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LOCALIZATION OF ION-TRANSPORT IN THE INTESTINE OF THE MIGRATING RIVER LAMPREY, *LAMPETRA FLUVIATILIS* L.

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INTRODUCTION

The intestine of the river lamprey, like that of teleosts, plays a major role in marine osmoregulation (Morris, 1958; Pickering & Morris, 1970). Monovalent ions from the ingested hyperosmotic environment can be transported across the intestine against a concentration gradient ultimately producing a net water flux from the gut lumen to the bloodstream. This water movement compensates for renal and extra-renal water losses. The swallowing and absorption mechanism is lost as the animal migrates into freshwater to spawn, a phenomenon that is dependent upon the maturation of the gonads (Pickering & Dockray, 1972).

Despite the similarities between marine osmoregulation in the lampreys and in the teleosts it has been argued that the mechanisms evolved independently in the two groups (Morris, 1958; Pickering & Morris, 1970). The lampreys, together with the hagfishes, are the only living representatives of the most primitive group of vertebrates, the Agnatha, and therefore their physiology is of phylogenetic importance.

The lamprey intestine consists of a straight tube between the oesophagus and anus, yet despite its apparent simplicity we know little of the regional localization of many of its functions. In the present investigation isolated intestinal preparations from migrating river lampreys were used to localize the regions of the intestine in which ion transport and water transport occur. A study of the intestinal mucosa at both light-microscope and electron-microscope levels has enabled a precise identification of the cell type involved.

MATERIALS AND METHODS

Animals

Migrating river lampreys, *Lampetra fluviatilis*, were taken from the estuary of the River Bela, Westmorland, and from the River Severn, Gloucestershire. In previous investigations it has been demonstrated that animals from these sources have the ability to swallow water and transport monovalent ions across the intestine when they are placed in dilute sea water (Pickering & Morris, 1970; Pickering & Dockray, 1972). The animals, of both sexes, were kept in circulating fresh water prior to the start of the experiment for a period not exceeding 5 days.

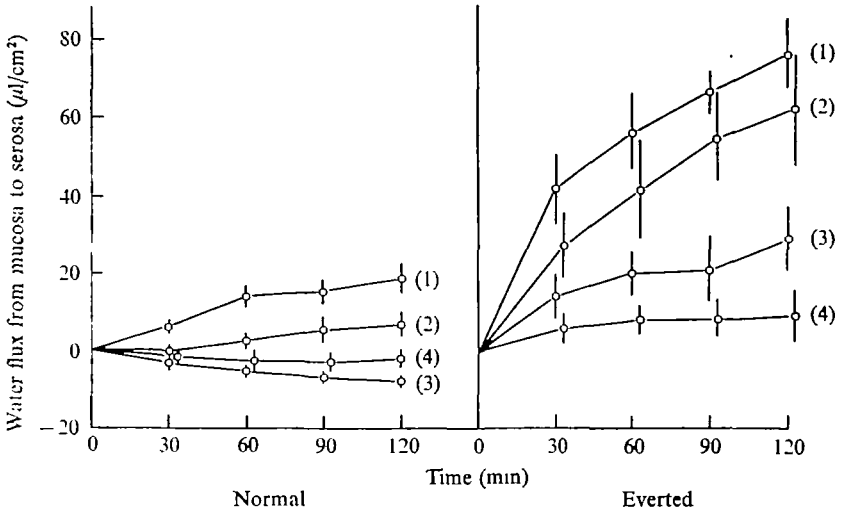
Measurement of ionic fluxes across the intestine

Lampreys were placed in 50% sea water for a period of 24 h before measurements were made of the ability of the intestine to transport salt and water. At the end of this period the animal was anaesthetized (MS 222 at 0·1 g/l) and the entire intestine was removed and flushed thoroughly on both surfaces with a Ringer solution based on the composition of lamprey serum (101 mM/l NaCl; 3·3 mM/l KCl, 2·5 mM/l $CaCl_2$, 2·5 mM/l $NaHCO_3$, 1·2 mM/l KH_2PO_4, 1·3 mM/l $MgSO_4$). The intestine was then divided into four segments of equal length and numbered from anterior to posterior. After further rinsing with Ringer solution one end of each intestinal segment was ligatured with nylon thread and a small polyethylene cannula was tied into the open end. Isotopically labelled Ringer solution (dyed with phenol red to detect leaks) was injected into the intestine via the cannula and the preparation was bathed in 10 ml of aerated Ringer solution. 0·5 ml samples of the bathing solution were taken for liquid scintillation counting at 2, 4, 6, 8 and 10 min in experiments using ^{22}Na and ^{36}Cl, and at 15, 30, 45 and 60 min in experiments using ^{35}S and ^{45}Ca. The ionic fluxes across the intestine were calculated from the slope of a plot of total radioactivity in the bathing fluid against time and the specific activity of the labelled Ringer solution on the inside of the preparation. In experiments with monovalent ions the graph was invariably in the form of a curve and therefore the tangent to the curve at 0 min was used to calculate the unidirectional flux rates. In the case of the slower-moving divalent ions there was a linear relationship between the radioactivity of the bathing fluid and time. At the end of each experiment the intestine was slit open and the serosal surface area of the preparation was measured.

