DISTRIBUTION OF DIGESTIVE ENZYMES

IN

PETROMYZON MARINUS

A STUDY OF DISTRIBUTION OF DIGESTIVE ENZYMES IN THE AMMOCOETE AND ADULT OF PETROMYZON MARINUS

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This dissertation deals with a study of certain aspects of the physiology of digestion of Petromyzon marinus with emphasis on the localization of certain enzymes. It was found that the physiology of digestion adjusted to the drastic change in feeding habits at metamorphosis.

ABSTRACT

Little is known about the adjustment in the physiology of digestion of the sea lamprey, Petromyzon marinus, during its metamorphosis from the larval stage with a diet of primarily carbohydrates to a parasitic stage with a proteinaceous diet. Such an adjustment is not only expected on purely theoretical grounds but is also based on comparative observations on species of fish differing in the nature of their diets. In approaching this problem it was decided to determine and localize the enzymatic activity in the gut of larval and adult P. marinus.

This dissertation presents evidence that the production of digestive enzymes in the ammocoete larva occurs predominantly, if not exclusively, in the anterior intestine and that this production is a function of the exocrine part of the pancreas. Further, that after metamorphosis the major production of proteolytic and possibly also lipolytic enzymes occurs in a part of the intestine posterior to the "pancreatic region", while production of amylase remains maximal in the anterior end of the parasite.

Supplementation of pancreatic trypsin, and possibly

also of pancreatic lipase at metamorphosis, by intestinal enzymes and absence of such supplementation for amylase are considered of adaptive significance for the particular diet of the parasite.

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INTRODUCTION

The sea lamprey (Petromyzon marinus) is a marine species which spends its larval life in fresh water and migrates after metamorphosis to the ocean. The animals used for the experiments discussed throughout this thesis are a landlocked form of the marine species. Instead of migrating to the ocean it has adjusted itself to spending its entire life cycle in fresh water.

The larvae live in the mud bottom of tributary waters of the Great Lakes, sucking microscopic organisms and detritus from the water and the mud. After about four years they undergo metamorphosis and migrate to the lakes to begin their parasitic existence, living on the blood and body juices of fish (Applegate, 1955).

This drastic change in feeding habits from a primarily carbohydrate food to a proteinaceous diet was expected to be accompanied by adjustment in the physiology of digestion from larval to parasitic stage. Such expectation was not only justified on purely theoretical grounds but was further supported by comparative observations on species of fish differing in the nature of their diets (Barrington, 1957).

This dissertation deals with certain aspects of the physiology of digestion in larva and adult of Petromyzon

marinus with emphasis on the localization of certain enzymes.

The physiology of digestion has been studied by a number of workers in recent years.

Krukenberg (1897) concluded, that the digestive secretion in fishes is much more diffused than in higher vertebrates and that the evolution of the digestive organs is both morphologically and functionally in the direction of a greater concentration and specialization, from a more diffused and less differentiated condition. He further suggested that the digestion of protein in primitive vertebrates is of the peptic rather than the tryptic type.

Alcock (1899) studied the digestive processes in the most primitive of the fishes, the cyclostomes. It so happened that her results agreed with Krukenberg's statements as she found proteolytic digestion in the ammocoete to be of the peptic type and the production of the enzyme concerned much more diffused than in the higher vertebrates. She did not find any evidence of either a tryptic or diastatic ferment.

Vonk (1927), working with adult Petromyzon, found an amylase in the intestinal mucosa. Because of its weakness he suggested that its activity may be ascribed to a tissue enzyme.

Barrington (1935) drew attention to Alcock's finding of proteolytic digestion of the peptic type in the ammocoete

and to the fact that the protease of the Tunicata is now known to be of the tryptic rather than the peptic type (Yonge, 1925; Berrill, 1929). In view of the phylogenetic relationship between these forms and the fact that the work on digestion in the ammocoete was carried out before the realisation of the importance of pH control, he demanded reconsideration of Alcock's work.

Barrington (1936) could not find in the ammocoete larvae of Lampetra planeri a protease of the peptic type as described by Alcock. According to Barrington proteolytic activity is due to an enzyme of the tryptic type and the production of this enzyme is localized at the anterior end of the intestine. It is of great interest in this connection that Brachet (1897) and Maskell (1930) have described the occurrence of "zymogen" cells of a specialized type in this region of the intestine in respectively Lampetra planeri and Geotria australis. Barrington's observations provided physiological proof for Brachet's and Maskell's assumptions as to the zymogenic nature of these cells.

In a later publication (1945) Barrington described zymogen cells in the anterior intestine of adult P. fluviatilis. In adult L. planeri these cells are not present, a result of the cessation of feeding at metamorphosis no doubt. The author compared the distribution of zymogen cells in adult Petromyzon, larvae of Lampetra and an American form

(probably Entosphenus) in order to determine changes of the alimentary canal during metamorphosis. Zymogen cells of the larva (Lampetra and Entosphenus) were decidedly different from those of adult Petromyzon. In Geotria and Mordacia (Maskell, 1930, 1931) these cells disappeared at metamorphosis and were not detected in the adult, presumably as a result of the cessation of feeding. Therefore, Barrington thought it possible that, in the metamorphosis of Petromyzon the zymogen cells of the adult were not necessarily derived from those of the larva, but that the larval zymogen cells disappeared and that new ones were developed to take their place.

Gage (1943) could not demonstrate zymogen granules in American cyclostomes and Amphioxus, although methods that revealed their presence in sharks and all higher forms were used on abundant material in all stages of growth and at all seasons of the year. He further reported however, that there are gland-like cells in the intestinal wall near the liver in the Cyclostomata (hags and lampreys) and that zymogen cells have been described for these forms in Europe (apparently a reference to Barrington's work).

The above review of the scarce literature on histological and physiological aspects of enzyme production in the digestive system of larva and adult of P. marinus clearly indicates the need for further investigation of certain

aspects. This is particularly so since the only pertinent investigation is that of Barrington (1936, 1945) which deals with comparison of distribution of zymogen cells in larva and adult of different species.

MATERIAL AND METHODS

In approaching the problem outlined in the introduction it was decided to determine and localize the enzymatic activity in the gut of larval and adult P. marinus.

Adult lampreys and ammocoetes, collected from the lakes Huron and Michigan and tributaries, were kept alive in aquaria with running well water. All larvae were in the pre-metamorphic stage and were kept in aquaria whose bottoms were covered with a 9-10 cm thick layer of organic mud. Splake (a hybrid of lake trout and brook trout) was found to be readily attacked by lampreys and therefore chosen as prey when feeding specimens were required.

Lampreys were considered "fasting" when they had been deprived of food for at least a month and when practically no contents were found in the intestine. All larvae were termed "feeding" when over the whole length of the intestine contents were found of external origin.

Preparation of enzyme extracts

Separate extracts were made of the oesophagus, various segments of the intestine and the liver. The number of segments of the intestine depended somewhat on the size

of the animal, but was at least four when all parts were rinsed or ten when the parts were alternately rinsed (see below).

Digestive juices in fasting lamprey are present in only very minute quantities. Isolation of such small quantities of juice from various parts of the digestive tract in sufficient quantities for an adequate comparative study of the enzyme contents would therefore have posed a very time consuming and difficult technical problem. Since it was felt that equally reliable results could be obtained by making extracts of the mucous membranes of the various parts of the digestive tract this latter method was adopted.

The lampreys were killed by decapitation. The whole alimentary canal was then removed, by carefully dissecting away the attachments and was then subdivided into various segments; these were weighed and slit longitudinally. Depending on the requirements of the experiment the segments were either rinsed or submitted to further treatment without rinsing. In the course of the rinsing procedure the mucous membrane was scraped off. Rinsing was done quantitatively with distilled water from a buret. It was then homogenized (Potter and Elvehjem, 1936) with addition of distilled water to make an aqueous extract of known segment to water weight-ratio.

To distinguish between the distribution of enzyme activity in the mucous membrane and the gut lumen, the segments of the intestine were alternately rinsed before homogenization. Since the segment immediately caudate to the oesophagus in P. fluviatilis is known to contain zymogen cells (Barrington, 1945) and the occurrence of such cells in P. marinus was therefore highly probable this part of the intestine was invariably rinsed, while the following, more posterior part, was homogenized without rinsing.

In the ammocoetes the very small oesophagus was not used for measurements. Since Barrington (1936) reported the occurrence of zymogen cells in only the anterior part of the intestine of L. planeri the intestine of the ammocoetes used in this experiment was subdivided into anterior and posterior parts. Both these parts were always washed and homogenized in toto. All extracts were stored in the refrigerator at approximately 4-5°C after addition of a few drops of toluene.

Measurement of H-ion concentration in the alimentary canal of adult lamprey

Measurements of the pH of the intestinal contents were carried out by means of the B.D.H. Capillator, using phenol-red as indicator. Small portions of the intestinal content were taken from a freshly decapitated lamprey, diluted with one to two drops of distilled water and mixed with

one drop of the indicator. The resulting colour was compared with those of a colour scale. This method does not allow for the observation of slight differences in pH values which might have existed between the two ends of the intestine, since the mucous content, which was always present in the posterior part of the canal of fasting lampreys, always had a greenish colour which interfered with the colour of the pH indicator.

Electrometric measurements of the pH of different samples of intestinal fluid could not be carried out with the available apparatus since only very small quantities of juice were present in the intestine of fasting lampreys.

In order to trace secretions responsible for a possible pH-distribution in the intestinal juice, electrometric pH measurements were done on water extracts of intestinal mucosa (and exocrine pancreatic tissue).

Measurements of enzyme activity

a. Proteolytic activity

Proteolytic activity was estimated by formol titration (Davis and Smith, 1955).

Tubes were prepared containing 0.3-0.9 ml extract as described above, 2 ml of 2% casein, 1 ml buffer (phosphate or borate, 0.1-0.2 M) and one drop of toluene. The tubes were left for 1-5 hours at 36-37°C. At the end of

this time one drop of a 0.1% phenolphthalein solution was added to each ml of digest and an aliquot part of each tube titrated to a definite pink colour with N/50 sodium hydroxide. One ml of neutral formol per 2.5 ml mixture was then added and the solution titrated to the same colour as before. The amount of amino acids liberated in terms of ml N/50 sodium hydroxide was taken as an index of proteolytic activity.

Blank values were obtained by titrating under identical conditions a mixture in which the active extract was replaced by a boiled extract or by titrating a mixture immediately after addition of the active extract.

b. Carbohydrase activity

Carbohydrase activity was estimated by the method of Folin and Wu (1920) after removal of proteins in the digestive mixture with cadmium sulphate and sodium hydroxide (Fujita and Iwatake, 1931).

Tubes were prepared containing 0.3 ml of enzyme extract, 0.6 ml of buffer (phosphate, 0.2 M), 2.1 ml substrate (0.3% soluble starch, 0.01% maltose or 0.001% sucrose) and one drop of toluene. The tubes were left for 1-6 hours at 35-37°C and at the end of this time proteins were removed with 2.4 ml of cadmium sulphate solution, followed almost immediately by addition of 0.3 ml of 1.1 N sodium hydroxide.

The contents were mixed and left standing for ten minutes; then centrifuged during ten minutes at 2000 r.p.m. Three ml of the centrifugate were pipetted into a 12.5 ml calibrated test tube, mixed with 2 ml of alkaline copper solution and heated during ten minutes in a boiling waterbath. After cooling for three minutes in cold water 2 ml of phosphomolybdic acid were added, the contents shaken vigorously and the volume brought to the 12.5 ml mark with distilled water. The intensity of the blue compound thus formed by the complexing of any cuprous oxide with phosphomolybdic acid was read on a Fisher Electrophotometer at 420 or 650 mμ and taken as a measure for the carbohydrase activity.

Blank values were obtained by measuring under identical conditions, a mixture from which proteins had been removed immediately after addition of the substrate.

Cadmium sulphate solution was prepared by dissolving 13 gr cadmium sulphate in 63.5 ml of 1 N sulphuric acid and diluting this to 1 liter with distilled water.

Alkaline copper solution was prepared by dissolving 40 gr of sodium carbonate in 400 ml of distilled water and adding to this 7.5 gr of tartaric acid and a solution of 4.5 gr copper sulphate in distilled water. This was then diluted to 1 liter, and the final solution filtered.

Phosphomolybdic acid was prepared by dissolving 94.2 gr of sodium molybdate and 10 gr of sodium tungstate in approximately 650 ml of distilled water and adding slowly, while

cooling, 240 ml of 85% phosphoric acid. This was then diluted to 1 liter.

c. Lipolytic activity

Lipases and esterases are unlike proteases and carbohydrases of low specificity since their only general requirement is that for an ester linkage. The enzyme can not be specified through the nature of the substrate used. Furthermore substrates such as olive oil, frequently used in the past, often present difficulties through the emulsification while this difficulty is not encountered with water-soluble esters. The latter are non-specific for lipases. Gomori (1945) and Archibald (1946) introduced the Tweens of which Tween 20 is highly specific for lipase activity.

Lipolytic activity was estimated by titrimetric determination of the fatty acids liberated from Tween 20, a polyoxyalkylene derivative of sorbitan monolaurate (Bier, 1955). Tween 20 was freed from free fatty acids by the method of Archibald (1946).

To eliminate free fatty acids from the substrate used, 90 ml of Tween 20 and a mixture of 50 ml of diethyl ether and 100 ml of petroleum ether were placed in a 100 ml glass-stoppered centrifuge flask and acidified with 0.75 ml of phosphoric acid. The contents were shaken and centrifuged.

