CXXVI. STUDIES ON THE CHEMICAL NATURE OF SECRETIN.

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ALTHOUGH it is now thirty years since secretin was first discovered by Bayliss and Starling [1902] very little is known as yet of its chemical nature. This is largely due to the fact that it is only comparatively recently [Weaver, Luckhardt and Koch, 1926; Mellanby, 1928] that the hormone has been separated completely from the depressor substance that is always present in acid extracts of the duodenum, as prepared by the original method of Bayliss and Starling, and obtained in a state approaching purity. The recent products of Mellanby [1928], Still [1930], and Ivy *et al.* [1930] have been shown by animal experiments to be physiologically pure, but chemical purity has not been claimed in any case.

Even before secretin had been separated from the depressor substance—vaso-dilatin—some evidence that it might possess a peptide character was obtained by Bayliss and Starling, who found that it was destroyed by proteolytic enzymes, and by hot acids and alkalis. They also showed that the molecule was dialysable. Lalou [1912] confirmed this observation and also showed that it passed through a thick collodion membrane.

Mellanby and Huggett [1926] suggested that secretin was a secondary proteose, since they found that it was precipitated from solution by complete saturation with ammonium sulphate, but not by half saturation. Later, Mellanby [1928] modified these views, suggesting that it was a polypeptide with a high organic phosphorus content. This was disputed by Takács [1928, 1, 2], Still [1930] and Ivy *et al.* [1930], all of whom claimed to have prepared highly active concentrates that were phosphorus-free. The presence of sulphur was reported by all these workers.

It is generally agreed that secretin is an ampholyte, although there is a divergence of opinion as to the nature of the acidic and basic groups present. Mellanby [1928] reported that his concentrate did not contain any free amino-groups; it was precipitated by acids, which he ascribed to the high content of

¹ The work described in this paper formed part of a thesis approved for the degree of Ph.D. in the University of London.

organic phosphorus. Nevertheless, he produced evidence that indicated that secretin formed salts with both acids and metallic bases.

The experiments described in this paper support the view that secretin is a secondary proteose.

EXPERIMENTAL.

Preparation of an active concentrate.

1. Since Mortimer and Ivy [1929] had reported that the method of Mellanby [1928] had failed in their hands, the "new secretin" of Weaver, Luckhardt and Koch [1926] was used as the starting material for purification.

The fresh intestine of a dog, excised within twenty minutes of death, was washed through with tap-water, filled with about 250 cc. of 0.4 % hydrochloric acid, and the ends were clamped. After half an hour at room temperature, the liquid was run out, strained through muslin, and the filtrate saturated with sodium chloride. The extraction was repeated, the two extracts being combined. The bulky precipitate produced by saturation with sodium chloride had the properties of the "new secretin" described by Weaver, Luckhardt and Koch. It was usually white, but sometimes coloured yellow by a trace of bile. Successive extracts were stored in the refrigerator until a large batch of crude material had been obtained. It was found that the activity did not deteriorate in the cold.

2. *Deproteinisation.* The precipitate was separated from the solution by centrifuging. The supernatant liquid, which contained most of the depressor substance, was poured off and rejected. The precipitate was mixed with enough 0.4 % hydrochloric acid to form a thin cream, and heated to boiling in a large dish. While boiling, the mixture was brought with 2*N* sodium hydroxide solution to approximately p_H 4.0 in accordance with the original method of Bayliss and Starling [1902] for preparing secretin, and then to approximately p_H 6.0 with 5 % sodium acetate solution. The solution was filtered hot through muslin and filter-paper and allowed to cool. The precipitate, which consisted of coagulated protein, was rejected, the activity having passed into the filtrate.

3. *Precipitation with trichloroacetic acid.* Solid trichloroacetic acid was added to the filtrate from step 2 to make a 5 % solution as described by Still [1930]. A white turbidity was produced, and after the mixture had stood for an hour in the refrigerator the precipitate was found to have flocculated. It was centrifuged off, washed with aldehyde-free acetone and ether, and dried. It contained all the secretin, together with some protein, but was free from depressor substance and bile pigments. It was found to retain its activity for periods of three months or more when stored in the refrigerator, and was therefore used as a standard for comparison with new products.