These techniques were used for both normal and everted preparations in an attempt to demonstrate the regional variation of the unidirectional fluxes down the length of the intestine. The conditions under which ion movement is measured in the everted preparation are likely to be different from those in the normal preparation. The everted preparation has more of the complex folding of the mucosa exposed and is less likely to be limited by oxygen availability at the mucosa. Thus this method is more suitable for a study of the changes in magnitude of the unidirectional ion fluxes down the length of the intestine than the accurate measurement of net fluxes by difference.

Inhibited preparations were obtained by pre-treating them for 15 min with 10^{-4} M dinitrophenol (DNP) in Ringer solution on both mucosal and serosal surfaces before the ionic flux measurements. Ouabain was also used at the same concentration in a similar manner, although in this case the bathing medium during the flux measurements also contained 10^{-4} M ouabain.

Net water fluxes were measured on normal and everted preparations by weight changes. The preparation was weighed to the nearest milligram on a torsion balance at intervals of 30 min for a period of 2 h. All experiments were performed at a temperature of 12 °C.



Text-fig. 1. The net water fluxes across the four regions of isolated lamprey intestine (mean ± S.E.). Figures in parentheses indicate the region of the intestine: (1) is anterior.

Microscopical techniques

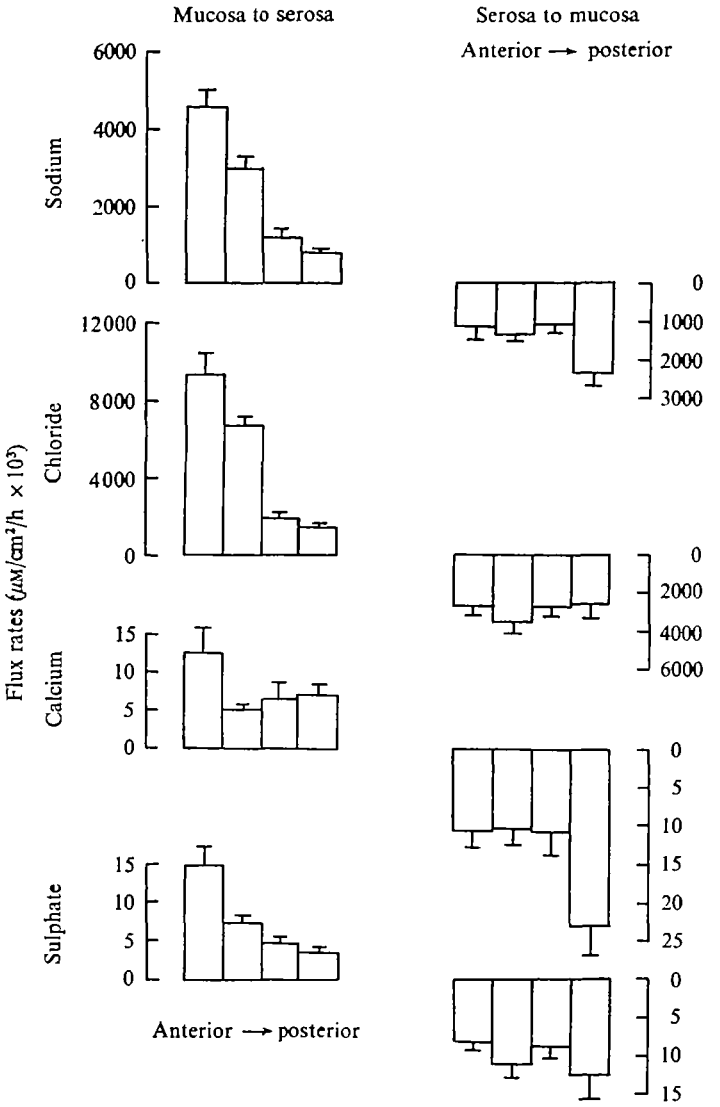
Mucosal tissue from all four regions of the intestine was taken from fresh-run, migrating river lampreys and fixed in Bouin's or Hellys' fluid. After embedding in 52 °C paraffin wax and sectioning at 6 μm the tissue was stained by the following techniques: Ehrlich's acid haematoxylin and eosin; Harris's haematoxylin and alcian blue; periodic acid–Schiff reaction (PAS); Cain's method for mitochondria (Cain, 1948); Ewen's aldehyde-fuchsin (Ewen, 1962) and sudan black with carmalum.

Anterior intestinal tissue from fresh-run, migrating river lampreys was also fixed in phosphate-buffered glutaraldehyde (Pease, 1964), post-fixed with phosphate-buffered osmium tetroxide, embedded in Araldite and sectioned with glass knives on a Reichart Om U2 ultramicrotome. The sections were mounted on naked copper grids, stained with 5 % aqueous uranyl acetate followed by lead citrate (Reynolds, 1963) and studied under an A.E.I. EM 6B electron microscope.