The etheric supernatant was rejected and the extraction repeated with 25 ml of the mixture. After removal of the ethers dissolved in the Tween by evacuation, the residue was neutralized with sodium hydroxide and finally decanted from the precipitate of sodium phosphate. The substrate was prepared by mixing 100 ml of buffer (sodium acetate 0.2 N), 50 ml of Tween, 10 ml of the indicator (an aqueous solution of phenyl red, 0.02%) and 90 ml of distilled water. The resulting pH of the mixture was about 7.2 and the substrate was suitable for measurements up to one week after its preparation.

Tubes were prepared containing 0.9 ml of the enzyme extract and 4.5 ml of the substrate preparation. They were incubated for 1-4½ hours at 37°C. To prevent foaming, a drop of octyl alcohol was added to the digest shortly before titration. Then 0.02 N NaOH was added from a buret till the indicator, which was yellow at the beginning, became red-violet (pH 8.3). During titration the contents of the tube were continuously mixed by a slow stream of nitrogen. This prevented at the same time absorption of carbondioxide.

Reference is made in the literature to the activating nature of octyl alcohol on lipolytic enzyme (Sobotka and Glick, 1934). The absolute inaccuracy resulting from the use of this alcohol in the above method was not deemed to be serious since the differences of enzyme activity

between samples were used in the evaluation of the results.

The substrate (4.5 ml) and the enzyme preparation (0.9 ml) were titrated separately. The sum of the two titration values was used as the control. The difference between the alkali used in the test and in the control was taken as an index of lipolytic activity.

EXPERIMENTAL RESULTS

Enzymatic activity in the digestive tract of the ammocoete

a. Proteolytic activity

Measurements of digestion of casein by extracts of the anterior and posterior parts of the intestine and of the liver, carried out by formol titration, indicated that the highest proteolytic activity occurred in extracts of the anterior intestine (Table I). The readings for the posterior intestine and liver demonstrated that, from the point of view of digestion, their proteolytic activity was negligible.

b. Amylolytic activity

Hydrolysis of starch was estimated for extracts of the anterior and posterior parts of the intestine and of the liver. The results, as shown in Table II, indicate that a strong amylolytic action occurred in extracts of the anterior intestine. Several experiments have demonstrated consistently slight amylolytic activity in extracts of the posterior intestine and of the liver.

c. Lipolytic activity

Table III shows the results of measurements on lipolytic activity of various parts of the digestive tract of the ammocoete. It was found that extracts of the anterior intestine have a high lipolytic activity compared with those of the posterior intestine, while extracts of the liver showed a very weak digestive action.

H-ion concentration in the alimentary canal of the parasite

The pH of the intestinal contents was found to range from 7.0-7.8.

The results of pH measurements of different segments of the intestinal mucosa in the feeding lamprey are represented in Table IVA, while Table IVB lists the results obtained in fasting lampreys. Initially a decrease in pH can be observed from the oesophagus caudate (6.65 to 6.60 in feeding, and 6.84 to 6.76 in fasting lamprey). Posterior of this region there is a noticeable increase in pH in both animals.

Enzymatic activity in the digestive tract of the parasite

a. Proteolytic activity

The estimation of proteolytic activity was carried out for extracts of intestinal mucosa, with or without intestinal juice, and of the liver. Some of the results are presented in Tables VA and B. It was found that proteolytic action occurs in extracts of the intestinal mucosa. No appreciable difference was found between activities of two adjacent segments (one rinsed, the other one not rinsed), indicating that only a small proportion of the proteolytic action of unwashed segments could have been exerted by adhering intestinal juice.

In presenting the results of enzymatic activity in different segments of the intestine throughout this thesis it was considered that it was not possible to make accurate length measurements of parts of the intestine once these were cut out. This is due in part to the always present peristaltic movements in the intestine of a freshly killed animal. Rather than expressing the position of the segments in the intestine as a whole in units of length it was decided to define this position in terms of percent of accumulative weight of the segment to the total weight of the intestine. In this way zero percent is the anterior end of the oesophagus while 100 percent coincides with the caudal end of the intestine.

The strongest proteolytic activity occurred between 55 and 90% (Plate I, Fig. 1 and 2). The distribution of activity appeared to be unaffected by the intake of food and was the same for different periods of incubation. No proteolytic activity was found in extracts of the liver, while such activity in extracts of the oesophagus was absent or negligible.

Effect of pH on proteolytic activity

In order to determine whether the proteolytic action was of the tryptic or the catheptic type the above experiments were repeated at different pH levels. Some of the results obtained are summarized in Tables VIA and B (Plate II, Fig. 1 and 2). They show that the activity increases with pH in all segments up to around the 80% position. Only in the very last part of the intestine a decrease rather than an increase in proteolytic activity could be observed. It is also seen that in extracts of the mucosa, the strongest increase with pH occurs at between 55 and 85%. A further series of experiments on the relationship between proteolytic activity of various extracts of the mucosa and the H-ion concentration in the digestive mixture confirmed the previous experiments. From the results given in Tables VIIA, B and C (Plate III, Fig. 1) it can be seen that tryptic activity is optimal at pH 8.

b. Amylolytic activity

Amylolytic activity was detected in extracts of the oesophagus, in all extracts of the various parts of the intestinal mucosa and in extracts of the liver. A significant difference was found in the pattern of distribution of intestinal amylase in the feeding and fasting lamprey.

Feeding lamprey

The results of the experiments on amylolytic activity of intestinal extracts of feeding lamprey (some of which are presented in Table VIIIA; Plate IV, Fig. 1)show that extracts of a small part of the mucosa, immediately posterior to the oesophagus, had a high amylolytic activity compared with those of other more posterior parts.

Directly behind this area of high amylase activity occurred a sudden decline in activity in rinsed extracts of the intestinal mucosa. This was followed by a gradual increase towards the end of the intestine, while extracts of unwashed mucosa showed a more gradual decrease in posterior direction over the whole length of the intestine.

Fasting lamprey

The initial high activity of the mucosa of feeding lamprey was absent (Table VIIIB; Plate IV, Fig. 2). Instead,

extracts of the mucosa showed right from the beginning of the intestine a gradual increase in activity, which roughly persisted over the whole length. On the whole the readings for the extracts of unwashed mucosa demonstrated a similar distribution of activity as those for extracts of washed mucosa. The strongest amylolytic activity occurred between 65 and 100%. In general, there were no appreciable differences between activity of extracts of washed and unwashed parts, although activity in the posterior half of the intestine was more pronounced.

The few measurements on starch digestion by extracts of the oesophagus pointed to the presence of a weak amylase activity, which increased when NaCl in optimal concentration (see below) was added to the digestive mixture (Table IX).

With one exception it was found that extracts of the liver showed amylolytic activity, which was considerably higher when sodium chloride was added (Table IX).

Effect of sodium chloride

The influence of sodium chloride on the amylolytic activity of extracts of the oesophagus, intestinal mucosa and liver from fasting lampreys was investigated. Addition of sodium chloride to the starch solution in amounts to make the concentration in the digestive mixture 0.05 M (Chesley, 1934a and b) gave a marked increase in activity for all

three types of extract (Table IX; Plate V, Fig. 1 and 2). Outstanding in this respect was the extreme activation obtained for that part of the intestinal mucosa, which followed the oesophagus.

c. Maltase activity

Maltase was found to be present in extracts of the mucosa from anterior as well as posterior regions of the intestine (Tables XA and B; Plate VI, Fig. 1 and 2). The readings for extracts of unwashed were much higher than those of washed mucosa. Posterior to the oesophagus the extracts showed a gradual increase in activity, which roughly persisted over the whole length of the intestine. The distribution of the acitivity appeared to be grossly the same for feeding and non-feeding specimens. It is noted, however, that in the case of feeding lamprey the activity in the extract of the very first washed segment was somewhat higher (0.046 mg glucose) than that of the next washed segment (0.026 mg glucose), while the reverse was true for fasting lamprey.

Maltase activity of extracts of the liver was very weak or absent.

d. Invertase activity

A weak invertase was shown to be present in extracts of the intestinal mucosa. The results, presented in Tables XIA and B (Plate VII, Fig. 1 and 2), showed a peak of activity at position 60%. The distribution of activity over the whole length of the intestine appeared essentially to be unaffected by the intake of food.

e. Lipolytic activity

The action of extracts of oesophagus, intestinal mucosa (with or without juice) and liver on Tween 20 was investigated. The composition of the digestive mixtures can be seen from Tables VIIA and B, in which some of the results are summarized.

Lipolytic activity of extracts of intestinal mucosa was shown by all segments of the intestine; the strongest action was found between 65 and 90%. Figures 1 and 2 of Plate VIII represent the distribution of lipolytic activity in the intestinal mucosa in feeding and fasting lamprey respectively. The results are not significantly different.

Lipolytic activity in the oesophagus was absent or negligible.

The occurrence of lipolytic enzyme in the liver was noticeable.

DISCUSSION

Enzymatic activity in the digestive tract of the ammocoete

a. Proteolytic activity

1. Intestine

Barrington (1936) described in the larva of L. planeri "two vesicular, oval-shaped areas which externally mark the anterior part of the intestine". These areas are "sharply separated from each other in the mid-dorsal line, but with ill-defined ventral and posterior boundaries". As in the larvae of Lampetra the anterior end of the intestine of the ammocoete of P. marinus is similarly marked.

Although the presence of zymogen cells has been reported in the larvae of L. planeri (Barrington, 1936) and Geotria australis (Maskell, 1930), and in adult Petromyzon fluviatilis (Barrington, 1945), no such reference is available in the literature for either larval or parasitic Petromyzon marinus. In the presentation of the results in this thesis it is assumed that such cells do also occur in the latter species. Thus reference to "anterior intestine" bears, as described by Barrington, on the region containing

zymogen cells. It should be emphasized that Barrington's results indicate that no such zymogen cells could be found immediately behind the vesicular regions. The term "posterior intestine" refers to that part of the intestine which does not contain zymogen cells.

In the furrow between the oesophagus and the tip of the intestine follicles are located (Boenig, 1929). To these follicles pancreatic function has been ascribed (Cotronei, 1927; Barrington, 1942 and 1945). Since Barrington (1936) found that the presence of the pancreas does not affect proteolytic activity in larvae of L. planeri and Cotronei (1927) concluded that the pancreas is exclusively endocrine, the pancreas was included in the tissues extracted in all the experiments on the anterior intestine described in this thesis.

Table I not only clearly indicates the occurrence of proteolytic activity in both the anterior and posterior parts of the intestine but also demonstrates that this activity is very considerably higher in the anterior part. It is concluded that this high activity in the anterior part is due to the presence of zymogen cells since, as already has been pointed out, the endocrine function of the pancreas is not expected to affect proteolytic digestion. This conclusion is supported by Barrington's observations (l.c.) on larval Lampetra, which also indicated higher proteolytic activity in the anterior part of the intestine of that species at the

same pH value of the experiment reported in Table I.

In the present investigation no attempt was made to determine the nature of the proteolytic activity in both parts of the intestine. This activity could be either tryptic or catheptic.

Proteolytic activity shows a maximum at the anterior end of the intestine at a pH near neutrality.

2. Liver

No proteolytic activity was found in liver extracts (Table I). Barrington (1936) reported slight proteolytic activity of the liver of Lampetra larvae in an acid medium, even with HCl present, but no activity in an alkaline medium. Absence of activity in present experiments might have been due to the pH of the digestive mixture, which was near neutrality.

b. Amylolytic activity

1. Intestine

Miss Alcock (1899) failed to find any trace of a "diastatic ferment" in the digestive system of ammocoetes. Since this author did not describe the method applied, it is difficult to discuss this result.

Barrington (1936) did not have any difficulty in demonstrating amylase in the larvae of L. planeri, and stated that the amylase was less localized in its distribution than the proteases. This is in strong contrast with the results presented in this thesis (Table II) which indicate a comparatively strong amylolytic activity in extracts of the anterior intestine, while the posterior intestine shows this activity only slightly. While no experimental evidence is available these results suggest that amylolytic activity is related with the occurrence of zymogenic cells in the anterior part of the intestine.

The weak amylase activity of the posterior intestine might be attributed to enzyme production throughout this region. However Vonk (1927, 1937) assumed that low carbohydrase activity observed in the intestinal mucosa of fish does not result from the mucosa but is brought about by absorption by the mucosa of the pertaining enzymes produced by the pancreas. The slight activity in the posterior intestine of the ammocoete could similarly be due to back absorption of enzyme, secreted by the anterior presumably pancreatic region, on to the intestinal mucosa. At any rate this amylolytic activity of the posterior intestine is too weak to be of significance in digestion.

2. Liver

Amylolytic activity was found in extracts of the liver. Activity in such extracts may be expected but there is no reason to consider it of significance in digestion unless pancreatic tissue is included in the organ (Barrington, 1957). Since the liver of the ammocoete was virtually free from pancreatic tissue, the activity may be ascribed to tissue amylase alone.

c. Lipolytic activity

1. Intestine

The results obtained in the present study regarding lipolytic activity (Table III) indicate a much stronger activity in the anterior than in the posterior intestine. The area of strong activity corresponds with that occupied presumably by zymogen cells, which suggests that this activity is a function of these cells. The weak lipolytic activity in the posterior intestine might be ascribed to back absorption or slight local production of enzyme as discussed for the proteolytic and amylolytic activity.

It should be emphasized here that lipolytic activity in the digestive system of any of the Cyclostomata has as yet not been described in the literature.