4. *Extraction with alcohol.* The precipitate from step 3 was extracted with alcohol under a variety of conditions in order to find the maximum concentration of the alcohol and the optimum temperature to ensure that the

secretin went into solution. The 90 % acid alcohol solution advocated by Still [1930] failed to dissolve the secretin at room temperature, and was somewhat unreliable at 40°. The solution finally adopted was 85 % neutral alcohol (1 cc. for every 10 mg. of solid product), which dissolved the secretin nearly completely; further dilution resulted in the passing into solution of some of the protein impurities of the trichloroacetic acid precipitate. Extraction was carried out for an hour at 40–50°, which was found to be the optimum temperature; at lower temperatures solution did not take place very readily, while above 60° there was a risk of destruction of secretin by the alcohol. At the conclusion of the extraction, the insoluble residues, consisting chiefly of proteins, were centrifuged off. The removal of impurities with brucine and pyridine as recommended by Still [1930] was tried, but resulted in a serious loss of activity. The solution was therefore treated immediately after centrifuging with one volume of aldehyde-free acetone and five volumes of ether as in Still's procedure, and kept for an hour in the refrigerator. The precipitate, which contained the secretin, was then centrifuged off, washed with acetone and ether, and dried. This product was free from insoluble protein, but contained some sodium chloride.

5. *Precipitation as picrate.* This step was adapted from the method used by Dudley and Starling [1924] for the purification of insulin. The precipitate from step 4 was dissolved in distilled water to make a 1.5 % solution, and a trace of insoluble material centrifuged off. The clear, faintly yellow solution was treated with half its volume of saturated picric acid solution and stirred thoroughly. The preparation was stored in the refrigerator for twenty-four hours, by which time the supernatant liquid had become almost clear. It was then decanted from the precipitate, and the small portion of the precipitate still suspended in it was centrifuged down. The clear solution, on addition of saturated picric acid solution, gave no further precipitate.

The precipitate in the beaker was rubbed up with a quantity of a dilute solution of picric acid—5 cc. of saturated solution in 100 cc.—and centrifuged. The washing of the precipitate with dilute picric acid was repeated twice to free the picrate completely from sodium chloride; the use of the dilute picric acid minimised the risk of secretin passing into solution as was observed by Takács [1928, 1]. The washed picrate was dissolved in a volume of alcoholic HCl solution—1 cc. 3*N* HCl to 3 cc. absolute alcohol—approximately equal to the original volume of saturated picric acid used. The precipitate in contact with the acid formed brown, sticky drops, which were triturated in the solution until they were decolorised. The small quantity of insoluble material was centrifuged off and discarded. The solution was then diluted with ten volumes of aldehyde-free acetone. The white, flocculent precipitate was centrifuged off, washed twice in the centrifuge-cup with acetone and twice with ether, and dried over sulphuric acid *in vacuo*.

The secretin concentrate prepared by this method was free from depressor substance, insoluble proteins, sodium chloride and picric acid.

Physiological assay.

Cats were used for the experiments. The animals were anaesthetised by an intraperitoneal injection of urethane (1.0–1.25 g. per kg. body-weight).

Pancreatic flow was observed by noting the movement of a small bubble in a graduated 1 cc. pipette, clamped horizontally, filled with normal saline, and connected to the pancreatic cannula. For a few of the earlier experiments, an electric drop-recorder was attached to the open end of the pipette, but the use of this was found to be impracticable, since the protein present in the secreted juice was coagulated by the heat generated by the heavy current required to operate the signal, and fouled the platinum contacts. Throughout half of this work, the pipette alone was used for measurement of flow. For the latter half of the work there was added a thermionic drop-recorder devised by Dr D. T. Harris, the use of which was not hampered by the coagulation difficulty. The contacts were included in the grid circuit, where only a few amperes were necessary, while the signal was placed in the plate circuit, where there was ample power to operate it. The mechanism of this recorder has already been described [Harris, D. T., 1931].

While the pipette alone was in use, preparations were tested with a view to determining the minimum quantity of solid material necessary to evoke any secretion, which was defined as the threshold dose. After the introduction of the thermionic drop-recorder, the unit adopted was the "threshold dose" of Ivy *et al.* [1930], who define it as "that amount of dried material in solution which, when injected intravenously, will cause a 10 drop (0.4 cc.) increase in the rate of flow of pancreatic juice within a ten-minute period following the time of injection, as compared with the preceding ten-minute period."

The products from the various stages of purification were each assayed a large number of times. The activity was found to vary with the cat, the same preparation sometimes evoking secretion of twice or three times as much juice with one animal as with another. With the cruder products, the activities of different batches also varied.

The figures below give approximate values of the threshold doses for different stages of purification.

