physiol.* 5^{e} *Série*, VII. p. 351. 1895.

[2] There are histological changes in the cells, and Mr Dale, who is now working at the subject, has been able to detect signs of exhaustion.

unable to do. It does not seem, therefore, that secretin disappears in this way. Nor is it to be found in the lymph, or urine, even after repeated doses.

We think that it disappears by oxidation; it has already been mentioned that it is easily destroyed in this way, and it is an obvious means by which the necessary destruction can be provided for.

IX. The properties of the juice produced by secretin.

The juice as secreted is clear and colourless. On standing it becomes slightly turbid, probably owing to escape of carbon dioxide and precipitation of calcium carbonate. It is alkaline in reaction even to phenolphthalein, and it therefore owes its alkalinity to carbonates and not bicarbonates.

It becomes nearly solid on boiling.

The juice obtained from an animal in the saline bath is more dilute than that from one on the table in the ordinary way, and at the end of a long experiment, in which considerable quantities have been collected, it is more dilute than at the commencement. This fact explains the results of Stassano[1] as regards its diminution of proteolytic activity during the course of an experiment. The total solids contained in a specimen secreted at the commencement of a saline bath experiment amounted to 1·8 %, and after the collection of 70 c.c. of juice to 1·4 %. In a later experiment in which the animal was merely kept warm on the table the total solids amounted to 3·6 %, a number closely agreeing with those found in pancreatic juice collected by the older methods.

The most interesting facts are naturally those relating to its ferment action.

On boiled starch it acts almost instantaneously, converting it to reducing sugar.

On boiled white of egg, and on gelatin it has no effect, at any rate within 48 hours, raw fibrin is digested in about 24 hours. When a few drops of succus entericus, or of a saline extract of duodenal mucous membrane, are added to the juice it is at once converted into a very active tryptic fluid which dissolves fibrin in a quarter-of-an-hour. Now these added substances contain the body investigated in Pawlow's laboratory by Chepowalnikoff[2] and known as *enterokinase*, which

[1] *C. R. Société de Biologie*, 31 Mai, 1902.

[2] Thèse. St Petersburg, 1899. See also Pawlow, *Le travail des glands digestives*. Paris, 1901, p. 257.

converts inactive trypsinogen into active trypsin, as the current theory would express the fact. The reason why raw fibrin is slowly attacked without the addition of enterokinase is that it has attached to it a small quantity of that body derived from leucocytes, as Delezenne has pointed out[1].

The fact that our secretin-juice is inactive, as regards its tryptic power, until acted upon by enterokinase, may raise a doubt as to whether it is to be looked upon as the normal juice. Now the juice used in the original enterokinase experiments was obtained from a permanent fistula by the introduction of acid into the stomach, and regarded by Pawlow as normal, but like the juice obtained by the intravenous injection of secretin was inactive until mixed with enterokinase. We are justified, then, in regarding the secretin juice as natural in this respect. Are we, however, to look upon all naturally secreted juice as being non-tryptic until combined with enterokinase? We think so, but are at present engaged in the investigation of this question, more especially as regards the nature of the influence of enterokinase. The old observations, such as those of Heidenhain, made before the discovery of enterokinase are clearly of no value in deciding this point, since we have no means of knowing how far they were obtained uncontaminated by contact with intestinal mucous membrane. It seems possible that stagnation in the ducts of the pancreas may result in activation of the juice, but since Delezenne[2] has shown that enterokinase is contained in lymphoid tissue generally, it is quite likely that, if such stagnated juice is active, it is on account of its having come into contact with tissue containing enterokinase in small amount.

Camus and Gley state[3] that the juice they obtained by intravenous injection of peptone was active of itself, but it seems to us that the explanation may lie in the circumstance that the amount collected was small, and being so in all probability the secretion appearing first was not rejected, but mixed with the rest. But we will not discuss this part of the subject further since it is now under investigation.

The fact that secretin-juice does not contain active trypsin serves to disprove a hypothesis as to the mode of action of secretin that occurred to us at the beginning of our work. Secretin might be either enterokinase itself, or a body similar to it, which, on contact with zymogen

[1] *C. R. Société de Biologie*, 30 Mai, 1902.
[2] *C. R. Société de Biologie*, 14 Mars, 1902, p. 283.
[3] *C. R. Société de Biologie*, 7 Mars, 1902, p. 241.

in the gland-cells, converts it into trypsin, which might conceivably necessitate its immediate rejection from the cells and a formation of secretion. That secretin is not enterokinase is shown by the fact that it withstands boiling, which enterokinase does not, and the whole hypothesis is overthrown by the fact that the juice secreted contains the zymogen and not active trypsin.