RESULTS

Net water fluxes across the isolated intestine

The fluxes of water across normal and everted preparations (measured by weight change) are illustrated in Text-fig. 1. This series of experiments illustrates one of the limitations of isolated intestinal techniques, since it is clear that eversion of the intestine enhances the movement of water when compared with the non-everted preparation. However, the results from both types of preparation show that the most important region for water transport is the anterior half of the intestine (regions 1 and 2). Net water fluxes from mucosa to serosa in the posterior region of the intestine are small or absent. Since there was no net osmotic gradient or diffusion gradient between the solutions on the mucosal and serosal surfaces at the start of the experiment, it is probable that these water fluxes depend ultimately upon the active transport



Text-fig. 2. Unidirectional ion fluxes down the length of the isolated lamprey intestine (Mean+S.E.).

of ions. Thus it can be concluded that the active transport of ions is likely to occur mainly in the anterior region of the intestine and that further posteriorly this mechanism gradually disappears.

Ion fluxes across the isolated intestine

Uninhibited preparations

The mean unidirectional fluxes of the ions sodium, chloride, calcium and sulphate are illustrated in Text-fig. 2. The relative magnitudes of the fluxes calls for some comment because of differences in the scale of Text-fig. 2. Chloride moved at approximately

Table 1. *The effect of inhibitors on the fluxes of sodium and calcium across the isolated lamprey intestine*

(Flux rates in μM/cm^2/h $\times 10^2$; mean $\pm$ S.E. (n).)

	Uninhibited	10^{-4} M DNP	10^{-4} M ouabain
Na			
Region 1 m→s	4493 ± 428 (11)	1028 ± 123 (6)	1782 ± 333 (4)
Region 2 s→m	1353 ± 170 (10)	2971 ± 300 (6)	2962 ± 247 (4)
Ca			
Region 3 m→s	6·8 ± 1·9 (9)	10·3 ± 1·8 (6)	—
Region 4 s→m	22·9 ± 3·8 (6)	28·6 ± 4·1 (6)	—

twice the rate of sodium, whereas the divalent ions moved slowly, approximately 500–1000 times slower than chloride. With all four ion species the mucosa-to-serosa flux was greatest in the most anterior region and progressively decreased in magnitude towards the posterior region. The distribution of ion fluxes was therefore similar to the distribution of water fluxes across the intestine and supports the conclusion that the majority of ion-transport occurs in the anterior intestine.

There was little variation between the fluxes from serosa to mucosa for all the ions studied in the first three regions of the intestine although the results for region 4 (the most posterior region) suggest an increased flux rate.

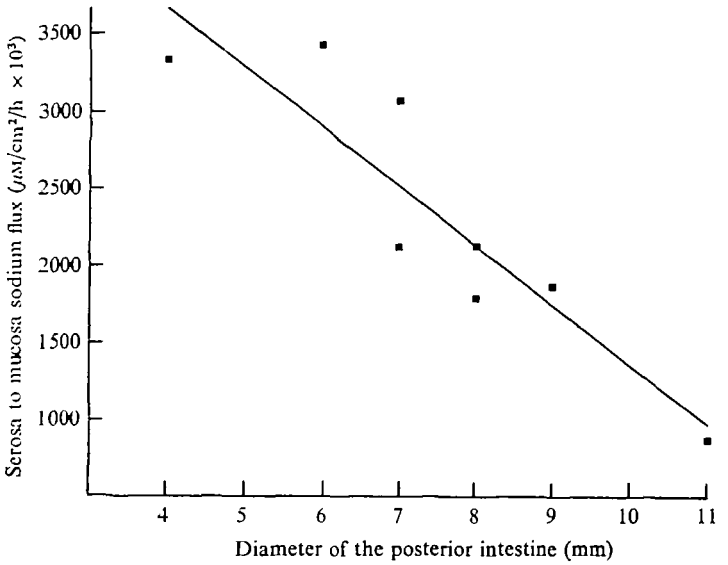
Inhibited preparations

The effects of 10^{-4} M DNP and 10^{-4} M ouabain on the fluxes of sodium in the anterior regions and the fluxes of calcium in the posterior regions were studied. Table 1 presents the data obtained from these experiments as well as the corresponding fluxes from uninhibited preparations.

Dinitrophenol acts by uncoupling oxidative phosphorylation in the tissue, thereby reducing the energy supplies to the ion-transporting cells. Ion fluxes which are ultimately dependent upon active transport should therefore be inhibited. From Table 1 it is clear that dinitrophenol significantly reduced the mucosa-to-serosa flux of sodium in the anterior intestine (region 1) by a factor of 4 ($P < 0{\cdot}001$). Furthermore, the serosa-to-mucosa flux in the anterior intestine (region 2) was significantly increased by a factor of 2 ($P < 0{\cdot}001$). We interpret this as indicating that in the uninhibited preparation approximately 50% of the back flux (from serosa to mucosa) is re-transported by the active mucosa-to-serosa flux before it has chance to appear in the mucosal fluid. In the posterior intestine neither unidirectional flux of calcium is affected by dinitrophenol and therefore the divalent-ion fluxes in this region are presumably non-active. That dinitrophenol acts on the energy supply of the cells rather than by changing the permeability of the intestine is supported by this lack of effect on the posterior calcium fluxes.