	Threshold dose (after Ivy)
Precipitate from step 3	8 mg. (approx.)
Precipitate from step 4	<u>2 mg. (approx.)</u>
Final product	0.5 mg.

The final product of Still [1930] was active in doses of 0.2–1.0 mg. per kg. in a 10 kg. dog, while Ivy *et al.* stated that the average threshold dose of their concentrate was 0.6–0.7 mg. These workers used methods of extraction closely related to the present method, which yields a product of activity comparable with those of their products.

The acid extraction method, however, was unsatisfactory in many respects compared with Mellanby's method, as was shown by the assay, with the above

technique, of a sample of Mellanby's own preparation, kindly supplied by him. The Ivy threshold dose for this preparation was found to be 0.05–0.1 mg.

Chemical properties of the concentrate.

The final product—"hydrochloride"—was a fine white powder, dissolving slowly in water to form a colourless solution. It was soluble over the whole range of p_H , but normally its aqueous solution had a p_H of 3.0 or less, whereas Mellanby's product in solution had a p_H of 8.0.

The preparation gave positive tests for the biuret (pink colour), xanthoproteic, Hopkins-Cole, Millon, Pauly (histidine colour) and sulphur reactions. The ninhydrin and Molisch reactions were negative.

A trace of precipitate was produced by half saturation of the aqueous solution with ammonium sulphate, while complete saturation produced a large precipitate.

Precipitates were produced by picric, tannic, and phosphotungstic acids, but not by sulphosalicylic or trichloroacetic acid.

The aqueous solution produced no coagulation when heated, and did not reduce Benedict's solution.

The activity was destroyed by boiling with 2*N* acid or alkali, and also by hot methyl or ethyl alcohol.

These results suggest that the secretin molecule is about the size of that of a peptone, and contains no carbohydrate or other reducing group. The sulphur must therefore be present in the disulphide form.

When kept in the refrigerator, the protein-free products generally retained their activity for long periods. There were, however, a few cases where the dried precipitate from step 4 of the purification process deteriorated somewhat rapidly even in cold storage. The cause of this has not been ascertained. In connection with this stability question, it may be mentioned that two active samples of Ivy's product, supplied by him to Dr Brocklehurst, were found to deteriorate slowly when kept at room temperature.

Behaviour of secretin with precipitated proteins.

In the early stages of the working-out of the above method of purification, attempts were made to extract the secretin from the sodium chloride precipitate of step 1, which contained both proteins and the depressor substance, by the method of Weaver [1927], which consisted in redissolving the precipitate as far as possible in distilled water, filtering off the undissolved material and saturating the filtrate again with sodium chloride. For this purpose the precipitate was suspended for an hour in a volume of distilled water about three-quarters that of the original acid solution. At the end of this time the insoluble slimy residues were filtered off, the filtrate was saturated with sodium chloride, and kept overnight in the refrigerator. The flocculent precipitate was centrifuged, washed with acetone and ether, and dried in air. Assay showed that this

product still contained a large quantity of protein, which resulted in a low activity.

The isoelectric precipitation procedure used by Ivy *et al.* [1929] for the purification of cholecystokinin was added to this method in order to remove proteins. The solution in distilled water, after the insoluble proteins had been filtered off, was adjusted to p_H 5.0–6.0 with $N/10$ sodium hydroxide solution. A white precipitate appeared, and was centrifuged off and discarded. Saturation of the solution with sodium chloride, or addition of trichloroacetic acid, then produced a second precipitate, which was centrifuged off, washed and dried as before.

Assay showed that products prepared by this method were either inactive or considerably less active than those prepared by the same method with the isoelectric precipitation step omitted. A considerable quantity, but not all, of the protein present had been removed by the isoelectric precipitation. This result suggested that the precipitated protein had adsorbed all or part of the secretin, and so removed it from solution.

Further evidence in confirmation of this view was obtained from examination of the slimy protein material that had failed to dissolve when the sodium chloride precipitate from the intestinal extract was suspended in distilled water. This material was extracted by the Bayliss-Starling procedure as in step 2 of the final method of preparation, and the extract evaporated down to about 25 cc. in a current of air at 40°. This solution when injected was found to be very highly active, 0.5 cc. evoking a steady flow lasting twenty minutes. This showed that very little of the secretin was dissolved by suspending the crude material in distilled water, even though it contained a large quantity of 0.4 % hydrochloric acid, the greater part of the active material remaining adsorbed on the protein.