The secretin-juice contains a fat-splitting ferment, but we cannot as yet speak positively as to whether it is active at once, or needs an "enterokinase" to make it so.

X. The action of secretin on some other glands.

Salivary glands. Secretin does not excite secretion in these. In one experiment we noted a slow thick secretion from the cannula in the submaxillary duct, but this was abolished at once on cutting the sympathetic on the same side of the neck. This result shows the caution necessary in these experiments and the absolute necessity of taking a blood-pressure tracing, since the explanation of the phenomena was given at once by the blood-pressure curve. The secretin preparation used contained a considerable admixture of depressor substance, and there can be no doubt that the fall of blood-pressure was sufficient to excite the nerve-centre by anæmia, and so produce the flow of sympathetic saliva.

Stomach. No effect, so far as we were able to make out from the absence of gastric juice after a number of injections of secretin.

Succus entericus. Also no effect.

Bile. Since the secretion of bile is very much influenced by the blood-pressure, the depressor substance in our ordinary secretin preparation would tend to obscure any simultaneous excitatory effect of the secretin itself; and on the other hand the bile-salts contained in it would increase the rate of secretin of bile, so that it was necessary to use a purified preparation. Neglect of this precaution makes the results of Victor Henri and Portier[1] of little value. Fig. 15 shows the effect of a bile-free secretin preparation on both the pancreas and liver.

The preparation used was made from mucous membrane which was ground up with sand, and extracted by several changes of alcohol in the cold. It was then boiled in alcohol, and a secretin preparation made in

[1] *C. R. Société de Biologie*, 6 Juin, 1902, p. 620.

the usual way from the dried membrane by extraction with acid, etc. The alcohol from the last extraction was evaporated to dryness, and

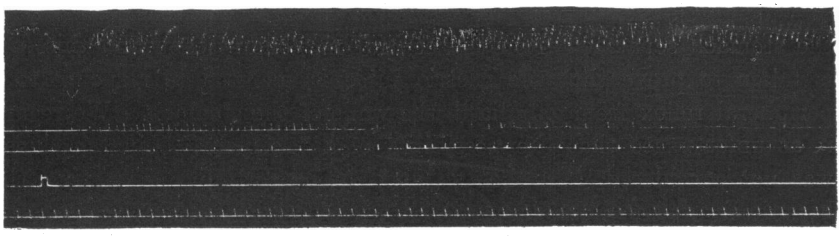

Fig. 15. *Action of bile-free secretin on the secretion of bile.* Upper tracing blood-pressure. Upper of four bottom lines—pancreatin secretion, next one—bile secretion, then signal and time in 10″. During the break in the pancreatic record the drops were not falling on the lever. Blood-pressure zero 27 mm. below time-marker.

the residue tested for bile-salts by Pettenkofer's test with no result. It will be noted that there was still present a small amount of depressor substance, and no doubt it is owing to this that the first effect of the injection is a slowing of the bile-flow. In order to neutralize the effect of this it is advisable to count the drops for a considerable time before the injection, and also afterwards. In this case there were in the 900 secs. preceding the injection 27 drops, and in the 700 secs. afterwards 42 drops, that is, the rate of secretion was almost doubled. We have made several similar experiments and obtained more or less acceleration of the secretion in all.

The question arises whether the substance exciting the liver is the same as that exciting the pancreas. It would be appropriate that the same body should perform both functions, but we must leave the question at present undecided.

Other "secretins." The point raised in the last paragraph leads naturally to the thought that there are similar mechanisms in relation to other secretions. We have tested the question to some extent with respect to the salivary glands, stomach, and succus entericus, by investigating the action upon them of extracts made in various ways from the tongue, salivary glands, pyloric and cardiac ends of stomach, and various parts of the small intestine, but have hitherto obtained no definite results. It is quite possible that in the case of the anterior portions of the alimentary canal the nervous mechanism is the most important one. In the case of the succus entericus, according to Pawlow's investigation, the pancreatic juice acts as an excitant; in our

language we should say then that pancreatic juice is the "secretin" for the succus entericus, but the subject needs further work. As regards the stomach, an article of interest was published recently by Popielski[1], in which it is shown that the digestion of meat proceeds normally in the stomach separated from the central nervous system, and this fact is taken to prove that the gastric juice is secreted in response to a peripheral reflex from ganglia in the walls of the stomach. We regard it as unfortunate that the possibility of a "secretin" being produced was not considered, and that so good an opportunity of testing the hypothesis was neglected.