Ouabain had quantitative effects on the unidirectional fluxes of sodium in the anterior intestine similar to those of dinitrophenol. This cardiac glycoside appears to act primarily by inhibiting Na- and K-activated ATPase, an enzyme complex which can be specifically involved in the active transport of sodium (see Skou, 1965).

Thus from these experiments it is concluded that the large monovalent ion flux

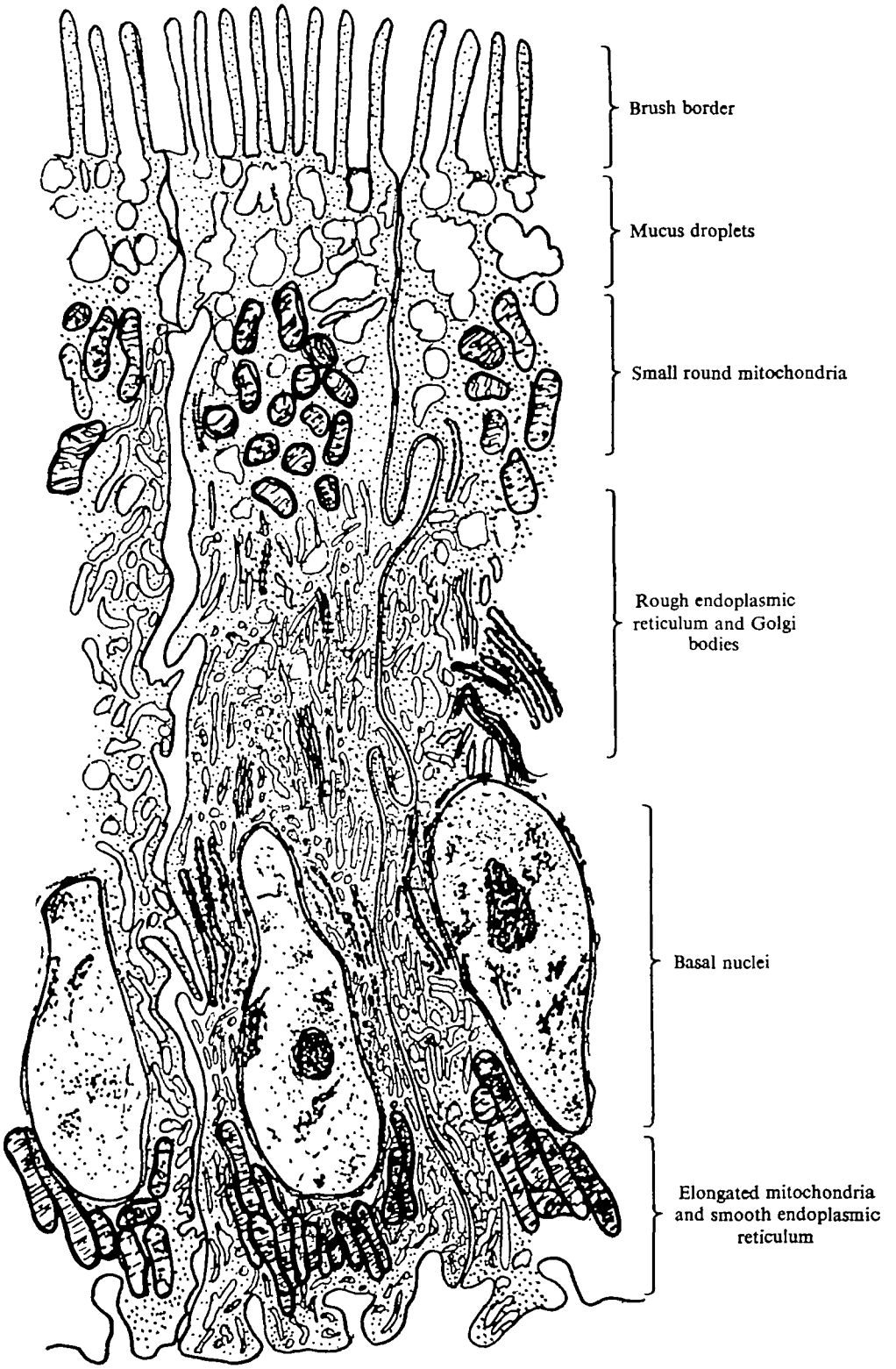


Text-fig. 3. The inverse relationship between the diameter and the serosa-to-mucosa flux of sodium in the posterior intestine (region 4). Regression, $y = -385{\cdot}7 + 5220$. Correlation coefficient, 0·90.

from mucosa to serosa in the anterior intestine is dependent upon active transport and is responsible for the large net water flux in this region. It seems likely that the poorly absorbed divalent ions are carried along with the fluxes of monovalent ion and water to some extent, thus explaining the similarities in the variation of the mucosa-to-serosa fluxes of calcium and sulphate down the length of the intestine with those of sodium and chloride. There are, however, very large differences in the magnitudes of the fluxes monovalent ions and divalent ions.