2. Liver

The finding of lipolytic activity in the ammocoete liver is of interest (Table III). Schlottke (1940) found a strong lipase in the liver of Lota vulgaris and since this did not occur in the bile or elsewhere in the digestive system in a significant concentration he suggested that it might serve to split fats brought to the liver by the portal artery. Assuming that Tween 20 is not hydrolyzed by esterases the liver may be the site of lipase production in the ammocoete.

Due to technical difficulties derived from the extremely small amounts of bile present in the liver, no attempt has been made to examine the bile separately on lipolytic activity. The question therefore whether this activity observed in the liver is of digestive importance, remains unanswered. However comparing the level of activity of the liver (including the bile) with that of the anterior intestine it seems improbable that the activity in the latter is the result of secretion of bile into the intestine.

Since the presence of bile salts in the bile could be demonstrated it may be concluded that activation of the lipase by the bile takes place in the intestine.

The oesophagus is too small to allow for observations on its enzymatic activity.

Enzymatic activity in the digestive tract of the parasite

a. Proteolytic activity

No reference is made in the literature by previous workers on the occurrence of proteases in the intestine of sea lamprey in the parasitic stage or any other species of Cyclostomata in the post-metamorphic stage.

It was pointed out in the "Results" that proteolytic activity occurs throughout the intestine of the parasite. An analysis of this activity in relation to the pH at which optimal digestion is obtained (Table VIIA, B and C; Plate III, Fig. 1) clearly indicates that from the anterior end to a point shortly anterior to the anal pore the proteolytic activity is of a tryptic nature with an optimal pH of approximately 8. On the other hand this proteolytic activity in the remainder of the intestine must be catheptic since it does not display an optimum in dependence of the pH of the medium.

It should be emphasized that the region of tryptic activity does not coincide with the occurrence of zymogen cells as described by Barrington (1945). In the larva of L. planeri however Barrington found coincidence of tryptic proteolytic activity and the occurrence of zymogen cells. Information on the occurrence of tryptic enzyme in the post-metamorphic phase of any species of cyclostomes is absent

as pointed out earlier so that no direct comparison can be made at this time. It seems justified to speculate that the occurrence of tryptic protease in the parasitic stage and in the region described is an adaptation to the highly specialized proteinaceous diet which is quite unlike that of the larval stage.

Barrington (1957) comments that "trypsinogen" in fish is commonly and perhaps always, formed in the pancreas, but there remains doubt as to how far, if at all, the latter is also secreted by the intestine. By analogy with mammals intestinal secretion would not be expected, but Al-Hussaini's (1949) study on the intestines of cyprinids showed proteolytic activity which was highest in the posterior third of the tract. While in the larva the production of trypsin may be wholly attributed to pancreatic zymogen cells, in the parasite production of a tryptic enzyme by pancreatic zymogen cells can certainly not be the sole source of this enzyme. It is concluded, as a result of this study, that the production of trypsin in the parasite is at least supplemented by an intestinal enzyme of the same nature.

It should be reminded here that both histologically and physiologically the stomach is considered to be absent in the Cyclostomata. Whether the absence of the stomach is phylogenetically a primary condition or whether it has resulted from a degenerative process is as yet unsettled.

Should the latter interpretation prevail (Al-Hussaini, 1949) the occurrence of a tryptic enzyme in the region described might well be considered as an adaptation to the loss of a stomach which in many vertebrates is the seat of pepsin production.

Barrington (1957), however, considers the condition of the digestive tract in Cyclostomata primary from a phylogenetic point of view. This view is based on his observations on the pancreas which in the lampreys is still an integral part of the intestinal wall but in most higher vertebrates forms a fully separate organ. Should Barrington's view be correct then tryptic activity in the intestine could not be interpreted as an adaptation in the above sense. The same author (1942), studying the evolutionary aspects of the stomachless condition concluded that peptic digestion is absent in the ammocoete (Barrington, 1936) and probably also in the adult. He suggested that the appearance of the stomach in the evolution from Cyclostomata to Pisces may be correlated with the change from microphagy to macrophagy.

In contrast to this view, considering the absence of the stomach from the Cyclostomata as a primitive rather than a secondary character stands the current interpretation of these animals as degenerative survivors of a group in which, for example, bone was originally present (Romer, 1955). Of particular importance here is the fact that, in a number of

fishes, there is evidence of a secondary loss of the stomach. In different genera of the same family the stomach may be either present or absent (Hirsch, 1950).

In the light of the direct evidence available for either point of view (primary versus secondary phylogeny of the stomach) it seems as yet difficult to settle this very important problem.

Observations on the pH of the intestinal tract

In the present work the optimum for trypsin in adult lamprey was found to be between 7.5 and 8.0. Since the pH within the intestine of the parasite approximately ranges between 7.0 and 7.8, the protease is well adapted for functioning in the environment provided (a conclusion which was also drawn by Barrington (1936) for the ammocoete of Lampetra) for even at the lower pH limit of 7.0 there is an ample margin of proteolytic activity.

Bayliss (1935) described the occurrence of trypsin in the plaice (Pleuronectes platessa). The pH of the intestine of the plaice was found to range from 7.43 to 8.65; the there occurring trypsin had a pH optimum between 7.5 and 8.5. The increased alkalinity in the intestine of the plaice would provide a better correlation between enzyme and environment than in the ammocoete. According to

Barrington (1936) this difference may be related to the improvement in the organization of the digestive system, which is of such considerable importance in the evolution from Cyclostomata to Pisces. The results presented in this thesis (Table VIIA and B; Plate III, Fig. 1) regarding trypsin-like activity in parasitic Petromyzon similarly show an optimum (about 8) which is slightly higher than the pH (7.0-7.8) of the environment. This difference is less pronounced than that found by Barrington (l.c.) in the ammocoete of Lampetra.

b. Amylolytic activity

From the results of the experiments dealing with these enzymes (Table VIIIA, and B; Plate IV, Fig. 1 and 2) in the parasite it could be concluded that there is a production of amylase in the anterior end of the intestine and probably also in more posterior regions. It has further been shown that in lampreys feeding shortly before decapitation, there is a significantly higher activity in the anterior end than elsewhere, while the reverse seems to be true for fasting lamprey. Since the high activity in the anterior end was found only in lampreys which were feeding shortly before decapitation, the question presented itself whether this was due to an increased production of amylase at the time of food intake, to an activation of enzyme already present in

an inactive form or to both these events.

When NaCl was added to extracts of washed intestinal mucosa of fasting lamprey in amounts to make the concentration 0.05 M in the digestive mixture it was found that the anterior end of the intestine showed a larger rise in activity than any other region (Table IX; Plate V, Fig. 1 and 2). This resulted in an activity distribution which was similar to that found in the washed mucosa of feeding lamprey (Table VIIIA; Plate IV, Fig. 1). It is therefore very likely that the high activity in the anterior end of the intestine in feeding lamprey is primarily the result of activation of amylase already present in large amount before feeding started.

The literature refers to a number of observations on such activation of fish amylase. Chesley (1934a and b) found that optimal activation occurred when the concentration of NaCl in the digestive mixture was 0.05 M. Bayliss (1935) observed in the intestine of Pleuronectes a weak amylase, probably secreted by the intestine itself, which was activated by addition of 0.05 to 0.10% NaCl to the digestive mixtures. Keddis (1956) noticed some activation of amylase in the intestinal juice and pancreas of Tilapia nilotica.

It is of interest in this connection that values of serum chloride for brown trout lie between 100 and 200 m.

equiv./l. (Gordon, 1958). Members of the trout family seem to be preferred as prey by the sea lamprey according to field and laboratory observations. Maximal activation of amylase in the lamprey gut by chloride from the blood of the prey animal is therefore a distinct possibility. It must be remembered, however, that in analogy with mammals an activation of amylase by amino acids derived for example from the blood of the prey animal is also possible.

It was pointed out earlier that the ammocoete displays strong amylolytic activity in the anterior intestine where zymogen cells are expected to occur, while the posterior intestine shows a weak amylase activity. Also in the parasite a strong amylase production occurs in the anterior end of the intestine where zymogen cells were described by Barrington (1945) for P. fluviatilis. The remainder of the intestine shows a weak to moderate activity (Table VIIIA and B; Plate IV, Fig. 1 and 2). This moderate activity may partly be the result of back absorption of enzyme from the pancreatic region (see page 37). In Table VIIIA (Plate IV, Fig. 1) for example there are striking differences between readings of extracts of washed and unwashed parts of the intestinal mucosa in the posterior region of the intestine. These are almost certainly the result of transportation of amylase. That the presence of amylase in the posterior region of the intestinal mucosa would be due to absorption alone is

however not in line with the distribution of activity since there is a rise in activity in the second half of the intestine in posterior direction. It is therefore concluded that a production of amylase also occurs in the posterior regions of the intestine.

Since the presence of amylase in the posterior intestinal mucosa of the ammocoete is uncertain and in the parasite the production of amylase in the posterior regions is weak in comparison to that in the anterior end of the intestinal mucosa most amylolytic digestion in these animals occurs in the region containing pancreatic zymogen cells. A comparison between the relative importance of the region of zymogen cells in relation to the posterior regions of the intestine in proteolytic and amylolytic digestion is of interest. While the major proteolytic activity in the adult can be attributed to an enzyme of non-pancreatic origin, amylolytic digestion in the parasite is similar to that of the larva and due primarily to an enzyme from the region of pancreatic zymogen cells. There is no indication of a similar supplementation of pancreatic amylase by intestinal amylase at metamorphosis in the parasite. This seems to be of adaptive significance in relation to the particular diet of the parasite.

The only reference in the literature to amylolytic digestion in Petromyzon is that of Vonk (1927) who found a weak amylolytic activity in extract of the intestinal mucosa

of adult Petromyzon. Since the activity was very low, it was difficult to decide whether it should be ascribed to a tissue or a digestive amylase. Vonk added that from a teleological point of view it would not be surprising to find only a weak digestive amylase, or none at all, in a blood sucking parasite. His finding of only a weak activity may be ascribed to the fact that he made extracts of the whole length of the intestine, in which the intense activity of a particular region would inevitably be masked by the weak activity of the remainder of it, to the omission of NaCl from the digestive mixture and possibly also to the absence of food intake at the time that the animals were to be used for the experiment.

c. Maltase activity

From the results of measurements of maltase activity (Table XA and B; Plate VI, Fig. 1 and 2) two main conclusions are to be drawn: (1) that there is a production of maltase throughout the entire length of the intestine, and (2) that the production of the enzyme in the pancreatic zymogen cell region is of the same order of magnitude as that in other parts of the intestine.

The very low carbohydrase activity in the intestinal mucosa of fishes suggests that the enzymes are not produced by the mucosa itself but result from the pancreatic secretion.

Their presence in the mucosa would then be due to absorption (Vonk, 1927, 1937).

This interpretation is however not in line with the distribution of activity for maltase (and amylase) in the parasite since there is a rise in activity in the second half of the intestine in posterior direction. The level of maltase activity found in the posterior end of the intestine can not be explained exclusively by absorption of enzyme from the anterior end of the intestine, particularly since the activity in the latter is low.

In fact, maximal activity of maltase occurs in digests from the posterior half of the intestine while amylolytic activity is maximal in those from the anterior end. This would suggest a stepwise degradation of carbohydrates, a view which is not supported by Vonk (1937). This author emphasized that, in mammals, amylase is secreted by the pancreas (and salivary glands) with only a trace of maltase; maltase, lactase and invertase are produced in the intestine which suggests that there is a "chain" of carbohydrase activity. According to Vonk a considerable maltase activity occurs in pancreatic extracts of fish and since he had already stated that carbohydrases are not produced in the intestinal mucosa the "chain" of carbohydrase activity would not be found in fish.

The findings presented in this thesis therefore do not support Vonk's views in this matter. In the sea lamprey,

at least, there is evidence for the chain of carbohydrase activity as described for mammals.

d. Invertase activity

The above considerations with respect to carbohydrase degradation can be supplemented by observations on invertase. There is evidence for the existence of invertase in the intestine of the parasite (Table XIA and B; Plate VII, Fig. 1 and 2). The activity is however too weak to allow conclusions about its distribution other than that it occurs in extracts from regions of the intestine which do not contain pancreatic zymogen cells.

e. Lipolytic activity

Results of investigations on lipolytic activity in the parasite indicate a production of enzyme in the mucosa of the entire intestine (Table XIIA and B; Plate VIII, Fig. 1 and 2). The secretion, if any, from the anterior end is very low; there is a gradual increase of activity in posterior direction followed by a decline shortly before the anal pore. As for maltase it can therefore be argued that the activity in extracts of the posterior half of the intestine is not merely the result of absorption of enzyme from a pancreatic secretion on to the intestinal mucosa.

When compared with the distribution of activity in the ammocoete it is seen that the main centre of production of lipolytic enzyme has shifted from the anterior intestine before metamorphosis to the posterior half of the intestine in the parasite. This may mean that during metamorphosis there is a decline in contribution from the presumably pancreatic region or an additional development resulting into a much increased participation in enzyme production by the posterior half of the intestine.

A comparison between the levels of activity in these two regions in the larva and the parasite (Tables III and XIIA) lends support to the second hypothesis, namely that an increased production of enzyme in the posterior intestine is brought about during metamorphosis.

Reviewing the results of this comparative study on the digestion in the larval and parasitic stages of Petromyzon marinus the important conclusion stands out that the production of enzymes in the ammocoete occurs predominantly, if not exclusively, in the anterior intestine and that the available evidence indicates this production to be a function of the exocrine part of the pancreas. After metamorphosis the major production of proteolytic and possibly also lipolytic enzymes occurs in a part of the intestine posterior to the

"pancreatic region". Production of amylase however remains maximal in the anterior end of the gut of the parasitic stage.