In the subsequent steps of the purification process, some further observations were made on the behaviour of secretin with precipitated protein. In step 3, the active material was precipitated from solution with trichloroacetic acid, the resultant precipitate still containing a considerable quantity of protein. The aqueous solutions of the precipitate from step 4 and the final product, however, which were protein-free, produced no precipitates with trichloroacetic acid; the success of step 3 therefore was dependent on the presence in the solution of protein, which was precipitated by the trichloroacetic acid, and carried the secretin down with it by adsorption. Step 1 also depended on protein adsorption. In connection with this, it may be mentioned that Necheles and Lim [1927], having recovered a pancreatic secretory excitant from the circulating blood of a dog by *vivi*-dialysis, expressed doubts as to whether this was secretin, since it could not be precipitated from solution by saturation with sodium chloride. Since dialysis was carried out through goldbeater's skin membranes into Locke's solution, their dialysate could not have contained any protein, so that precipitation with sodium chloride was impossible.

Observations were also made on the limitations of the adsorbing power of precipitated proteins. It was evident from step 2 that coagulation of the

proteins by heat also destroyed their adsorbing power to a great extent. (A small quantity of secretin could be recovered by extracting the coagulated protein with alcohol.) The dried precipitate from step 3, when required for assay, was dissolved in distilled water or normal saline, and the solution brought to p_H 6.0–7.0. The protein thus precipitated could be filtered off without loss of activity, the secretin remaining in solution. This suggests that the acetone and ether that were used to wash the precipitate had a denaturing effect on the protein, which destroyed its adsorbing power; it was a more profound change than mere dehydration, since it could not be reversed by water.

Adsorption of secretin on benzoic acid.

Mellanby [1928] attempted to absorb secretin on benzoic acid, but was unsuccessful, since his product was precipitated from solution by acids. Since in the present research there had been no evidence of precipitation by acids, the behaviour of the present product with benzoic acid was investigated with a view to its possible application to the purification of secretin.

The solution from step 2 was used for the first experiments. It was mixed with about a quarter of its volume of a 10 % solution of commercial sodium benzoate, and the mixture acidified by slow addition of 2*N* hydrochloric acid until the benzoic acid was completely precipitated. This was then filtered off on a hardened filter, washed with water, and pressed as dry as possible. It was then spread in thin layers on glass plates, which were stacked in a vacuum desiccator, and dried for two or three days over calcium chloride and phosphorus pentoxide. When dry, the benzoic acid was rubbed off the plates, powdered in a dry mortar, and dropped into a quantity of ether that had been dried over sodium. The benzoic acid dissolved, leaving a few mg. of a semi-transparent solid of a light brown colour, which was filtered off on a hardened filter, washed with the dried ether, collected and assayed.

Results of the physiological assay of the adsorbate and of the filtrate from the benzoic acid precipitation showed that the whole of the activity had been adsorbed from the solution by the benzoic acid. The adsorbates were all free from the depressor substances, which had remained in the filtrates. The adsorbate still contained a small quantity of protein, and was also contaminated with some black specks that were considered at the time to have come from the commercial sodium benzoate. The evidence suggested that the method would give more promise when applied to a protein-free product.

The precipitate from step 4, which was protein-free, was later subjected to similar tests. For these experiments the commercial sodium benzoate was replaced by benzoic acid, which was recrystallised from hot water before use. It was then dissolved in excess of 2*N* sodium hydroxide, and the solution filtered. The secretin preparation, dissolved in distilled water, was added to the benzoate solution, the mixture stirred for a second, and the benzoic acid then precipitated as rapidly as possible by addition of concentrated hydrochloric acid with constant stirring. From this point, the procedure was the same as in

the earlier experiments, except that the adsorbate was centrifuged from the ether solution, since hardened filters were found to adsorb secretin [*cf.* Mellanby, 1928].

Assay of this product showed beyond doubt that secretin was adsorbed by benzoic acid. The adsorbate in general was found to have double the activity of the original precipitate from step 4. It was, however, always contaminated with the insoluble black specks that had been noticed in the earlier experiments, however carefully the benzoic acid had been purified. These specks settled very readily from the aqueous solution and could be centrifuged off.

The great drawback to the application of this process to the purification of secretin was its very small yields. From 50 mg. of the original material only $\frac{1}{8}$ of its weight of adsorbate was obtained, yet the activity was only doubled. This suggested that only a limited quantity of secretin could be adsorbed by a given weight of benzoic acid. An attempt was made to avoid loss due to this cause by using excess of benzoic acid, but the adsorbate was found to be contaminated with a large quantity of sodium chloride, which was probably occluded by the benzoic acid on precipitation.

