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THE *IN VITRO* ABSORPTION OF WATER AND SOLUTES FROM THE INTESTINE OF GOLDFISH, *CARASSIUS AURATUS*

BY M. W. SMITH

From The A.R.C. Institute of Animal Physiology, Babraham, Cambridge

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It has been shown *in vivo* that the intestine of the goldfish can absorb water (Smith, 1930) but at a rate which is too low to account for its output of urine (60–150 ml./kg/day). Goldfish, like other fresh-water fish, ingest only small amounts of water, and this infrequently, so that the bulk of its water intake must be through the oral membranes or the gills. The goldfish gills are able to extract chloride and sodium from very dilute solutions of these ions and transfer them to the blood (Krogh, 1937; Maetz, 1956), so that the intestine may play only a minor role in the absorption of water and ions. But preliminary observations have shown that the everted intestinal bulb of the goldfish maintains an initial transmural potential difference of 7 mV and is able to concentrate glucose at its serosal surface (Smith, 1964). These findings suggest the presence of active transport mechanisms. This work was started to establish the capacity of the goldfish intestine to transfer water and solutes and then to determine whether the adenosinetriphosphatase known to be present in the mucosal cell membranes (Hollands & Smith, 1964) was implicated in any of these processes.

METHODS

Goldfish 7–10 cm in length were killed by decapitation and the intestine exposed by removal of the abdominal wall. The intestine is convoluted and partly obscured by a bilobular liver which extends down the whole length of the body cavity. The intestinal loops were separated carefully with the aid of a dissecting microscope and the tract when straight was found to be 2–3 times the length of the whole fish. The tract appeared as an undifferentiated tube which widened at the oral end to form the intestinal bulb. The piece of intestine used for experiments was taken immediately below this bulb to within 2 cm of the end of the tract and consisted of the anterior and posterior intestine and possibly part of the rectum. These areas merge with one another and are defined by the pattern of the mucosa seen through the thin muscle layers (McVay & Kaan, 1940). The intestine to be used was washed several times with medium, lightly blotted on filter paper, weighed and transferred to Warburg vessels. To make an intestinal sac both ends of the intestine were cannulated, the sac was filled with medium and both ends were then sealed with pins made of glass.

Extra washings from the intestine were shown, by culturing on nutrient aerobic and anaerobic media, to contain a mixed growth of coliform organisms. Washings containing these bacteria did not lower the concentration of glucose during incubation over a 240 min period at 25° C and it was concluded that their presence was of little consequence in experiments of short duration.

The amount of medium added to an intestinal sac is expressed as $W_2 - W_1$ where W_1 is the weight of the empty intestine and W_2 the weight of intestine plus medium. At the end of incubation the amount of medium remaining was measured directly by expelling the contents into a tared vessel. This was preferred to weighing the sac after incubation (W_3) and then representing the amount of medium remaining as $W_3 - W_1$ because the intestine changed weight under certain experimental conditions. All fluxes were calculated per unit length of intestine.

TABLE 1. Comparison of carp serum with a physiological saline medium

	Concentration (mM)						
	Na	K	Ca	Cl	Inorganic P	Glucose	pH
Carp serum	130	6·3	2·9	—	0·94	6·2 5·3–9·2 (2)	7·67 (1)
Medium	155	5·5	3	161	3	11	7·1

(1) Field, Elvehjem & Juday (1943).
(2) Golovatsky, Avdosyev & Nazarkevich (1963).

Incubation medium. A medium similar to that of Robinson (1949) was used. The composition of this medium is compared with that of carp serum (Table 1), a cyprinoid whose blood has been previously investigated by other workers. The pH of the 3 mM-K phosphate was 7·4 but the final pH of the medium was 7·1, even though CO_2-free water was used to prepare the incubation medium. Preliminary experiments (see results section) showed that the goldfish intestine did not deteriorate obviously during a 6 hr incubation in the K-phosphate medium.

Analytical procedures. Glucose estimations were made by the method of Hansen (1962) and with chemicals supplied by Boehringer & Soehne, Mannheim. Sodium and potassium analyses were carried out with a flame photometer (Evans Electro Selenium, EEL) with an accuracy of approximately ±2%.