In the case of the fluxes from serosa to mucosa it is concluded that these are not active. The relatively large fluxes of both monovalent ions and divalent ions in the posterior region may be explained by the following hypothesis. It has been shown that in the regions where active transport is occurring the serosa-to-mucosa flux appears small, an artifact resulting from back transport. Since it appears that the posterior intestine does not have a large, active, mucosa-to-serosa flux of ions, back transport will be reduced and consequently the isotopically measured flux from serosa to mucosa will be large.

The absence of a large active flux of ions in the posterior intestine presents an opportunity for studying changes in permeability of the lamprey intestine during the anadromous migration. During this migration the intestine atrophies, a process which involves a decrease in the diameter of the alimentary tract (Larsen, 1969), and a reduction of the epithelial cell height and the thickness of the muscular layers. The diameter of the posterior intestine (region 4) was taken as an indicator of the degree of degeneration and plotted against the serosa-to-mucosa flux of sodium (Text-fig. 3). There is an inverse relationship between the diameter of the intestine and the magnitude of this flux, suggesting that the permeability of the intestine increases during its degeneration. This factor may well be important in the breakdown of the osmoregulatory role of the intestine in seawater-adapted animals.



Text-fig. 4. A diagrammatic representation of the ultrastructure of the columnar cell of the anterior intestine.

Light microscopy

The above results show that active ion transport is located in the anterior intestine of the migrating river lamprey. At least three cell types may be found in the mucosa of this region. *Zymogen cells* (Barrington, 1945) are characterized by a marked granulation and affinity for haematoxylin and aldehyde-fuchsin. Experimental evidence for the enzymic function of these cells has been provided by Barrington (1936). *Ciliated cells* are easily recognized by their long cilia at the apex of the cell and the apical nucleus (Plate 1). The remaining cell type will be termed the *columnar cell.* This cell has a median or basal nucleus, prominent nucleolus and very variable quantities of alcian-blue-positive and PAS-positive material in the apex of the cell. The free border has a striated appearance, and under conditions of phase contrast two discrete groups of inclusions may be seen, one in the apical cytoplasm and the other basal to the nucleus (Plate 1). The acid-fuchsin method of Cain (1948) showed these inclusions to be mitochondria. These columnar cells occasionally contain large vacuoles and the cytoplasm around and basal to the nucleus has an affinity for eosin, a staining response not unlike that of the 'chloride cells' of teleost gills.

Since the zymogen and ciliated cells have obvious, specific functions it is suggested that the columnar cell of the anterior intestine is responsible for active transport of monovalent ions. This cell type was therefore studied at the ultrastructural level to provide supporting evidence for its function.

Electron microscopy

A diagrammatic representation of the ultrastructure of the columnar cell illustrates many of the characteristic features (Text-fig. 4). The apical cell membrane is folded into a regular brush border which is no doubt identical with the striated border seen at the light-microscope level. Beneath this brush border is an area of apparent mucous droplet release (Plate 2), thus supporting the observations with alcian-blue staining. In the supranuclear area are small, rounded mitochondria and granular endoplasmic reticulum. Golgi bodies may also be found in this area (Plate 3). Perhaps the most characteristic feature of this cell type is the tubular, branching, smooth-membraned endoplasmic reticulum which is particularly well developed in the basal area of the cell (Plate 4). Large, elongated mitochondria occur in this subnuclear region and are orientated on the long axis of the cell. The columnar cell rests on a convoluted basement membrane and the lateral cell membranes are often folded in a complex manner. Occasional large intercellular spaces may be found, but at the tips of the cells there are tight junctions (Plate 2).

DISCUSSION

In investigations of this type it is important to recognize the limitations of isolated intestinal preparations. Essentially, the blood supply of the normal animal has been removed, so that diffusion must play an important role in carrying transported solutes away from the transporting sites. Thus quantitative data from these preparations may be different from data obtained from intact animals and from data obtained by using different *in vitro* techniques. This is exemplified in the present investigation where

version of the preparation caused a marked increase in the rate of transport of ions and water when compared with the non-everted preparation. This effect is probably due to an increase in the exposure of the mucosal folds coupled with an increase in the available oxygen at the mucosa. The Ringer solution used in the present experiments did not contain glucose or any other nutrient; any active transport must therefore have obtained its energy from sources within the intestinal tissue.