SUMMARY

1. The expected adjustment in the physiology of digestion of Petromyzon marinus to the change from primarily carbohydrate food in the larva to a proteinaceous diet in the parasitic stage was investigated. The distribution of proteolytic, carbohydrase and lipolytic activity in various parts of the digestive system of larva and adult was determined.

The ammocoete

2. Strong proteolytic, amylolytic and lipolytic activity could be demonstrated in extracts of the anterior intestine.
3. Much weaker activity was observed in extracts of the posterior intestine and liver.
4. It is concluded that the production of enzymes in the intestine is predominantly, if not exclusively, a function of the anterior intestine in which zymogen cells have been described for the ammocoete of L. planeri.

The parasite

5. Proteolytic activity in extracts of the mucosa, from the anterior end of the intestine to a point shortly anterior to the anal pore, was of a tryptic nature while the activity for the remainder of the intestine was catheptic.

6. While in the larva the production of trypsin may be wholly attributed to pancreatic zymogen cells, production of trypsin in the parasite is at least supplemented by an intestinal enzyme of the same nature.

7. Since the pH within the intestine approximately ranges between 7.0 and 7.8, the trypsin is well adapted for functioning in the environment provided (pH optimum 8).

8. The slightly better correlation between enzyme and environment in the plaice than in the parasitic stage of the lamprey may be related to the improvement in the organization of the digestive system, which is of such considerable importance in the evolution from Cyclostomata to Pisces.

9. No proteolytic activity was observed in extracts of the liver while such activity in extracts of the oesophagus was negligible or absent.

10. Strong amylolytic activity was detected in extracts of the anterior end of the intestinal mucosa of

the feeding animal, while in the fasting animal the activity in extracts of the same region was weak.

11. Amylolytic activity in extracts from posterior regions of the intestinal mucosa showed a gradual increase towards the anal pore in both the feeding and fasting animal, but never attained the high level observed for extracts from the anterior end of the intestinal mucosa of the feeding animal.

12. There is evidence that the high activity in the anterior end of the intestine in the feeding animal is primarily the result of activation by NaCl of amylase already present in large amount before feeding started. Maximal activation of amylase in the gut by chloride from the blood of the prey animal is a distinct possibility.

13. As in the larva, amylolytic digestion in the parasite is primarily due to an enzyme from the region of pancreatic zymogen cells.

14. A weak amylase was found in the oesophagus and liver which could be activated by NaCl.

15. Maltase activity in extracts from the pancreatic region was found to be of the same order of magnitude as in other parts of the intestine.

16. Invertase activity was observed in extracts from the anterior as well as the posterior end of the intestine.

17. While maximal activity of amylolytic enzyme was found for extracts from the anterior end of the intestine, maximal activity of maltase was found in those from the posterior end. This suggests a stepwise degradation of carbohydrates. The finding of invertase activity posterior to the pancreatic region supports this consideration.

18. Major lipolytic activity was observed for extracts from the posterior regions of the intestine.

19. Comparison between the levels of lipolytic activity in anterior respectively posterior intestine of the larva and anterior respectively posterior regions of the intestine of the parasite lends support to the hypothesis that an increased production of enzyme in the posterior intestine is brought about during metamorphosis.

20. Extracts of the liver showed a notable lipolytic activity while those of the oesophagus showed little activity or none at all.

21. Supplementation at metamorphosis in the parasite of pancreatic trypsin, and possibly also of pancreatic lipase, by intestinal enzymes of the same nature and absence of such supplementation for amylase are considered of adaptive significance for the particular diet of the parasite.

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TABLE I — Petromyzon marinus

Proteolytic activity in extracts of the anterior and posterior intestine and liver.

7 Feeding ammocoetes				
Sample 0.3 ml, casein solution 2% 2 ml, buffer (phosphate M/5) pH 6.9 1 ml; 37°C.				
Extract	Weight of tissue	Dilution of extract	Activity in ml 0.02 N NaOH after:	
			1½ hours	4 hours
Ant. int.	62.5 mg	1:75	0.60	0.93
Post. int.	177.1	1:75	0.08	0.09
Liver	99.8	1:75	0.00	0.00

TABLE II — Petromyzon marinus

Amylolytic activity in extracts of the anterior and posterior intestine and liver.

7 Feeding ammocoetes			
Sample 0.3 ml, starch solution 0.3% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 1½ hrs at 36°C.			
Extract	Weight of tissue	Dilution of extract	Activity increase in mg glucose
Ant. int.	62.5 mg	1:75	0.107
Post. int.	177.1	1:75	0.015
Liver	99.8	1:75	0.022

TABLE III — *Petromyzon marinus*

Lipolytic activity in extracts of the anterior and posterior intestine and liver.

7 Feeding ammocoetes				
Sample 0.9 ml, substrate-buffer mixture pH 7 4.5 ml; 37°C.				
Extract	Weight of tissue	Dilution of extract	Activity in ml 0.02 N NaOH after:	
			1½ hours	4 hours
Ant. int.	104.0 mg	1:40	0.17	0.26
Post. int.	305.0	1:40	0.04	0.08
Liver	202.0	1:40	0.01	0.03

TABLE IV — *Petromyzon marinus*

H-ion concentration in extracts of different segments of the intestinal mucosa and liver.

	Extract	Weight of tissue	Dilution of extract	pH
A	**Feeding parasite**			
	I	0.848 gr	1:3	6.65
	II	0.449	1:3	6.60
	III	0.341	1:3	6.70
	IV	0.187	1:3	6.82
	V	0.184	1:3	6.85
	Liver	0.372	1:3	7.15
B	**Fasting parasite**			
	I	1.103	1:7	6.84
	II	1.027	1:7	6.80
	III	1.001	1:7	6.76
	IV	0.634	1:7	6.98
	V	0.356	1:7	7.00
	Liver	0.687	1:7	7.37

TABLE V — Petromyzon marinus

Proteolytic activity in extracts of the intestinal mucosa, liver and oesophagus.

A

Feeding parasite				
Sample 0.9 ml, casein solution 2% 2 ml, buffer (phosphate M/10) pH 6.9 1 ml; 37°C.				
Extract	Weight of tissue	Dilution of extract	Activity in ml 0.02 N NaOH after:	
			1 hour	5 hours
I	0.225 gr	1:40	0.01	0.84
Ia	0.306	1:40	0.26	0.90
II	0.370	1:40	0.15	0.88
IIa	0.286	1:40	2.08	3.04
III	0.290	1:40	2.06	3.33
IIIa	0.289	1:40	3.88	5.31
IV	0.307	1:40	1.11	3.29
IVa	0.267	1:40	0.66	1.29
Liver	0.351	1:40	0.00	0.00

B

Fasting parasite				
Sample 0.9 ml, casein solution 2% 2 ml, buffer (phosphate M/10) pH 6.9 1 ml; 37°C.				
Oesophagus	0.034 gr	1:100	0.00	0.00
I	0.490	1:25	0.30	1.13
Ia	0.462	1:25	0.38	1.02
II	0.535	1:25	0.80	3.11
IIa	0.449	1:25	3.09	3.82
III	0.490	1:25	3.31	5.06
IIIa	0.420	1:25	4.14	5.94
IV	0.465	1:25	1.75	3.45
IVa	0.339	1:25	1.55	2.83
Liver	0.576	1:16	0.00	0.00

TABLE VI — Petromyzon marinus

Proteolytic activity in extracts of the intestinal mucosa and liver at different pH levels.

A

Feeding parasite					
Sample 0.9 ml, casein solution 2% 2 ml, buffer (phosphate M/10) 1 ml; 37°C.					
Extract	Weight of tissue	Dil. of extr.	Activity in ml 0.02 N NaOH after:		
			1½ hrs	4 hrs	
			pH 6.9		pH 5.8
I	0.301 gr	1:40	0.24	0.36	0.27
Ia	0.416	1:40	0.13	0.29	0.13
II	0.317	1:40	0.61	0.83	0.31
IIa	0.279	1:40	1.98	3.29	1.86
III	0.356	1:40	1.70	2.78	0.59
IIIa	0.286	1:40	3.31	3.67	1.87
IV	0.205	1:40	1.18	2.18	1.01
IVa	0.178	1:40	0.90	1.91	1.99
Liver	0.640	1:40	0.00	0.00	0.00

B

Fasting parasite				
Sample 0.9 ml, casein solution 2% 2 ml, buffer (phosphate M/5) 1 ml; 4 hrs at 37°C.				
Extract	Weight of tissue	Dil. of extr.	Activity in ml 0.02 N NaOH	
			pH 8	pH 6
I	0.865 gr	1:24	1.93	0.42
II	0.563	1:24	4.38	2.46
III	0.653	1:24	6.15	2.62
IV	0.406	1:24	1.26	3.54

TABLE VII

Influence of the H-ion concentration on the proteolytic activity in extracts of the intestinal mucosa of fasting parasites.

A

Extract I		
Weight of tissue 0.887 gr (0-32.22%). Dilution of extract 1:30. Sample 0.9 ml, casein solution 2% 2 ml, buffer 1 ml; 3 hrs at 37°C.		
Buffer M/5	pH	Activity in ml 0.02 N NaOH
Phosphate	6.0	0.08
,,	6.3	0.08
,,	7.0	0.70
,,	7.5	1.01
Borate	8.3	1.06
,,	8.8	0.90
,,	9.2	0.73

B

Extract III		
Weight of tissue 0.653 gr (57.42-84.04%). Dilution of extract 1:24. Sample 0.3 ml, casein solution 2% 2 ml, buffer 1 ml; 4 hrs at 37°C.		
Phosphate	5.8	0.38
,,	7.4	2.47
,,	8.0	2.64
Borate	8.1	2.67
,,	8.6	2.44
,,	9.0	2.36

C

Extract IV		
Weight of tissue 0.396 gr (85.62-100%). Dilution of extract 1:30. Sample 0.6 ml, casein solution 2% 2 ml, buffer 1 ml; 3 hrs at 37°C.		
Phosphate	6.0	1.81
,,	6.3	1.71
,,	7.0	1.67
,,	7.5	1.27
Borate	8.3	0.46
,,	8.8	0.42
,,	9.2	0.44

TABLE VIII — *Petromyzon marinus*

Amylolytic activity in extracts of the intestinal mucosa and liver

A

Feeding parasite			
Sample 0.3 ml, starch solution 0.3% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 2 hrs at 36°C.			
Extract	Weight of tissue	Dilution of extract	Activity increase in mg glucose
I	0.892 gr	1:16	0.475
Ia	1.345	1:16	0.748
II	1.213	1:16	0.070
IIa	1.277	1:16	0.375
III	1.317	1:16	0.080
IIIa	1.132	1:16	0.396
IV	0.700	1:16	0.108
IVa	0.577	1:16	0.254
V	0.508	1:16	0.103
Va	0.315	1:16	0.190
Liver	2.105	1:8	0.066

B

Fasting parasite			
Sample 0.3 ml, starch solution 0.3% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 1½ hrs at 36°C.			
I	0.517 gr	1:16	0.001
Ia	1.079	1:16	0.023
II	0.713	1:16	0.032
IIa	0.683	1:16	0.040
III	1.211	1:16	0.080
IIIa	0.980	1:16	0.072
IV	0.913	1:16	0.068
IVa	0.607	1:16	0.115
V	0.632	1:16	0.070
Va	0.398	1:16	0.082
Liver	1.402	1:8	0.000

TABLE IX — *Petromyzon marinus*

Influence of sodium chloride on the amylolytic activity in extracts of the intestinal mucosa, liver and oesophagus.

Fasting parasite				
Sample 0.3 ml, starch solution 0.3% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 4 hrs at 36°C.				
Extract	Weight of tissue	Dilution of extract	Activity increase in mg glucose	
			NaCl	-
Oesophagus	0.150 gr	1:16	0.076	0.027
I	2.633	1:16	0.416	0.017
II	1.864	1:16	0.042	0.008
III	1.985	1:16	0.052	0.038
IV	1.985	1:16	0.068	0.046
V	1.465	1:16	0.108	0.044
VI	0.757	1:16	0.098	0.062
Liver	1.876	1:16	0.074	0.024

TABLE X — Petromyzon marinus

Maltase activity in extracts of the intestinal mucosa and liver

A

Feeding parasite			
Sample 0.3 ml, maltose solution 0.01% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 4 hrs at 36°C.			
Extract	Weight of tissue	Dilution of extract	Activity increase in mg glucose
I	0.892 gr	1:16	0.046
Ia	1.345	1:16	0.069
II	1.213	1:16	0.026
IIa	1.277	1:16	0.100
III	1.317	1:16	0.038
IIIa	1.132	1:16	0.135
IV	0.700	1:16	0.052
IVa	0.577	1:16	0.102
V	0.508	1:16	0.082
Va	0.315	1:16	0.112
Liver	2.105	1:8	0.004

B

Fasting parasite			
Sample 0.3 ml, maltose solution 0.01% 2.1 ml, buffer (phosphate M/5) pH6.9 0.6 ml; 1½ hrs at 36°C.			
I	0.517 gr	1:16	0.011
Ia	1.079	1:16	0.035
II	0.713	1:16	0.022
IIa	0.683	1:16	0.075
III	1.211	1:16	0.056
IIIa	0.980	1:16	0.076
IV	0.913	1:16	0.045
IVa	0.607	1:16	0.092
V	0.632	1:16	0.075
Va	0.398	1:16	0.072
Liver	1.402	1:8	0.000

TABLE XI — *Petromyzon marinus*

Invertase activity in extracts of the intestinal mucosa and liver

A

Feeding parasite			
Sample 0.3 ml, sucrose solution 0.001% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 5 hrs at 36°C.			
Extract	Weight of tissue	Dilution of extract	Activity increase in mg glucose
I	0.892 gr	1:16	0.004
Ia	1.345	1:16	0.020
II	1.213	1:16	0.001
IIa	1.277	1:16	0.023
III	1.317	1:16	0.004
IIIa	1.132	1:16	0.026
IV	0.700	1:16	0.002
IVa	0.577	1:16	0.016
V	0.508	1:16	0.001
Va	0.315	1:16	0.012

B

Fasting parasite			
Sample 0.3 ml, sucrose solution 0.001% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 1½ hrs at 36°C.			
I	0.517 gr	1:16	0.004
Ia	1.079	1:16	0.018
II	0.713	1:16	0.014
IIa	0.683	1:16	0.020
III	1.211	1:16	0.032
IIIa	0.980	1:16	0.034
IV	0.913	1:16	0.024
IVa	0.607	1:16	0.030
V	0.632	1:16	0.012
Va	0.398	1:16	0.014
Liver	1.402	1:8	0.000

TABLE XII — *Petromyzon marinus*

Lipolytic activity in extracts of the intestinal mucosa, liver and oesophagus.

