The dry powder weighed about 1·5 g. The substance was readily soluble in distilled water and dilute acids. It was fairly stable in the dry state at room temperature, although some loss in activity did occur when it was stored for some months.

The substance is of protein nature and is free of histamine, as evidenced by blood-pressure experiments. When 1 mg. was injected intravenously into a cat, as described by Jalling & Jorpes (1947), it induced in most animals a copious flow of a highly acid gastric juice. The amount secreted by different animals, however, showed great variations. We found values ranging from scarcely any secretion at all in a few animals to 32 ml. 0·1 N-HCl in a single instance. The mean response was about 10 ml. 0·1 N-HCl/mg. gastrin, corresponding to a potency of 10 units/mg., as defined by Jalling & Jorpes. The most potent preparations previously reported are those of Komarov (1942*b*), having an activity of 0·4 unit/mg., and those of Uvnäs (1942), usually with about 0·5 unit/mg. (Kahlson, 1948), and in some instances claimed to vary between 1 and 10 units/mg. (Uvnäs, 1945).

SUMMARY

1. Gastrin has been extracted from boiled hog pyloric mucosa with 0·1 N-hydrochloric acid in 95 % methanol.

2. Impurities were removed on neutralizing the crude extract to pH 5–5·5. The active principle flocculated in the methanolic extract almost quantitatively at pH 7.

3. Further purification was achieved through precipitation from water at pH 7. Inorganic salts were removed through dialysis.

4. 1 mg. given to an anaesthetized cat with vagi cut caused a gastric secretion containing about 10 ml. of 0·1 N-hydrochloric acid, i.e. it contained about 10 secretory units (Jalling & Jorpes, 1947).

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REFERENCES

Edkins, J. S. (1906). *J. Physiol.* **34**, 183.
Jalling, O. & Jorpes, J. E. (1947). *Acta physiol. scand.* **13**, 231.
Kahlson, G. (1948). *Brit. med. J.* **2**, 1091.
Komarov, S. A. (1942*a*). *Rev. canad. Biol.* **1**, 191.
Komarov, S. A. (1942*b*). *Rev. canad. Biol.* **1**, 377.
Uvnäs, B. (1942). *Acta physiol. scand.* **4**, Suppl. 13.
Uvnäs, B. (1945). *Acta physiol. scand.* **9**, 298.
Uvnäs, B., Munch-Petersen, J. & Rönnow, G. (1944). *Acta physiol. scand.* **7**, 389.

The Preparation of Secretin

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Friedman & Thomas (1950) described a method for the preparation of secretin on a large scale, and in a state of purity permitting its intravenous administration in man. Their method compares favourably with the older ones. Briefly it is as follows: the sodium chloride precipitate of an acid extract of hog intestine is extracted with methanol and the active material precipitated with acetone. Further purification is achieved by means of trichloroacetic acid. The final freeze-dried product has an activity of 100–130 cat units/mg.

We found the method of Friedman & Thomas very useful, although the yield of secretin was fairly low. Friedman & Thomas noted that though vigorous stirring of the intestines during extraction increased the total yield of secretin, so much inert material was extracted at the same time that the secretin activity per unit weight of the final product was lower. We have confirmed this, but prefer to use the more efficient method of extraction, removing the inert protein at a later stage.

The principle introduced by these authors of extracting the secretin from the sodium chloride precipitate with methanol is very valuable. The same cannot, however, be said about the procedure of purification by means of trichloroacetic acid, which precipitates most of the inert proteins together with the active material. In our attempts to avoid using trichloroacetic acid we soon found that the bulk of the inert material was removed on neutralizing the methanolic extract. On adding sodium hydroxide in methanol to pH 7 more than half of the protein matter present in the extract precipitated, while the secretin remained in solution.

The method described below gives, when properly adhered to, a secretin with an activity of from 600 to 900 cat units/mg. ash-free substance, and a yield of 1000–1600 cat units/m. hog intestine.

METHODS

Method of standardization

The method used was that of Wilander & Ågren (1932). Since the technique of the operation has a marked effect on the response of the animals to secretin, we have described our technique in detail.

Cats weighing 2·5–4·0 kg. and starved for 24 hr. were used. 2 hr. before operation the animals were anaesthetized by intramuscular injection of 8·0 ml./kg. body wt. of a warm 1 % solution of chloralose in physiological saline.

The abdomen was opened by a 5 cm. longitudinal incision in the mid-line, 2 cm. distal to the sternum. The pylorus was ligated and a 5–6 cm. long loop of intestine, starting from the pylorus, was fixed at each end to the abdominal wall at the ends of the incision.