Despite these limitations it is interesting to note that the value of the mucosa-to-serosa sodium flux of the isolated anterior intestine of the river lamprey (4·49 μM/cm^2/h) is similar to that measured across the isolated anterior intestine of the winter flounder *Pseudopleuronectes americanus* (5·25 μM/cm^2/h) (Huang & Chen, 1971). Calculations based on the net water fluxes across the everted isolated intestine indicate that the rate of water movement is approximately half the value measured in intact animals (Morris, 1958; Pickering & Morris, 1970; Pickering & Dockray, 1972) because of the difficulties mentioned previously.

The major fluxes of ions and water occur in the anterior intestine of the migrating river lamprey and decrease progressively down the length of the intestine. Since these fluxes are expressed in terms of the serosal surface area of the intestine it might be possible to explain the decrease in magnitude of the mucosa-to-serosa flux of ions down the intestine as a result of a decrease in the complexity of mucosal folding and consequently a decrease in surface area. This seems unlikely, because the mucosal folding appears to be more complex towards the posterior end of the alimentary canal. Thus the decrease in ion transport down the length of the intesine would be even more obvious if it could be expressed in terms of mucosal surface area.

Our data show that sodium and chloride are the major ions transported, with relatively small amounts of the divalent ions calcium and sulphate carried across with the monovalent ions. There is no evidence for a significant ion flux in either direction in the posterior intestine. From the present investigation it is not possible to state categorically which of the monovalent ions is actively transported, since we have no data concerning the electrochemical potential across the lamprey intestine. It does seem likely that sodium is actively transported since this is inhibited by ouabain, a cardiac glycoside that is known to inhibit active sodium transport (Skou, 1965; Conte, 1969). However, it must be remembered that Rolleston & Newsholme (1966), working on guinea-pig cerebral cortex, found that ouabain (10^{-4} M) could inhibit glycolysis in a manner which was not directly dependent upon the inhibition of Na^+- and K^+-activated ATP ase. Simple electrostatic attraction of chloride by the transported sodium ions cannot completely explain the large chloride fluxes we obtained and therefore it may well be that chloride is transported separately in the mucosa of the anterior intestine, although experimental verification is needed. Chloride fluxes which are higher than sodium fluxes may be found in the mammalian intestine (Parsons, 1967) and in teleosts (Huang & Chen, 1971), where they are possibly the result of a sodium-independent Cl^-–HCO_3^- exchange mechanism.

A histological survey of the intestine of the migrating river lamprey prompted the suggestion that the columnar cells of the anterior intestine are responsible for active transport of ions and consequently for water transport. Electron-microscope studies support this conclusion and also suggest that this cell type has the additional function of mucus production.

The brush border of the apical cell membrane suggests an absorptive function since this greatly increases the surface area of the cell exposed to the fluid in the lumen of the gut. The basal area of the cell is characterized by large numbers of elongated mitochondria associated with the abundant, tubular, smooth-membraned endoplasmic reticulum. This association is characteristic of ion-transporting cells in teleost gills (Philpott, 1962), crustacean gills (Copeland, 1967) and insect anal papillae (Copeland, 1964). This is therefore the strongest ultrastructural evidence for an ion-transporting role for the columnar cells of the anterior intestine of the migrating river lamprey.

The extensive system of intercellular spaces found in the mammalian gall bladder (Diamond & Tormey, 1966) are thought to be important components of the pathway for water movement. It is currently believed that water transport in the vertebrates occurs because of local osmotic gradients within these extracellular spaces. Active ion transport is needed to maintain these gradients in a dynamic equilibrium (see Maetz, 1968). Intercellular spaces were present between the columnar cells of the anterior intestine in the present investigation. However, these spaces were not abundant and this could be a consequence of the osmoregulatory condition of the intestine. The tissue for electron microscopy was obtained from freshly caught river lampreys taken from brackish water, and under these conditions the intestine may not have been transporting water at its maximum capacity. Consequently many of the intercellular spaces were reduced.

Alcian-blue-positive granules in the tips of the columnar cells gave the appearance of mucous droplets under the electron microscope. It is suggested that these droplets represent mucus in the process of synthesis and release since abundant rough endoplasmic reticulum, Golgi bodies and mitochondria occur in the area of cytoplasm beneath the mucous droplets. Thus the columnar cell would seem to have a dual function, at least during part of its life-history. It is interesting to note that the distinction between 'chloride-cells' and mucous cells in the teleost gill has caused considerable controversy in the past (Bevelander, 1935).

We do not know whether ion transport in the lamprey intestine is hormonally controlled in a similar way to that of marine teleosts. Circumstantial evidence suggests that different controlling mechanisms may occur in the two groups. In the teleosts evidence is accumulating that corticosteroids stimulate transport across the intestine (Hirano & Utida, 1968; Epstein, Cynamon & McKay, 1971) but in the cyclostomes a recent investigation has failed to detect significant levels of these steroids in the migrating sea lamprey *Petromyzon marinus* (Weisbart & Idler, 1970). Clearly, further work is needed on steroidogenesis in the lamprey before this problem can be resolved.

SUMMARY

1. Isolated intestinal preparations from migrating *Lampetra fluviatilis* re-adapted to 50% sea water were used to localize ion transport.