Other chemical sympathies. It will suffice to call to mind the well-known relation between the uterus and mammary glands, as also the production of a lactase in the pancreatic juice of adult dogs after feeding with milk, and we do this to call attention to the advisability of a renewed investigation of these facts from the point of view of the production of bodies analogous to our pancreatic secretin.

XI. The action of drugs and other extracts on the secretion of the pancreas.

In the course of our experiments we have tested the effect of the intravenous injection of a large number of substances on the pancreas. The only bodies found to be active were pilocarpin and physostigmin, as indeed has been long known. The bodies found to be inactive were pancreatic juice itself, succus entericus, gastric juice, extracts, neutral and acid, of both ends of the stomach, peptone, ether in solution in physiological saline, various extracts of submaxillary glands and tongue, and of the spleen.

As regards *peptone* Gley[2] has obtained secretory effects, but the doses needed were large and the effect small, 1 c.c. of juice in 5 to 10 minutes; the ordinary rate produced by secretin is, on the contrary, 6 c.c. in ten minutes, and this is often exceeded.

Pilocarpin produces a slow secretion of a thick juice, which is stated to be active without enterokinase, a point on which we are engaged at present. The maximum flow obtained by the injection of 7 c.c. of 1 % solution was 10 drops in 160 secs., that is at the rate of 1 c.c. in

[1] *Centralbl. f. Physiologie*, XVI. p. 121. June 7th, 1902.

[2] *Bull. du Muséum d'Hist. nat.* III. p. 244, 29 Juin, 1897; see also Camus and Gley, *C. R. Société de Biologie*, 7 Mars, 1902.

8 minutes, since 30 drops were equal to 1 c.c., but only 10 drops were obtained in all. The secretory effect of pilocarpin, as of physostigmin, is at once abolished by a small dose of atropin, a drug which has no influence on the secretory effects of secretin injections.

On two occasions we noticed the rapid flow of a few drops of secretion on injecting *curare*, but since a second dose had no effect we concluded that this effect was due to some subsidiary cause, perhaps contraction of ducts or squeezing out secretion by vascular dilatation.

Taking all the above facts into consideration we think we are justified in the conclusion that the mode of action of bodies like pilocarpin and peptone is of a totally different nature from that of secretin, which is a specific substance, acting only on the pancreas, and perhaps the liver, whereas all the other active substances produced an effect on all glands indiscriminately.

XII. On specific chemical vaso-dilators.

We took the opportunity afforded us by the preparation of extracts of various tissues to test the truth of a hypothesis which we have for

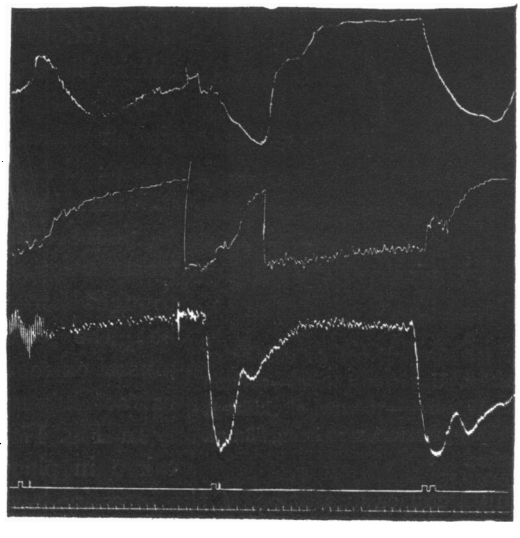

Fig. 16. *Effect of mussel extract and intestine extract on volume of limb and intestine.* Uppermost curve, volume of limb; next curve, volume of ileum; lowest curve, blood-pressure, of which the zero = level of time-marker. *A*, mussel extract. *B*, extract of ileum. *C*, extract of jejunum.

some time thought probable, namely, that the products of metabolism of certain tissues would be found to act as vaso-dilators only for certain tissues in functional relation to those in which they arise, or at all events would act to a greater degree on these tissues than on the rest of the body in general.