The intestinal loop was opened by a longitudinal incision 3 cm. in length. The edges of the intestine were folded over the abdominal wall and fastened to it with catgut, at a distance of not less than 6 cm. on each side of the mid-line, The flap of the opened intestine should completely cover the incision in the abdominal wall, and the edges of the latter should not be sewn together since undue pressure on the pancreas must be avoided. The ampulla of Vater should be situated near the middle of the flap of intestine. The great saphenous vein was cannulated.

The cats could be used almost immediately after operation, and were usually in good condition for 12 hr. or more.

The secretin, dissolved in about 1 ml. of physiological saline, was injected into the saphenous vein, followed by 2–3 ml. of warm physiological saline. After a lag of 2–3 min. the secretion of pancreatic juice started abruptly. The juice secreted for the next 10 min. was collected on wads of tissue paper. The wads were soaked in distilled water in an Erlenmeyer flask and brought to boiling with an excess of 0·1 N-HCl. The excess acid was titrated with 0·1 N-NaOH, using methyl red as indicator.

The secretin unit is that amount of secretin which, under the conditions described, induces a secretion of alkali equivalent to 0·1 ml. of 0·1 N-HCl.

Method of preparation

The uppermost 1 m. of the intestine was removed from the hogs not later than 1 hr. after slaughter, emptied of its contents and immersed in ice water. After cooling for 0·5 hr. the intestines were either worked up immediately or frozen and stored at −20°. Storage for several weeks at this temperature did not affect the yield. If frozen material was used it was brought to the laboratory and allowed to thaw in cold water.

The fresh or thawed intestines were everted, the mucus covering them was washed away with ice water and the material squeezed dry. It was then extracted for 0·5 hr. with 200 ml. ice-cold 0·13 N-HCl/m. intestine, with constant stirring during the extraction. When fresh material was used the extraction was performed at the slaughter house.

Volumes of 20 l. extract from 100 m. intestine were brought to the laboratory and left standing overnight in the cold room, care being taken to keep pieces of ice floating in the extract all the time. The supernatant was decanted from the coarse particles that had settled to the bottom, and filtered with suction in the cold room (+4°) after addition of 30 g. of Hyflo Super-Cel/l. The bottom layer, about 1 l., was likewise filtered with the aid of Super-Cel. To each l. of the combined filtrates 320 g. NaCl were added.

The precipitate was collected on a suction filter in the cold room, washed on the filter with cold saturated NaCl solution and pressed as dry as possible. It is essential that the mother liquor is carefully removed before the extraction with methanol. The precipitate weighed about 300–350 g. with 40–60 % moisture. It could amount to 500 g. after very thorough shaking during extraction. The precipitate was extracted for 12 hr. at +4° with 5 parts by weight of methanol. Only one extraction was made, as suggested by Friedman & Thomas. The methanol solution which had a pH of about 2·5 (glass electrode) was neutralized to pH 7 with N-NaOH in methanol. A heavy precipitate formed and was removed by filtration through fluted filter paper. It contained only 2–3 % of the secretin activity but more than 50 % of the original nitrogen extracted. The actual amount of precipitate varied considerably in different preparations due to the extent to which the intestines were stirred during extraction, and to the amount of moisture remaining in the NaCl precipitate. In four instances the amount of nitrogen precipitated when the methanol extract was neutralized made up 49, 40, 60 and 61 % respectively of the total amount.

The reaction of the filtrate was adjusted to pH 2·5 with HCl and 3 vol. of cold acetone were added. The precipitate containing the secretin was washed on a suction funnel five times with acetone and three times with absolute ether. Yield, 21–25 g. of air-dry substance. The secretin activity of the dry powder was approximately 10–15 cat units/mg. with a total yield of 2100–3750 cat units/m. of intestine. More than 90 % of the precipitate consisted of NaCl, and the N content was 0·5–1·0 %.

The acetone precipitate was suspended in 100 ml. ice-cold distilled water per 35 g. of precipitate. This dissolved the NaCl, leaving the secretin undissolved in the concentrated brine which was formed. The suspension was filtered with suction on hardened paper (Schleicher and Schüll no. 1575) and washed with cold concentrated NaCl solution. The filtrate, which contained about 30 % of the nitrogen originally present in the acetone precipitate but only a negligible secretin activity, was discarded. The filter cake was dissolved in about 25 ml. cold distilled water. If the solution was cloudy it was cleared by filtration with suction through hardened filter paper after the addition of 1 g. of Hyflo Super-Cel. The clear solution was saturated with NaCl, the precipitate filtered with suction, dissolved in a minute amount of distilled water and freeze-dried. The yield of the dry powder was about 1 g. from 100 m. of intestine. The activity was approximately 200 units/mg. or 2000 units/m.