2. A large, active, monovalent-ion flux was found in the anterior intestine and its magnitude decreased towards the posterior end of the intestine.

3. The monovalent-ion flux is responsible for a net water flux from mucosa to serosa in the anterior intestine.

4. It is tentatively suggested that both sodium and chloride are actively transported by the mucosa. The divalent ions may be carried along with the monovalent ions to a limited extent.

5. Studies at the light- and electron-microscope levels indicate that columnar cells of the anterior intestine are responsible for ion transport, and there is evidence that the same cells produce mucus.

We are indebted to Mr L. Stewart, M.Sc., M.I.Biol. (Lancashire River Authority), for permission to collect *L. fluviatilis* and to Professor E. J. W. Barrington, F.R.S., for the provision of facilities.

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EXPLANATION OF PLATES

PLATE 1

Mucosa of anterior intestine of a fresh-run, migrating river lamprey. (Helly fixed. Alcian blue and Harris's haematoxylin. Phase-contrast.) Note the ciliated cells (*cil*) with apical nuclei and the columnar cells (*col*) with median or basal nuclei. Alcian-blue-positive material (AB +ve) may be found in the apex of the columnar cells beneath the striated border (*str*).

PLATE 2

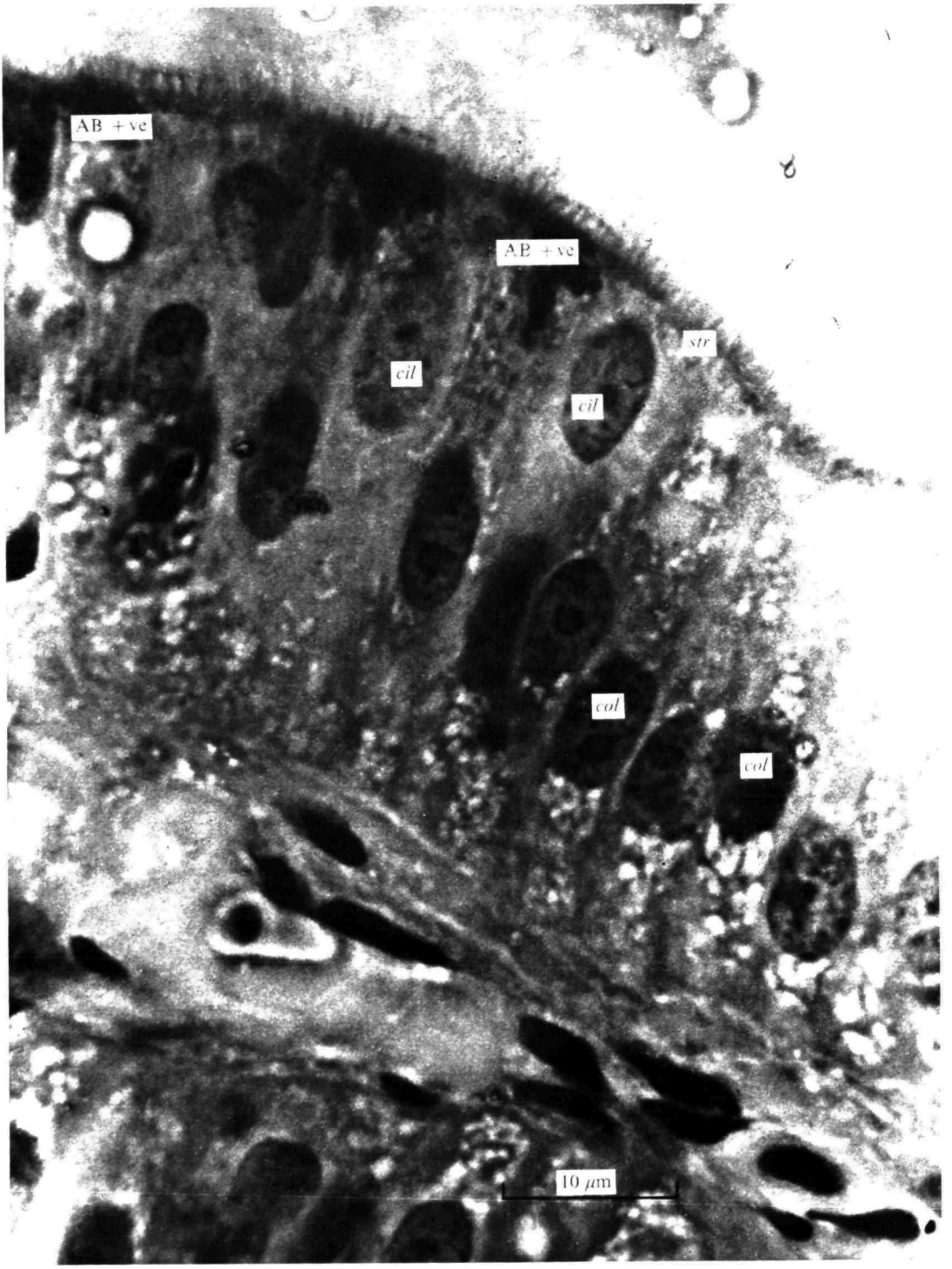
The apical cytoplasm of two columnar cells from the anterior intestine. Beneath the brush border (*bb*) is an area composed of mucus droplets (*md*). Small, rounded mitochondria (*srm*) are numerous, and tight junctions (*tj*) can be clearly seen between the apices of the cells.