Ultrafiltration.

Lalou [1912] and Takács [1928, 2] both succeeded in obtaining ultrafiltrates of secretin through thick collodion membranes, but over 50 % of the activity was lost. Their ultrafiltrates, however, still showed a measurable activity, especially that of Takács, which he showed by the sulphosalicylic acid test to be protein-free. This indicated that the secretin molecule was not as large as that of a protein. Further ultrafiltration experiments were carried out in the present research to obtain more definite evidence as to the size of the molecule.

Ultrafiltration through cellophane. The experiments were carried out in a Bechhold ultrafilter. A disc of cellophane, supported by a disc of 60 mesh silver gauze, was placed between two well-greased rubber rings, and introduced, with the cellophane uppermost, into the ultrafiltration chamber. The perforated plate at the bottom of the ultrafiltration chamber, on which the membrane rested, was plated with silver to avoid the possibility of metal being dissolved by hydrochloric acid.

The cellophane was first tested with 1 % solutions of egg-albumin and peptone. In both cases liquid passed through the membranes at the rate of one drop per minute. The ultrafiltrate from the albumin solution contained no protein as proved by the sulphosalicylic acid test, while that from the peptone solution gave precipitates with both tannic and phosphotungstic acids, showing that the cellophane was permeable to peptone but not to albumin.

A strong solution of the precipitate from step 4 (40 mg. in 20 cc. water) was then ultrafiltered through cellophane. On application of a pressure of 3–4 kg. per cm.², the solution passed through the membrane at the rate of one drop per minute, no visible residue being left above the membrane. The ultrafiltrate was assayed, together with the original solution.

In three experiments out of four, the ultrafiltrate was found to be active, the activity having been reduced to about $\frac{1}{8}$ of that of the original solution. It was observed that the fourth ultrafiltrate, which was inactive, was the only one of this series that gave a negative biuret reaction.

It can be concluded from these experiments that secretin has a larger molecule than a peptone, and can only pass through the largest pores of a cellophane membrane.

Collodion membranes. A series of graded collodion membranes were made by the method of Desai [1930].

The stock collodion solution was a 2 % solution in anhydrous alcohol and ether in the proportion of 1 : 3, obtained from British Drug Houses, Ltd. From this stock solutions were prepared containing 1, 2, 4 and 8 % by volume of ethylene glycol as swelling agent. Sets of membranes of four grades of permeability were then made from these four solutions. Desai had found that with the aid of membranes prepared from these solutions, peptones, secondary proteoses, and primary proteoses could be separated from one another by dialysis. Since the precipitation tests had been found to support the assumption of Mellanby and Huggett [1926] and others that secretin was a secondary proteose, it was considered that this range of membranes should serve for the identification of the secretin molecule.

The nitrocellulose/water ratios of the membranes were determined by the method described by Pierce [1927], several determinations being made for each grade of membrane, and the mean value taken as the correct ratio.

Collodion membranes.

Membrane series	% Ethylene glycol in solution	Nitrocellulose/water ratio	Desai's value
I	1	1 : 0.5323	1 : 0.510
II	2	1 : 1.4048	1 : 1.050
III	4	1 : 2.2852	1 : 2.083
IV	8	1 : 2.7640	1 : 4.102

Series IV was not prepared until several months after the other three series, the stock collodion solution meanwhile having been stored in the refrigerator. This may account for the high nitrocellulose/water ratio, compared with that of the corresponding membrane of Desai's series.

The membranes were first tested with albumin and peptone solutions. Both solutions filtered very rapidly through a membrane of series IV under a pressure of 2.25 kg. per cm.² The membrane was found to allow the passage of peptone, but to retain albumin. With membranes of series III, ultrafiltration proceeded at a similar rate, but all but a trace of the peptone was retained. Membranes of series I were found to be impermeable to all aqueous solutions, even under a pressure of 5 kg. per cm.².