A

Feeding parasite				
Sample 0.9 ml, substrate-buffer mixture pH 7 4.5 ml; 37°C				
Extract	Weight of tissue	Dilution of extract	Activity in ml 0.02 N NaOH after:	
			1 hr	4½ hrs
Oesophagus	0.026 gr	1:100	0.04	-
I	0.225	1:40	0.06	0.09
Ia	0.306	1:40	0.07	0.14
II	0.370	1:40	0.07	0.08
IIa	0.286	1:40	0.03	0.37
III	0.290	1:40	0.12	0.35
IIIa	0.289	1:40	0.33	0.84
IV	0.307	1:40	0.13	0.38
IVa	0.267	1:40	0.07	0.19
Liver	0.351	1:40	0.08	0.15

B

Fasting parasite				
Sample 0.9 ml, substrate-buffer mixture pH 7 4.5 ml; 37°C.				
Oesophagus	0.050 gr	1:60	0.04	-
I	0.460	1:16	0.10	0.15
Ia	0.772	1:16	0.15	0.19
II	0.512	1:16	0.14	0.19
IIa	0.610	1:16	0.44	0.69
III	0.444	1:16	0.18	0.26
IIIa	0.299	1:16	0.56	1.30
IV	0.212	1:16	0.05	-
IVa	0.140	1:16	0.10	-
Liver	0.666	1:16	0.06	0.09

PLATE I

Distribution of proteolytic activity in the intestinal mucosa

Fig. 1. Feeding parasite. Dilution of extract 1:40. Sample 0.9 ml, casein solution 2% 2 ml, buffer (phosphate M/10) pH 6.9 1 ml; 5 hrs at 37°C. Data are given in Table VA.

Fig. 2. Fasting parasite. Dilution of extract 1:25. Sample 0.9 ml, casein solution 2% 2 ml, buffer (phosphate M/10) pH 6.9 1 ml; 5 hrs at 37°C. Data are given in Table VB.

Plate I

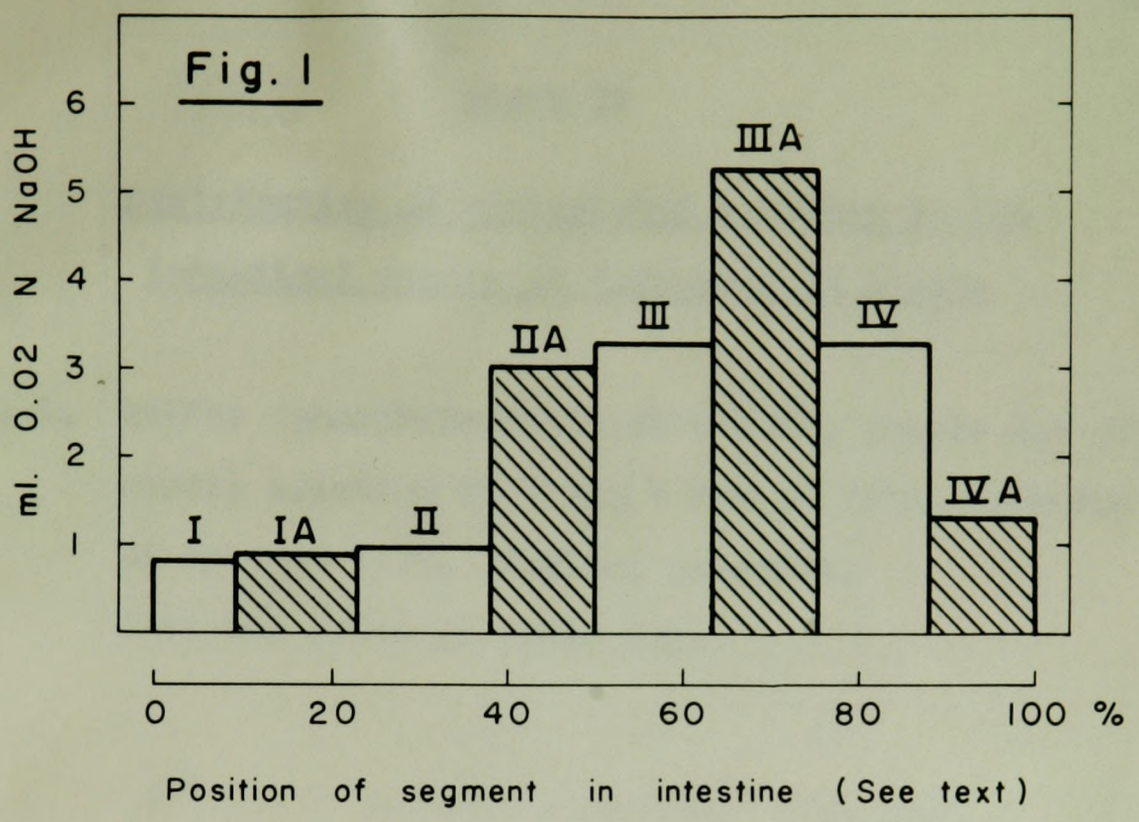
Fig. 1
ml. 0.02 N NaOH
1
2
3
4
5
6
I
IA
II
IIA
III
IIIA
IV
IVA
0
20
40
60
80
100 %
Position of segment in intestine (See text)

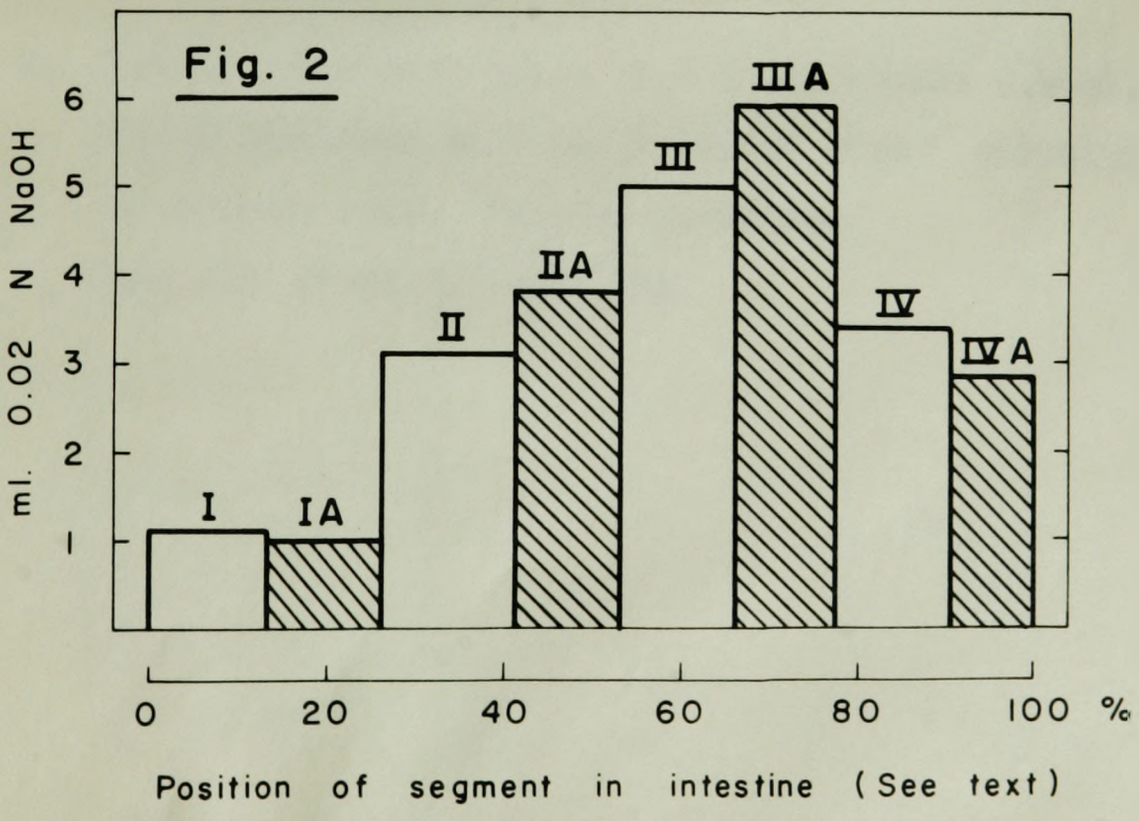
Fig. 2
ml. 0.02 N NaOH
1
2
3
4
5
6
I
IA
II
IIA
III
IIIA
IV
IVA
0
20
40
60
80
100 %
Position of segment in intestine (See text)

PLATE II

Distribution of proteolytic activity in the intestinal mucosa at different pH levels

Fig. 1. Buffer (phosphate M/10) pH 6 1 ml, sample 0.9 ml, casein solution 2% 2 ml; 4 hrs at 37°C. Dilution of extract 1:24. Fasting parasite.
Data are given in Table VIB.

Fig. 2. Buffer (phosphate M/10) pH 8 1 ml, sample 0.9 ml, casein solution 2% 2 ml; 4 hrs at 37°C. Dilution of extract 1:24. Fasting parasite.
Data are given in Table VIB.

Plate II

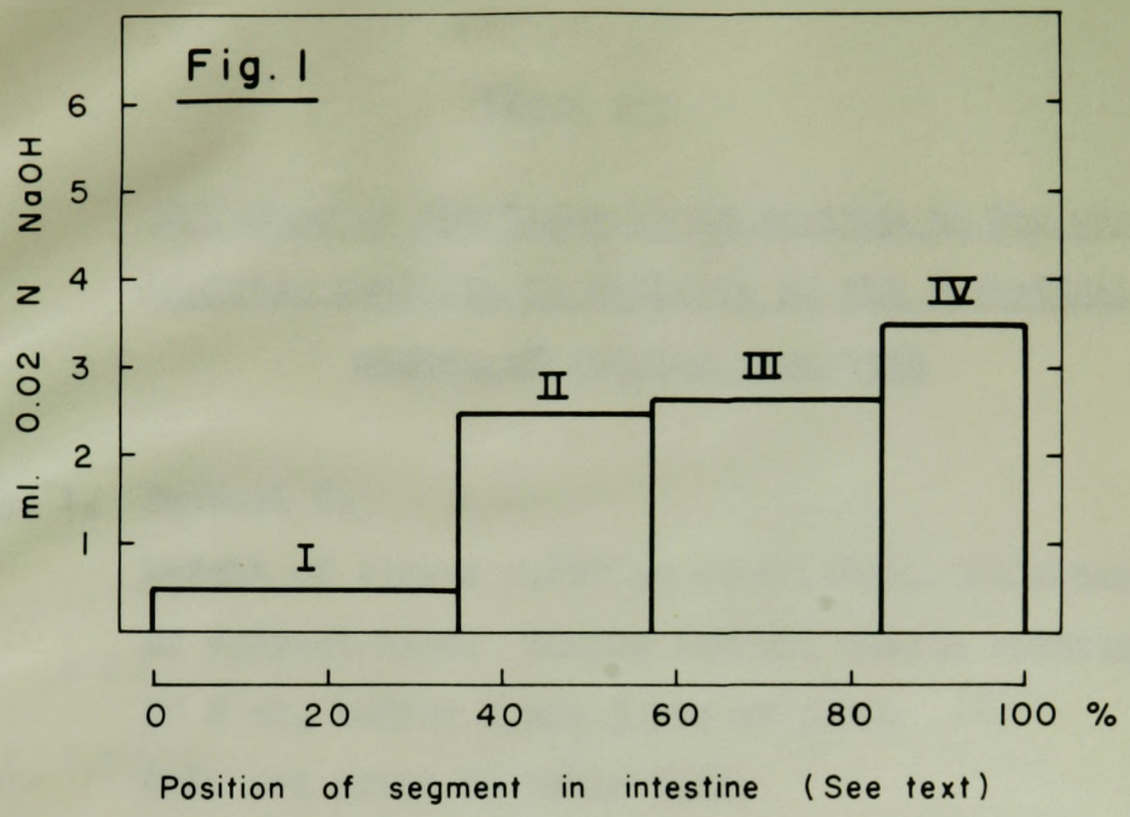
Fig. 1
ml. 0.02 N NaOH
1
2
3
4
5
6
I
II
III
IV
0
20
40
60
80
100 %
Position of segment in intestine (See text)