PLATE 3

The supranuclear cytoplasmic area of two columnar cells from the anterior intestine. On the left is the apical nucleus of a ciliated cell (*cil*). Rough endoplasmic reticulum (*rer*) and Golgi bodies (*g*) are found in the columnar cells. The convoluted cell membranes (*ccm*) can also be seen between the cells.

PLATE 4

The basal area of three columnar cells of the anterior intestine between the nuclei (*n*) and the convoluted basement membrane (*bm*). The most characteristic features of this region are the elongated mitochondria (*em*) and smooth, tubular endoplasmic reticulum (*ser*).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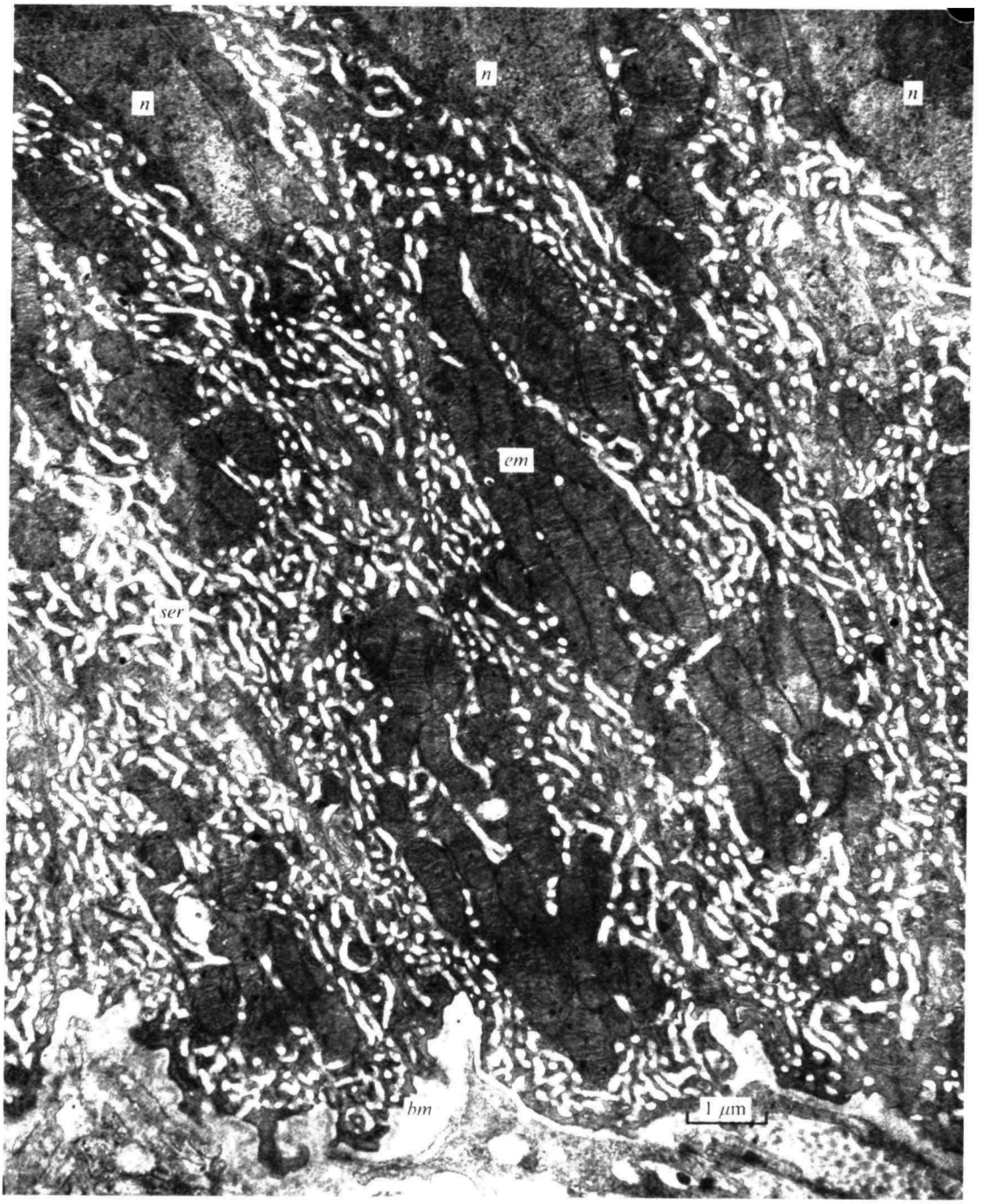

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