Results were obtained which tended to confirm our view, and since we are unable to continue these experiments it is worth while to record here what we found in the hope that the attention of other physiologists may be attracted to this question.

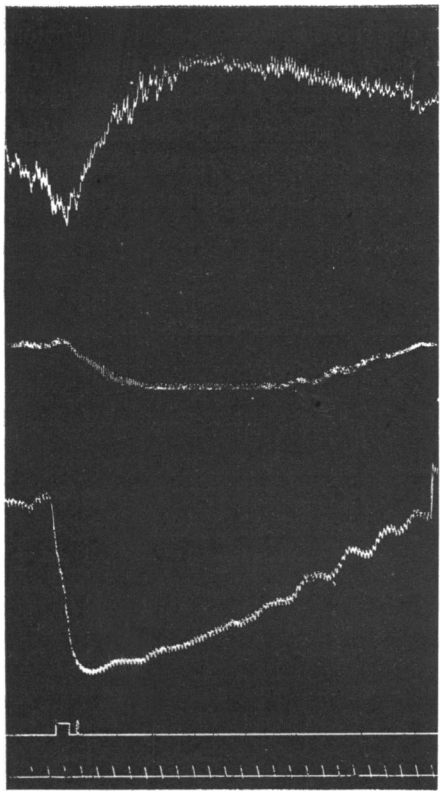

Fig. 17. *Effect of intestine extract on volume of kidney and intestine.* Uppermost curve—volume of intestine, next curve—volume of kidney, lowest curve—blood-pressure, zero being 23mm. below time-marker.

In Fig. 16 is shown at *A* the effect of mussel extract, which is known to be a general vaso-dilator, dilatation is seen in both limb and intestine; at *B*, extract of ileum produces dilatation in the intestine (ileum), the limb follows passively the fall in blood-pressure; at *C*, extract of jejunum has a similar effect to extract of ileum; there is no obvious difference between the effects of ileum and jejunum extracts on the ileum as we thought there might possibly be; the contrast between mussel extract and intestine extract, however, is obvious.

In Fig. 17 the kidney is taken in place of the limb. Extract of jejunum causes dilatation of the intestine, and has no effect on the kidney.

In both of the above experiments, which were made on Feb. 11th and 12th, 1902, the splanchnic nerves were cut to obviate nervous interference.

XIII. Summary of conclusions.

1. The secretion of the pancreatic juice is normally evoked by the entrance of acid chyme into the duodenum, and is proportional to the amount of acid entering (Pawlow). This secretion does not depend on a nervous reflex, and occurs when all the nervous connections of the intestine are destroyed.

2. The contact of the acid with the epithelial cells of the duodenum causes in them the production of a body (secretin), which is absorbed from the cells by the blood-current, and is carried to the pancreas, where it acts as a specific stimulus to the pancreatic cells, exciting a secretion of pancreatic juice proportional to the amount of secretin present.

3. This substance, secretin, is produced probably by a process of hydrolysis from a precursor present in the cells, which is insoluble in water and alkalis and is not destroyed by boiling alcohol.

4. Secretin is not a ferment. It withstands boiling in acid, neutral or alkaline solutions, but is easily destroyed by active pancreatic juice or by oxidising agents. It is not precipitated from its watery solution by tannic acid, or alcohol and ether. It is destroyed by most metallic salts. It is slightly diffusible through parchment paper.

5. The pancreatic juice obtained by secretin injection has no action on proteids until "enterokinase" is added. It acts on starch and to some extent on fats, the action on fats being increased by the addition of succus entericus. It is, in fact, normal pancreatic juice.

6. Secretin rapidly disappears from the tissues, but cannot be detected in any of the secretions. It is apparently not absorbed from the lumen of the intestine.

7. It is not possible to obtain a body resembling secretin from any tissues of the body other than the mucous membrane of the duodenum and jejunum.

8. Secretin solutions, free from bile-salts, cause some increase in the secretion of bile. They have no action on any other glands.

9. Acid extracts of the mucous membrane normally contain a body which causes a fall of blood-pressure. This body is not secretin, and the latter may be prepared free from the depressor substance by acting on desquamated epithelial cells with acid.

10. There is some evidence of a specific localized action of the vaso-dilator substances which may be extracted from various tissues.