The membranes of series III were tested with solutions of the secretin concentrate in normal saline (20 mg. in 10 cc.). In each case the solution

passed through the membrane very rapidly, leaving no visible residue. The ultrafiltrates, however, were all found to be inactive, showing that membranes of series III were impermeable to secretin. Membranes of series IV were then tested with similar solutions, the whole solution passing through the membrane on each occasion, as in the case of series III. These ultrafiltrates were also found to be inactive, showing that the secretin molecule is larger than that of a peptone, a conclusion borne out by the absence of any precipitate when most of the ultrafiltrates were tested with picric acid. On one occasion, however, the ultrafiltrate from one of this series of membranes was found to give a precipitate with picric acid; unfortunately, the assay of this particular ultrafiltrate was prevented by an accident. This result suggests that the permeability of the average membrane of series IV is just below the minimum permeability required to permit the passage of molecules of the size of secretin. A membrane of nitrocellulose/water ratio about 1 : 4 would probably be permeable to such molecules. Desai has shown that a membrane of this ratio allows the passage of all secondary proteoses, but not of primary proteoses; this points to secretin being a large secondary proteose, as first suggested by Mellanby and Huggett [1926].

DISCUSSION.

The adsorption of secretin from acid and neutral solutions by precipitated protein is an important factor to be considered in the purification of the hormone, and in the determination of its chemical nature.

Since the adsorbing power of native proteins is destroyed by their denaturation with organic solvents, loss of secretin by adsorption is reduced to a minimum by the denaturation of the protein as soon as possible after precipitation with sodium chloride. In the present research, the deproteinisation of the precipitate by the Bayliss-Starling method, and the subsequent precipitation with trichloroacetic acid were carried out before denaturation because these two steps resulted in a complete separation of secretin from the basic depressor substance, which remained in the acid solution.

Mellanby extracts the duodenal mucosa directly at room temperature with absolute alcohol, which destroys the adsorbing power of the proteins present. If the alcohol is sufficiently dilute to dissolve some of the protein, however, the adsorbing power of this dissolved protein is not destroyed; this is shown in the method of purification worked out by Ivy *et al.* [1930], in which the secretin was extracted from the crude sodium chloride precipitate of Weaver, Luckhardt and Koch [1926] with 75 % alcohol at room temperature. Since the active material in the resultant alcoholic extract could be precipitated with trichloroacetic acid after the removal of the alcohol, it follows that some of the proteins had passed into solution without undergoing loss of adsorbing power.

Ivy and his co-workers, by isoelectric precipitation at p_H 5.3–5.7, separated their secretin concentrate into two fractions, one of which was soluble in water, and the other only in 0.4 % hydrochloric acid, and which they suggested were

two different secretins. Since both these fractions were precipitated from solution by trichloroacetic acid, it follows that neither was protein-free; they were probably adsorbates of secretin on proteins of different isoelectric points. No other evidence has been obtained suggesting the existence of more than one secretin.

Mellanby's observation that secretin was precipitated from solution by acids was not confirmed, the product of the present research remaining in solution over the whole p_H range on the acid side of neutrality. Since, however, its potency was only about 1/10 of that of Mellanby's product, this may have been due merely to the small quantity present.

The adsorption of secretin by precipitated protein in acid and neutral solutions and by benzoic acid is in close agreement with the other cases of adsorption already stated by Mellanby, who found that in neutral solution adsorption took place on alumina and charcoal, but in alkaline solution the amount adsorbed diminished with rise of p_H . The evidence shows that adsorption takes place in acid or neutral solutions but is altered by addition of alkali.

The results obtained from the ultrafiltration experiments and precipitation tests suggest that secretin is a large secondary proteose rather than a polypeptide as suggested by Mellanby. Further evidence of its peptide structure is afforded by its rapid destruction by proteolytic enzymes, and by indications of salt formation observed by Mellanby [1928]. The active part of the molecule with respect to adsorption should therefore be the positive ion, for in accordance with the zwitterion constitution of amino-acids and proteins [see Harris, L. J., 1930], this ion retains its charge in acid and neutral solutions, but not in alkaline solution. The nature of this positive ion is an open question, for Mellanby has shown that the molecule contains no free amino-groups.

SUMMARY.

1. A method is described for preparing a secretin concentrate, free from depressor substances, insoluble proteins, and inorganic salts.
2. The threshold dose of this concentrate is about 0.5 mg. for a cat of 2.5 kg. body-weight.
3. Chemical and precipitation tests indicate that secretin is a secondary proteose.
4. Secretin is soluble in water over the whole range of p_H .
5. Secretin is adsorbed readily by precipitated proteins from acid and neutral solutions. The adsorbing power of the proteins is destroyed by denaturation with organic solvents.
6. Secretin can be adsorbed from solution on benzoic acid.
7. Secretin can be ultrafiltered through cellophane. It is retained by a collodion membrane permeable to peptones.
8. The above results are discussed fully and compared with those of other workers. It is suggested that the adsorption of secretin is associated with its positive ion.

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