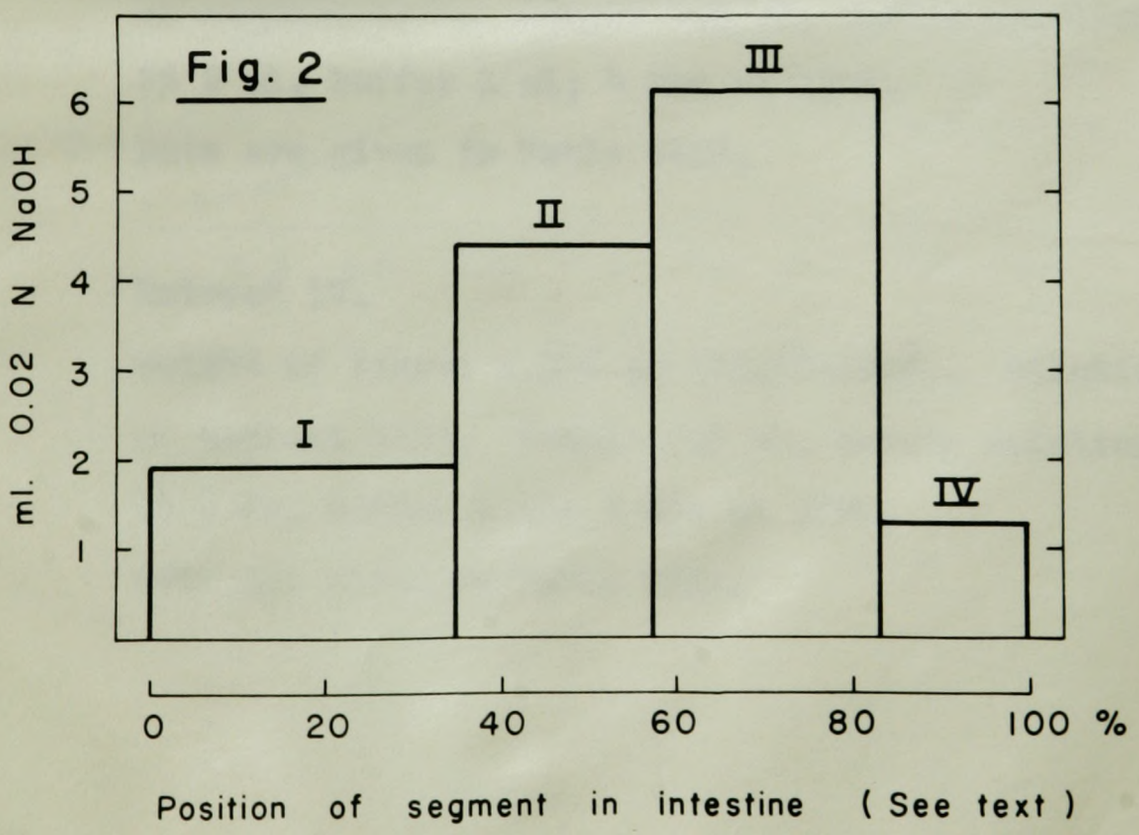
Fig. 2
ml. 0.02 N NaOH
1
2
3
4
5
6
I
II
III
IV
0
20
40
60
80
100 %
Position of segment in intestine (See text)

PLATE III

Influence of the H-ion concentration on the proteolytic activity in extracts of the intestinal mucosa of fasting parasites

Fig. 1. Extract I.
Weight of tissue 0.887 gr (0-32.22%). Dilution of extract 1:30. Sample 0.9 ml, casein solution 2% 2 ml, buffer 1 ml; 3 hrs at 37°C.
Data are given in Table VIIA.

Extract III.
Weight of tissue 0.653 gr (57.42-84.04%). Dilution of extract 1:24. Sample 0.3 ml, casein solution 2% 2 ml, buffer 1 ml; 4 hrs at 37°C.
Data are given in Table VIIB.

Extract IV.
Weight of tissue 0.396 gr (85.62-100%). Dilution of extract 1:30. Sample 0.6 ml, casein solution 2% 2 ml, buffer 1 ml; 3 hrs at 37°C.
Data are given in Table VIIC.

Plate III

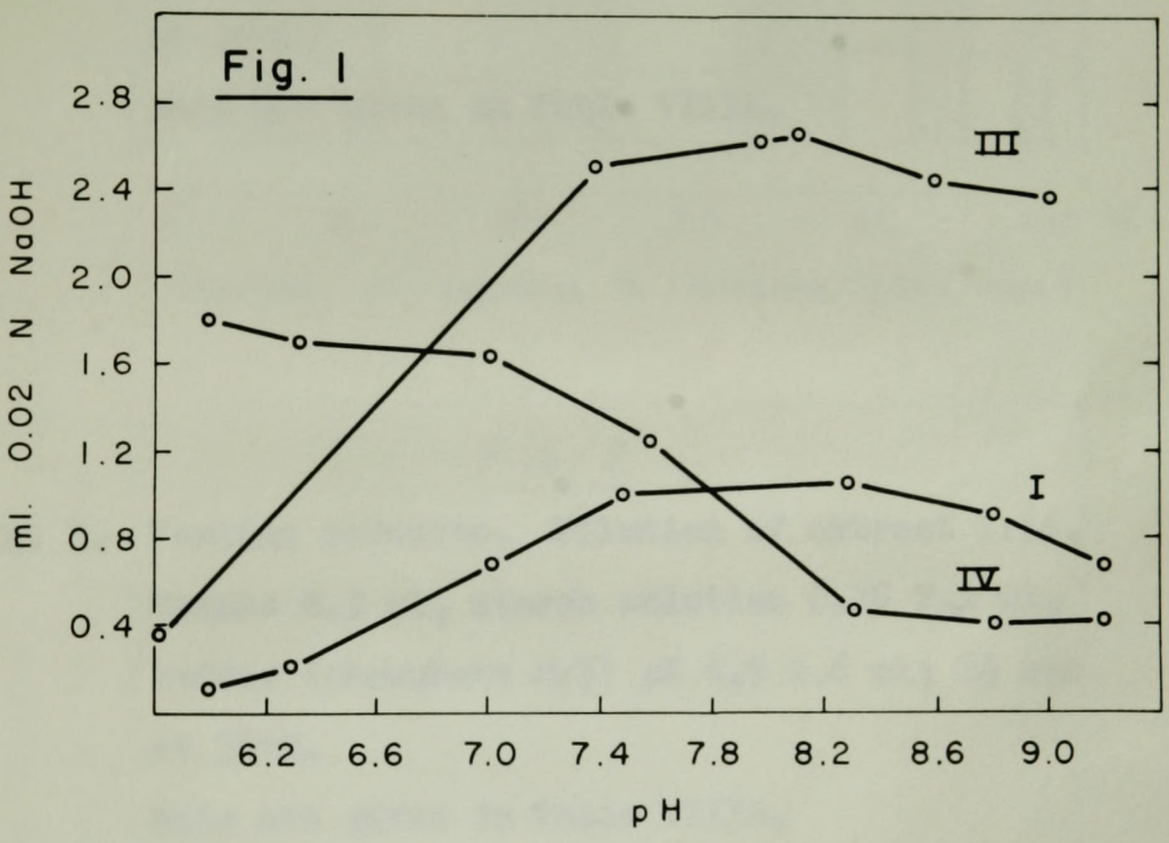
Fig. I
ml. 0.02 N NaOH
2.8
2.4
2.0
1.6
1.2
0.8
0.4
III
I
IV
6.2
6.6
7.0
7.4
7.8
8.2
8.6
9.0
pH

PLATE IV

Distribution of amylolytic activity in the intestinal mucosa

Fig. 1. Feeding parasite. Dilution of extract 1:16. Sample 0.3 ml, starch solution 0.3% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 2 hrs at 36°C.
Data are given in Table VIIIA.

Fig. 2. Fasting parasite. Dilution of extract 1:16. Sample 0.3 ml, starch solution 0.3% 2.1 ml, Buffer (phosphate M/5) pH 6.9 0.6 ml; 1½ hrs at 36°C.
Data are given in Table VIIIB.

Plate IV

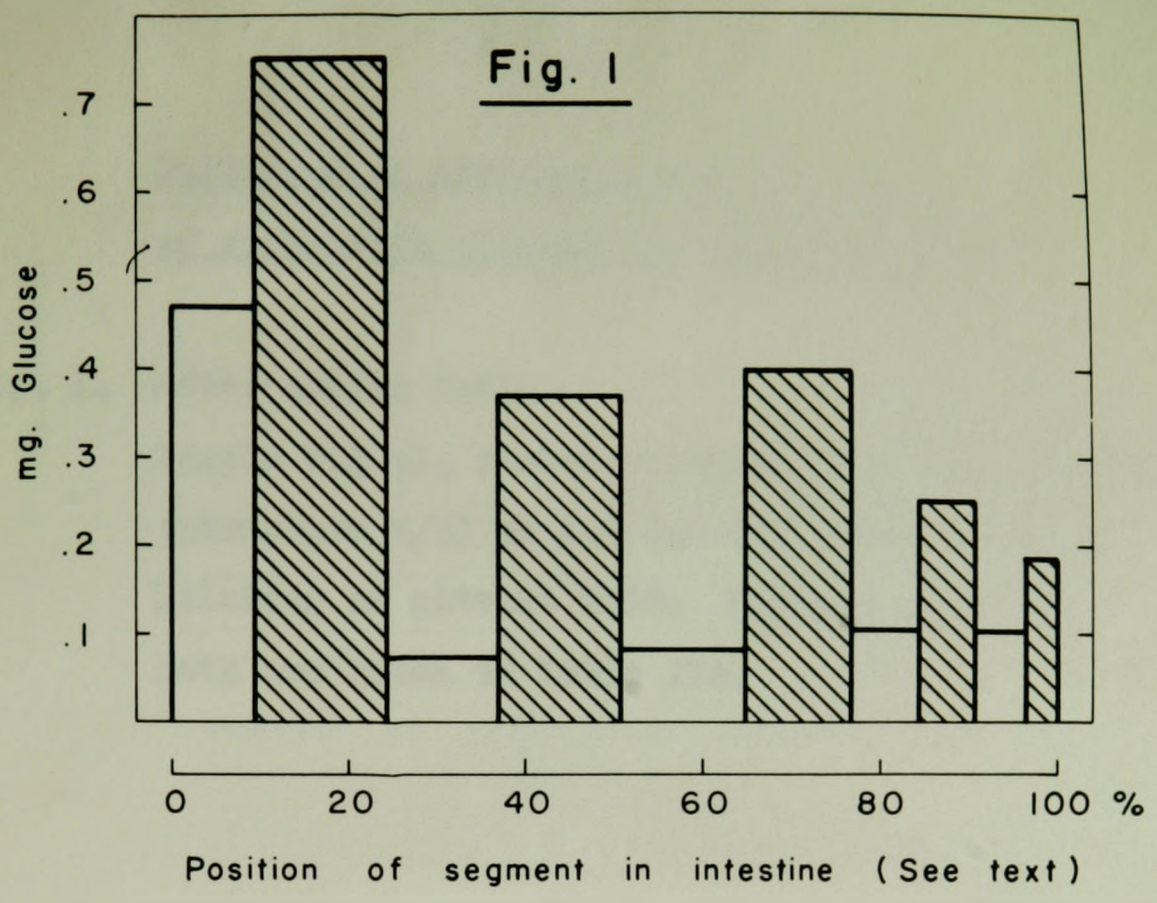
Fig. 1
mg. Glucose
.7
.6
.5
.4
.3
.2
.1
0
20
40
60
80
100 %
Position of segment in intestine (See text)

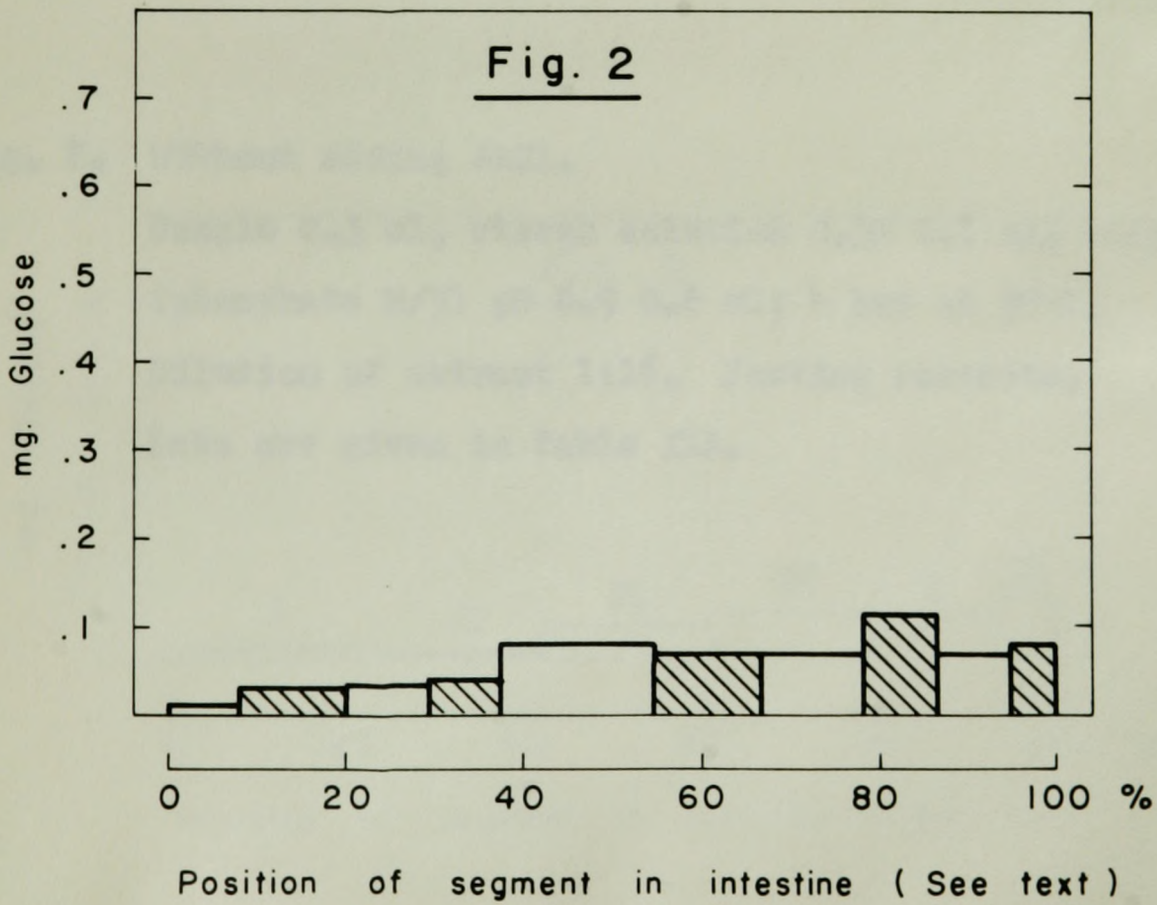
Fig. 2
mg. Glucose
.7
.6
.5
.4
.3
.2
.1
0
20
40
60
80
100 %
Position of segment in intestine (See text)

PLATE V

Influence of sodium chloride on the distribution of amylolytic activity in the intestinal mucosa

Fig. 1. After adding NaCl.
Sample 0.3 ml, starch solution 0.3% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 4 hrs at 36°C. Dilution of extract 1:16. Fasting parasite. Data are given in Table IXA.