Freeze-dried powder (10 g. from about 1000 hogs) was suspended in 1000 ml. of cold absolute methanol. Most of the substance dissolved within half an hour; no attention was paid to the undissolved matter. The pH of the suspension was brought to 6·5–7·0 with 0·1 N-NaOH in methanol. The precipitate was removed on a suction filter and dried with ether. It weighed about 6·5 g. and contained about 10 % of the total secretin activity of the freeze-dried material.

The pH of the filtrate was adjusted to 2·0–2·5 with 0·1 N-HCl in methanol, and 3 vol. of cold ethyl ether were added. The flocculent white precipitate formed was collected on a suction filter and washed on the funnel with ether. It was immediately dissolved in distilled water and freeze-

dried. Yield, about 2·0 g. with an activity of 400–600 units/mg. or seven to eight times higher than that of the impurities removed at pH 7·0.

The powder was almost water-free, but contained about 35% ash. The secretin activity/mg. organic dry substance is consequently 600–900 units/mg.

SUMMARY

1. Secretin has been extracted with methanol from the sodium chloride precipitate of an intestinal extract, as recommended by Friedman & Thomas.

2. Impurities were removed through repeated isoelectric precipitations from methanol at pH 7.

3. The final product had an activity of from 600 to 900 cat units/mg. ash-free substance, the yield being 1000–1600 units/m. of hog intestine.

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REFERENCES

Friedman, M. H. F. & Thomas, J. E. (1950). *Proc. Soc. exp. Biol., N.Y.*, **73**, 345.

Wilander, O. & Ågren, G. (1932). *Biochem. Z.* **250**, 489.

The Free Amino-acids of Invertebrate Nerve

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Many types of animal tissue possess an electrolyte composition which differs markedly from that of the body fluids in two important respects: first, the inorganic cations, largely sodium and potassium, outweigh the inorganic anions, chloride, sulphate and phosphate, i.e. there is an inorganic anion deficit; and, secondly, the concentration of potassium is greater (and, conversely, the concentrations of sodium and chloride are less) than in the extracellular fluid, i.e. there is an internal accumulation of potassium. This type of electrolyte distribution is highly developed in excitable tissues. Thus, the extruded axoplasm of giant squid nerves contains over 300 m-moles/l. of potassium and only about 50 and 70 m-moles/l. of sodium and chloride respectively, but squid blood contains only about 20 m-moles/l. of potassium and considerably more than 400 m-moles/l. of both sodium and chloride. In both vertebrate and invertebrate muscle tissue various phosphorus compounds account for most of the inorganic anion deficit; and this may be true also for vertebrate nerve which contains a very high concentration of phosphorus, much of which, however, may not contribute to the anion content of the axoplasm since it is combined with nitrogenous bases to form the phospholipins of the myelin sheath. But in the nerves of marine invertebrates such as the Crustacea and the cephalopod molluscs there is much less phosphorus, and here much of the deficit must be made up by purely organic anions.

The function of these internal anions is largely unknown. But they are almost certain to be important in connexion with the maintenance of a high internal potassium concentration, possibly by some mechanism of the type described by Boyle & Conway (1941). It is therefore important to know what the internal anions are, and this paper deals with the identification of the principal ones present in the nerves of certain marine invertebrates.

The first important attempt to identify the anions in these invertebrate nerves was made by Schmitt and his collaborators working first with extruded squid axoplasm (Bear & Schmitt, 1939) and later with aqueous extracts of lobster nerves (Schmitt, Bear & Silber, 1939; Silber, 1941). This work culminated in the isolation by Silber (1941) of large quantities of alanine and aspartic acid, the separate amounts of which he estimated from analyses of the lobster nerve extracts for total amino nitrogen and total amino-acid carboxyl groups assuming that no other amino-acids were present. The values he obtained—133 and 90 m-moles/kg. of alanine and aspartic acid respectively—left a large fraction of the anion deficit unaccounted for. But the presence of other amino-acids would invalidate these values; so a re-investigation of the amino-acid content of these nerves was essential before beginning a search for other anions.

A preliminary, qualitative survey showed that the nerves from all the seven marine invertebrate species tested contained appreciable amounts of