RESULTS

Incubation of goldfish intestines under control conditions

Goldfish intestines washed free of contents and rinsed in 0·32 M sucrose contained 5·70 ± 0·36 (S.E.) μ-mole/100 mg wet wt. (12)* of sodium and 8·00 ± 0·20 μ-mole/100 mg wet wt. (12) potassium. The water content of the intestine was 3·67 ± 0·20 g water/g dry wt. (10). These values have been taken to represent initial values though it is possible that some changes in ionic composition took place during the 10–20 min needed to dissect and clean the intestine. After incubation for 260 min in the medium at 25° C and in the presence of oxygen, the sodium concentration of the intestine fell to 4·09 ± 0·56 μ-mole/100 mg fresh wt. (12) and that of potassium to 6·03 ± 0·26 μ-mole/100 mg fresh wt. (12). After an incubation

* Figures in parenthesis indicate the number of experiments upon which mean values are based.

period of 380 min under the same conditions the intestine lost 16% (10) of its total nitrogen while the water content increased by 20·7% to 4·33 ± 0·18 g water/g dry wt. (13). This change could be accounted for partly by the loss of tissue solids.

Sections of intestine taken before and after incubation for 380 min under the conditions described above were stained with haematoxylin and eosin, with periodic acid–Schiff reagent and with phosphotungstic acid–haematoxylin. Sections taken after incubation and stained with phosphotungstic acid–haematoxylin showed some distortion of muscle fibres but there were no obvious changes in structure of the lamina propria or mucosa.

The thickness of the intestinal wall varied from 0·1 mm between villi to 0·4 mm where the length of a villus was added to the thickness of the muscular layers. It was assumed that through such thin tissue there would be an adequate diffusion of oxygen to all parts of the intestine during incubation. The oxygen consumption of the goldfish intestine measured at 25° C remained constant for 6 hr with oxygen consumption values of 45 ± 4·1 (16) and 50 ± 2·8 (15) μl./100 mg fresh wt./hr, for the first 30 min and last 60 min respectively of a 6 hr incubation period. Since the changes shown to take place as a result of incubation were small this medium, referred to as the normal K-phosphate medium, was adopted for all succeeding experiments. In some specified cases the concentration of glucose in this medium has been increased.

Effects of ouabain

An ATPase system sensitive to inhibition by ouabain has been demonstrated in the hamster intestine (Crane, Miller & Bihler, 1961) and in the mucosa of the guinea-pig intestine (Taylor, 1963). The following experiments were designed to test whether such an enzyme existed in the goldfish intestine. Goldfish intestines were incubated either in the presence of ouabain, 730 μM, or under control conditions for 380 min. Respiration was recorded throughout and the water content of the tissue determined at the end of incubation. The results (Fig. 1) show a slowly declining oxygen consumption when ouabain was present. This reached a stable level after approximately 120 min incubation. The mean oxygen consumption for intestines incubated with ouabain, measured from the third to the fifth hour of incubation was 30·9 ± 1·8 μl./100 mg fresh wt./hr (15). This was 36% less than that for the control intestine measured over the same period of time (oxygen consumption = 47·9 ± 1·3 μl./100 mg fresh wt./hr (48)). The concentration of ouabain was high and it is unlikely that a higher concentration would have a greater effect. Sixty-four percent of the tissue oxygen consumption was insensitive to ouabain, a figure very

similar to that obtained by Whittam & Willis (1963) for the rabbit kidney cortex slice. The water content of the intestines incubated with ouabain was 5·85 ± 0·61 g water/g dry wt. (5). This was 35 % greater than that of goldfish intestines incubated under control conditions (4·33 ± 0·18 g water/g dry wt. (13)). Judged by these two parameters there would seem to be an ouabain-sensitive ATPase enzyme present in the goldfish intestine. However, the concentration of ouabain was high and the results would have greater significance if repeated at low concentrations.