Fig. 2. Without adding NaCl.
Sample 0.3 ml, starch solution 0.3% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 4 hrs at 36°C. Dilution of extract 1:16. Fasting parasite. Data are given in Table IXA.

Plate V

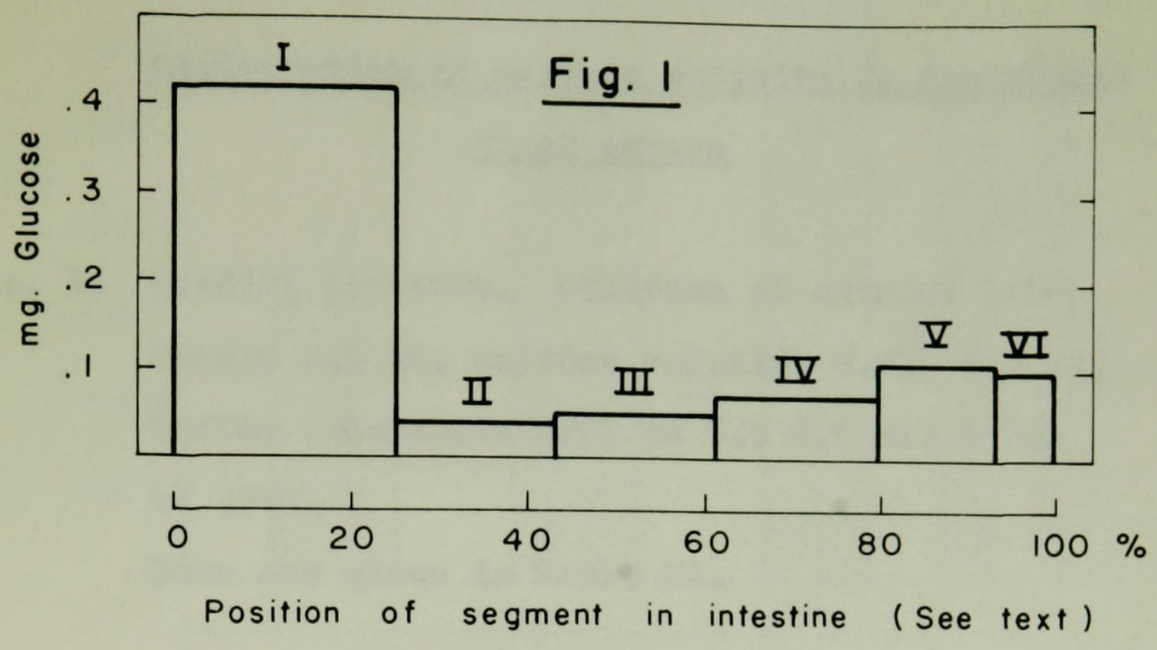
Fig. 1
mg. Glucose
.4
.3
.2
.1
I
II
III
IV
V
VI
0
20
40
60
80
100 %
Position of segment in intestine (See text)

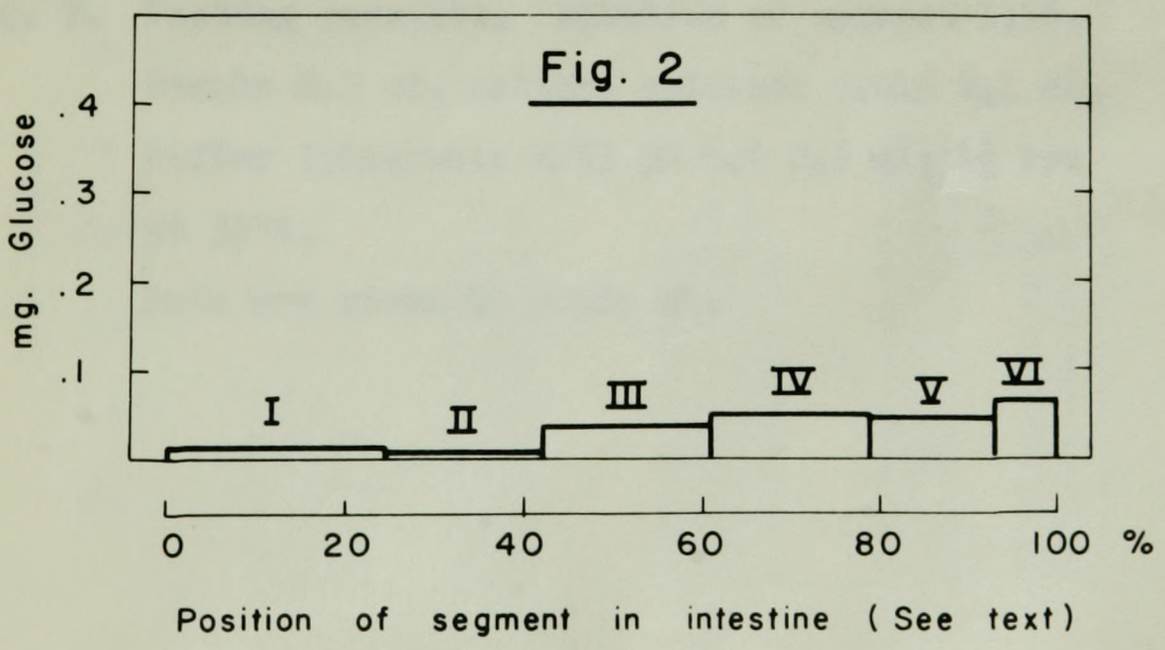
Fig. 2
mg. Glucose
.4
.3
.2
.1
I
II
III
IV
V
VI
0
20
40
60
80
100 %
Position of segment in intestine (See text)

PLATE VI

Distribution of maltase activity in the intestinal mucosa

Fig. 1. Feeding parasite. Dilution of extract 1:16. Sample 0.3 ml, maltose solution 0.01% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 4 hrs at 37°C.
Data are given in Table XA.

Fig. 2. Fasting parasite. Dilution of extract 1:16. Sample 0.3 ml, maltose solution 0.01% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 1½ hrs at 37°C.
Data are given in Table XB.

Plate VI

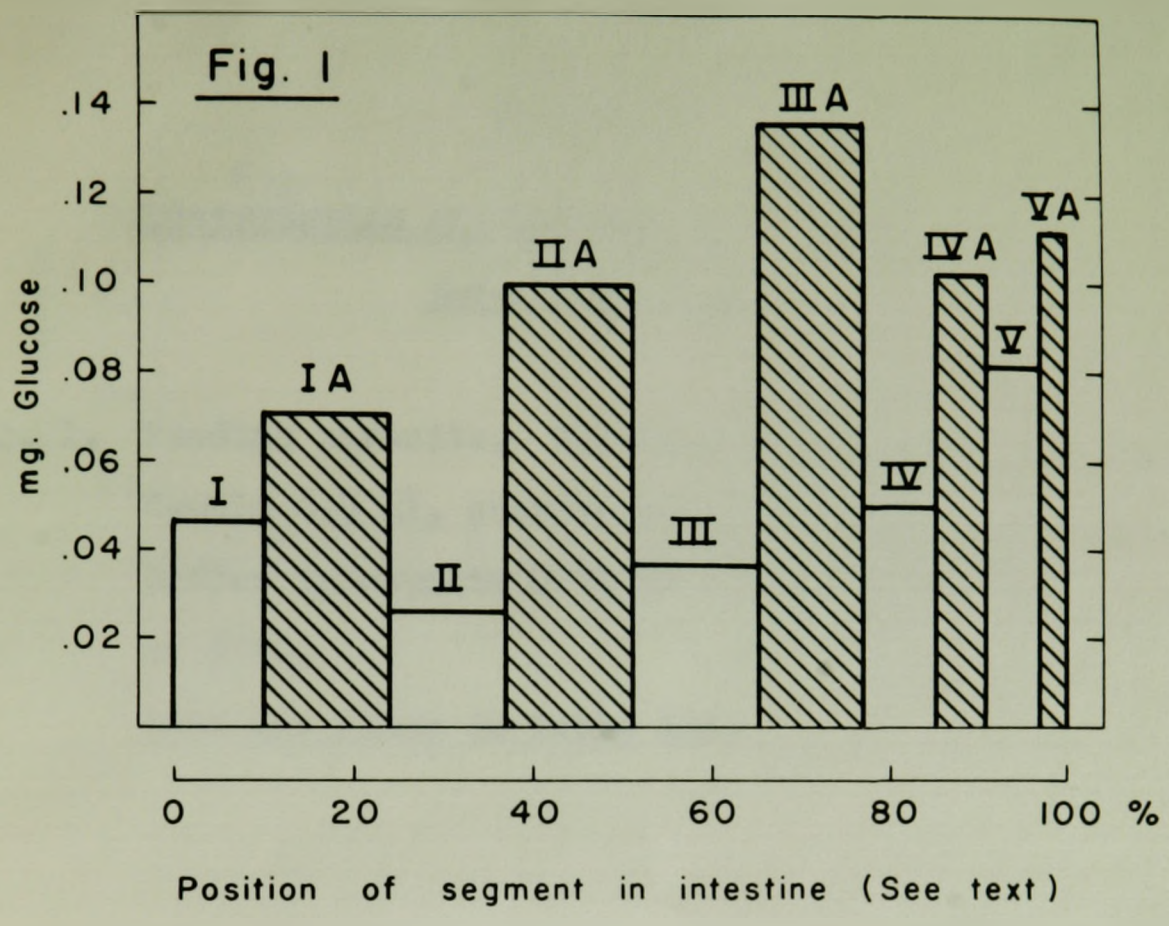
Fig. 1
mg. Glucose
.14
.12
.10
.08
.06
.04
.02
I
IA
II
IIA
III
IIIA
IV
IVA
V
VA
0
20
40
60
80
100 %
Position of segment in intestine (See text)

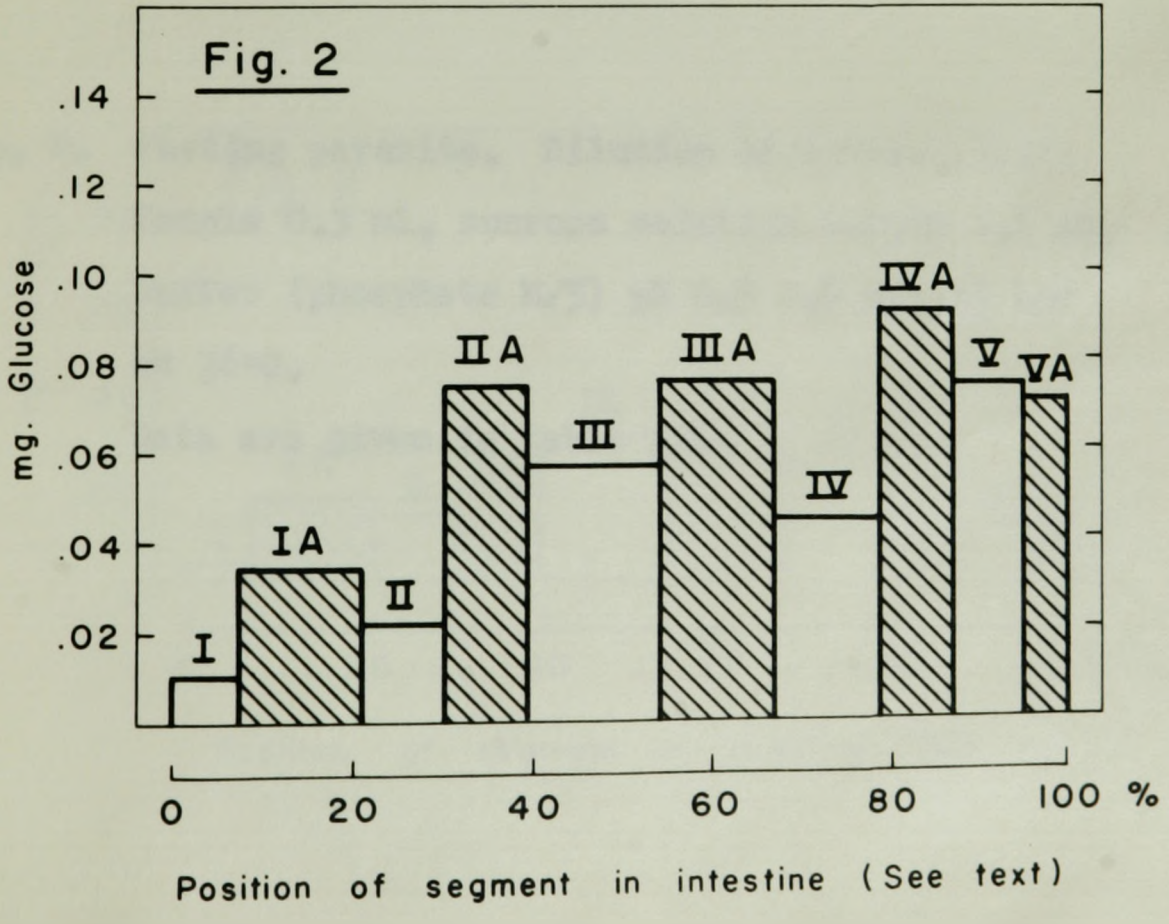
Fig. 2
mg. Glucose
.14
.12
.10
.08
.06
.04
.02
I
IA
II
IIA
III
IIIA
IV
IVA
V
VA
0
20
40
60
80
100 %
Position of segment in intestine (See text)

PLATE VII

Distribution of invertase activity in the intestinal mucosa

Fig. 1. Feeding parasite. Dilution of extract 1:16. Sample 0.3 ml, sucrose solution 0.001% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 5 hrs at 36°C.

Data are given in Table XIA.

Fig. 2. Fasting parasite. Dilution of extract 1:16. Sample 0.3 ml, sucrose solution 0.001% 2.1 ml, buffer (phosphate M/5) pH 6.9 0.6 ml; 1½ hrs at 36°C.

Data are given in Table XIB.

Plate VII

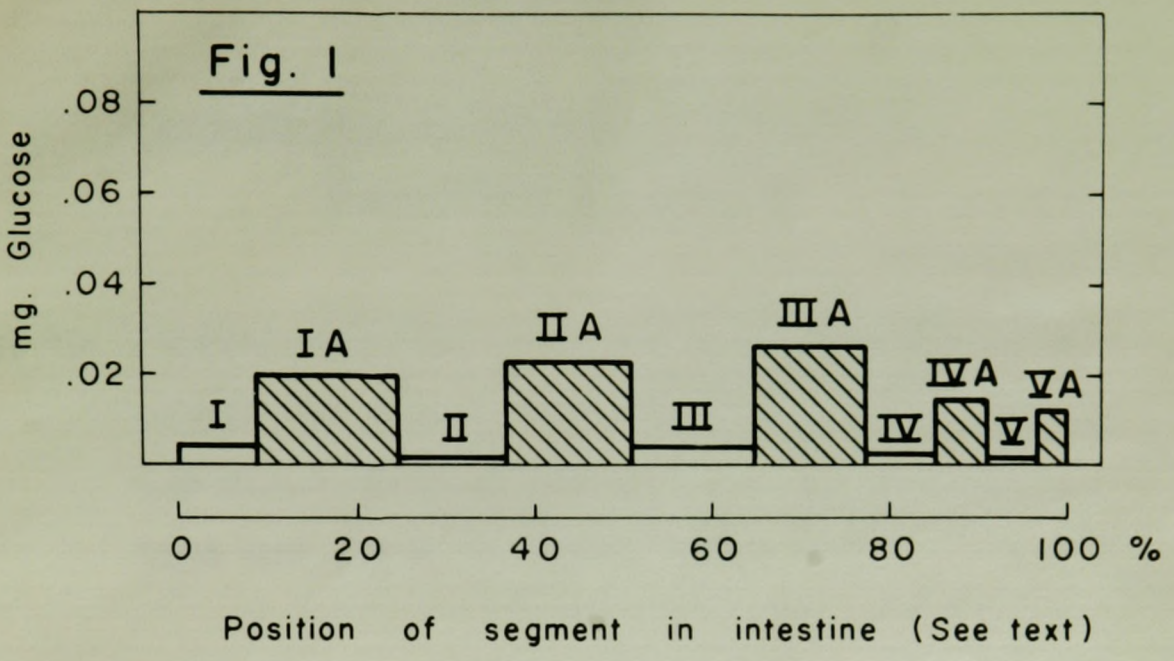
Fig. 1
mg. Glucose
.08
.06
.04
.02
I
IA
II
IIA
III
IIIA
IV
IVA
V
VA
0
20
40
60
80
100
%
Position of segment in intestine (See text)

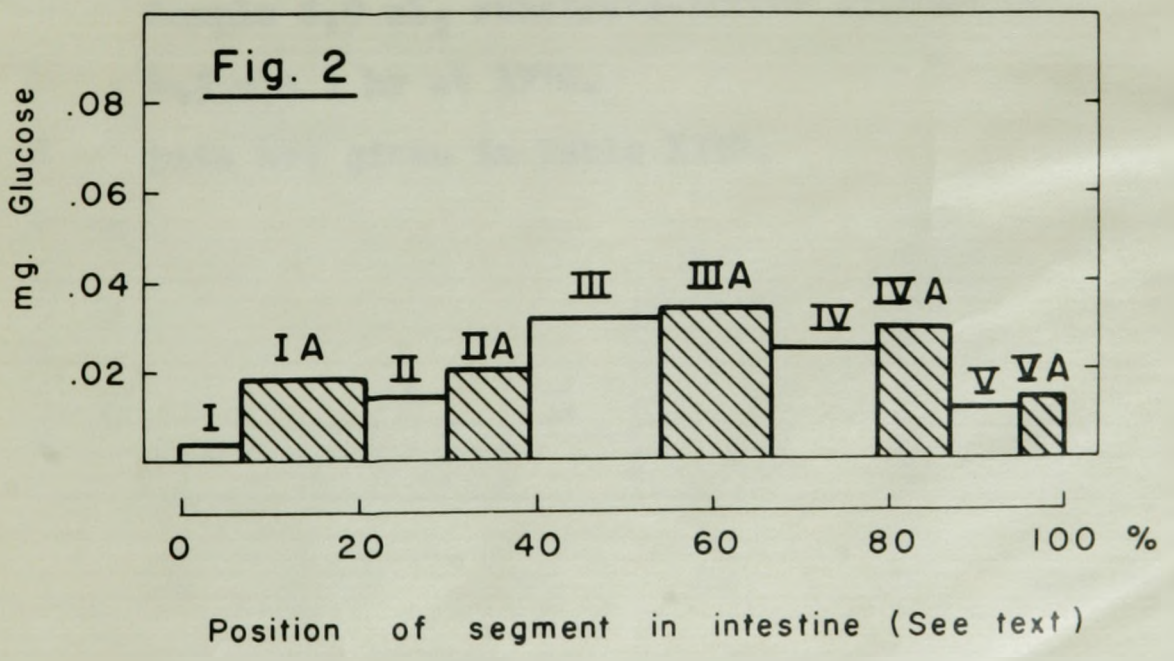
Fig. 2
mg. Glucose
.08
.06
.04
.02
I
IA
II
IIA
III
IIIA
IV
IVA
V
VA
0
20
40
60
80
100
%
Position of segment in intestine (See text)

PLATE VIII

Distribution of lipolytic activity in the intestinal mucosa

Fig. 1. Feeding parasite. Dilution of extract 1:40. Sample 0.9 ml, substrate-buffer mixture pH 7 4.5 ml; 1 hr at 37°C.
Data are given in Table XIIA.

Fig. 2. Fasting parasite. Dilution of extract 1:16. Sample 0.9 ml, substrate-buffer mixture pH 7 4.5 ml; 1 hr at 37°C.
Data are given in Table XIIB.

Plate VIII

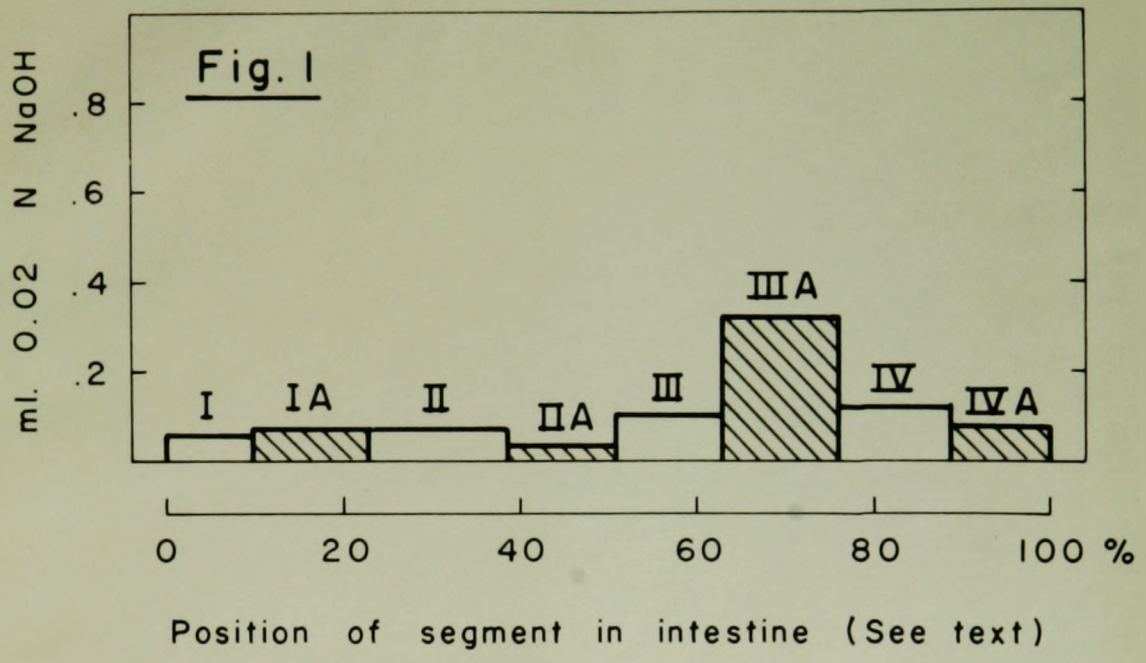
Fig. 1
ml. 0.02 N NaOH
.8
.6
.4
.2
I
IA
II
IIA
III
IIIA
IV
IVA
0
20
40
60
80
100 %
Position of segment in intestine (See text)

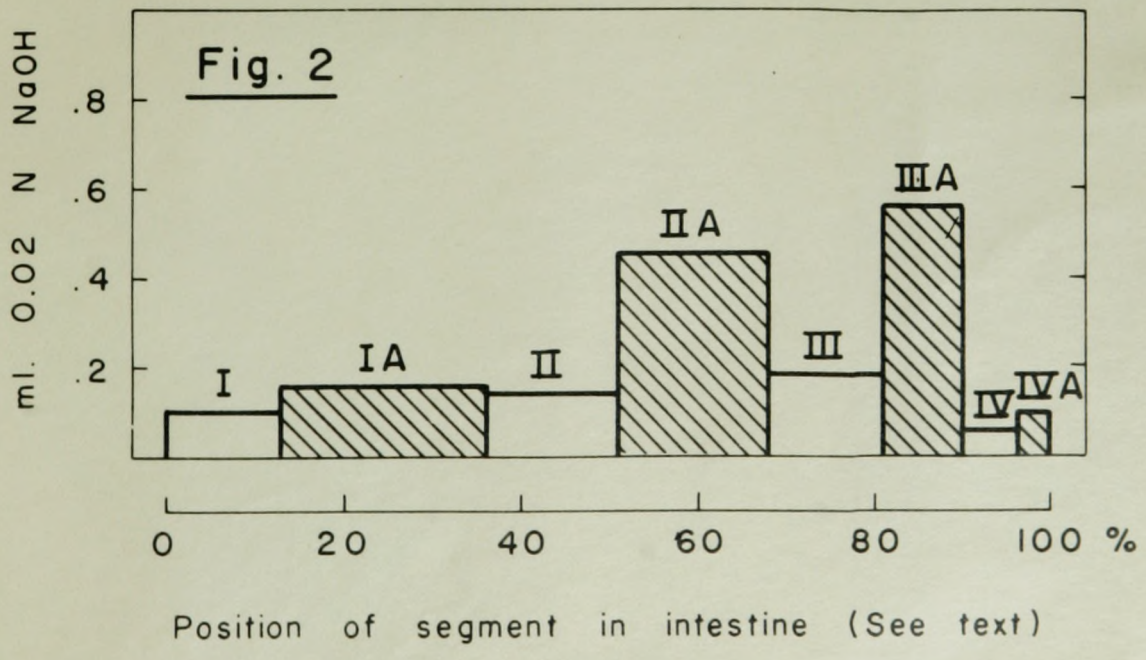
Fig. 2
ml. 0.02 N NaOH
.8
.6
.4
.2
I
IA
II
IIA
III
IIIA
IV
IVA
0
20
40
60
80
100 %
Position of segment in intestine (See text)