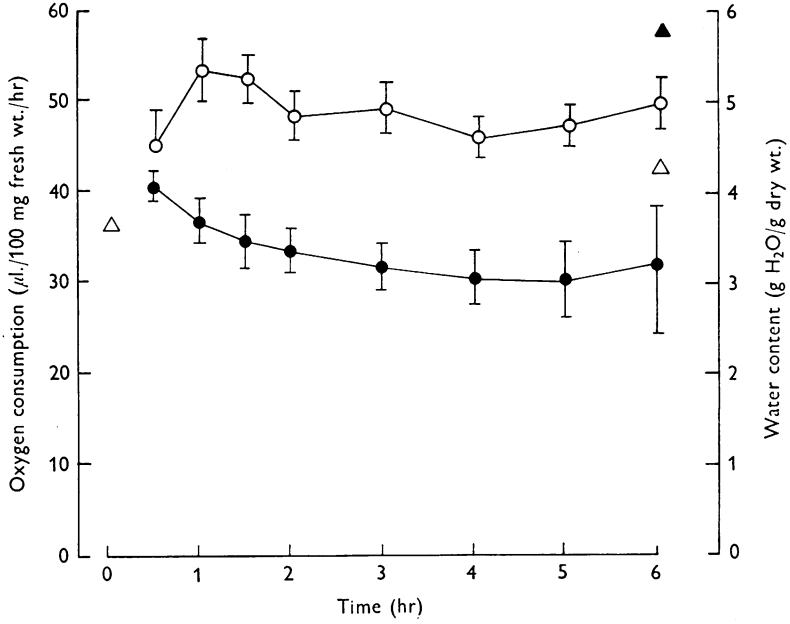

Fig. 1. Effects of ouabain on oxygen consumption and tissue water content of goldfish intestine incubated for 380 min at 25° C. O_2 consumption was measured for 6 hr after a 20 min equilibration when O_2 flowed through the flasks. Oxygen consumption, open circles, and water content, open triangles, of goldfish intestine incubated in normal K-phosphate medium; oxygen consumption, filled circles, and water content, filled triangles, of goldfish intestine incubated in the same medium plus 730 μM ouabain. Values represent means ± S.E. (5–16 observations).

Figure 2 shows the percentage inhibition of respiration and increase in water content over that of control intestines measured over a range of ouabain concentrations; the incubation period was again 380 min. The percentage inhibition of oxygen consumption was calculated as the difference between oxygen consumption values obtained from normal and ouabain-treated tissues measured from the third to fifth hour of incubation. This time period was chosen as one when the effect of ouabain was maximal and the error of individual estimates minimal (see Fig. 1).

There was a rough parallel between the magnitude of effect ouabain produced on respiration and that produced on the water content of the tissue. The size of these effects was dependent on the concentration of ouabain over the range 1–100 μM.

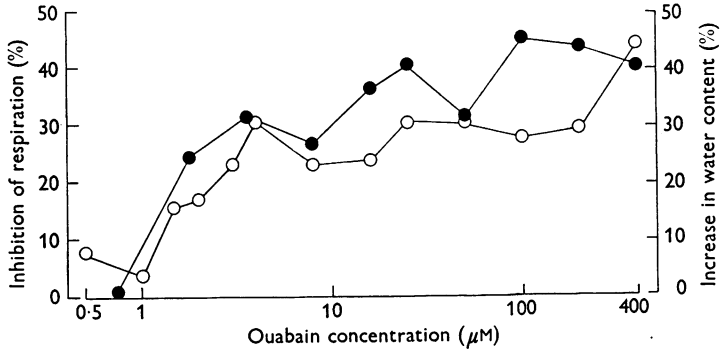

Fig. 2. Percentage inhibition of respiration and increase in water content of goldfish intestine incubated in normal K-phosphate medium containing different concentrations of ouabain. Incubation was for 380 min at 25° C with equilibration of the flasks as in Fig. 1. The percentage inhibition of respiration, filled circles, was calculated by comparison with respiration under control conditions from the third to fifth hr of incubation. Open circles, percentage increase in water content measured at the end of incubation. Points indicate the mean of 3–6 values.

Respiration and electrolyte content of goldfish intestine

Sacs of goldfish intestine, filled with the normal K-phosphate medium identical with that bathing the serosal surface, were incubated for 260 min at 25° C. The respiration was recorded and compared with that obtained when ouabain, 50 μM, was present in the lumen or in the medium bathing the serosal surface. Immediately after incubation the intestines were rinsed in sucrose and analysed for sodium and potassium. The results are shown in Fig. 3. When ouabain was initially present on the serosal side of the sac the tissue potassium fell to $2{\cdot}38 \pm 0{\cdot}14$ μ-mole/100 mg fresh wt. (9) which was less than half that found in the control tissue. At the same time the tissue sodium rose to $11{\cdot}0 \pm 0{\cdot}35$ μ-mole/100 mg fresh wt. (9) or twice the control value. During incubation the oxygen consumption was inhibited by 30%. None of these changes was seen when ouabain was placed inside the sac where it would be in direct contact with the mucosa.

Sodium and potassium content of smooth muscle from the goldfish intestine

The mucosa and lamina propria of the intestine can together be separated from the muscle coats by squeezing with a glass coverslip. Smooth muscle isolated in this way constituted 20% of the total wet weight of in-

testine and contained $4{\cdot}91 \pm 0{\cdot}43$ μ-mole/100 mg wet wt. (9) sodium and $0{\cdot}99 \pm 0{\cdot}23$ μ-mole/100 mg wet wt. (9) potassium. The oxygen uptake of this muscle in medium at 25° C was too small to be recorded accurately on

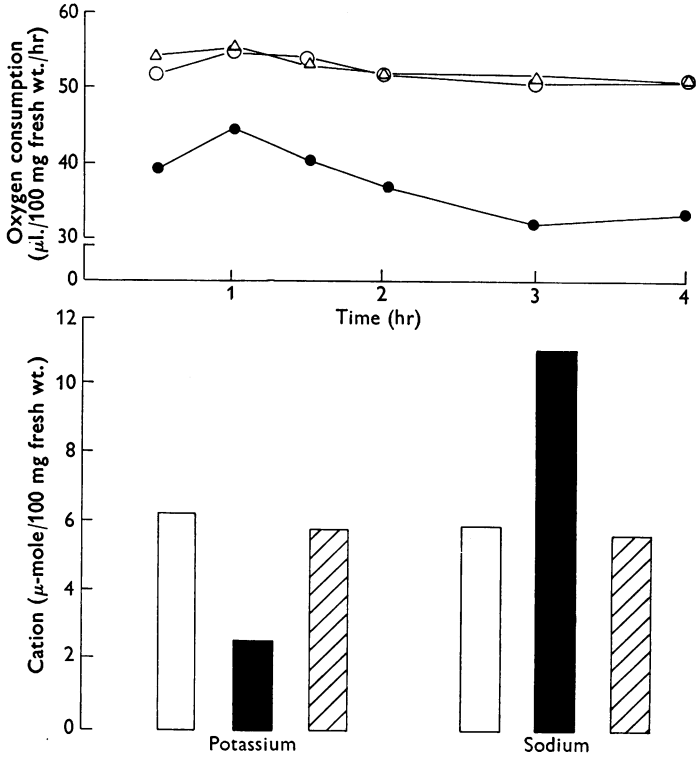

Fig. 3. Effects of ouabain on respiration and tissue potassium and sodium of goldfish intestinal sacs incubated at 25° C for 260 min in normal K-phosphate medium. Period of equilibration as in the previous figures. Open circles, oxygen consumption measured for intestines incubated in the normal K-phosphate medium; filled circles, with ouabain in contact with the serosa; open triangles, with ouabain confined to the lumen of the sacs. The open histograms show the sodium and potassium contents of intestinal sacs incubated in the normal K-phosphate medium. The sodium and potassium contents of intestinal sacs incubated with ouabain are shown as closed histograms where ouabain was in contact with the serosa and hatched histograms where the ouabain was confined to the lumen. The concentration of ouabain used was 50 μM. Points and histograms indicate the means of 9–14 values.

the Warburg apparatus. At the end of 260 min incubation the control muscle sheets contained sodium, $5{\cdot}17 \pm 1{\cdot}26$ μ-mole/100 mg fresh wt. (5) and potassium, $0{\cdot}94 \pm 0{\cdot}25$ μ-mole/100 mg fresh wt. (6). Ouabain present in a concentration of 50 μM during the incubation did not alter significantly

the concentration of sodium, $4{\cdot}57 \pm 0{\cdot}33$ μ-mole/100 mg fresh wt. (4), or potassium $0{\cdot}83 \pm 0{\cdot}26$ μ-mole/100 mg fresh wt. (5) but the error in these estimations was large and a small effect would pass unnoticed. Clearly, however, the main site of ouabain action must lie either in the lamina propria or the mucosa.

Luminal transfer of water and solutes in the goldfish intestine

Sacs of goldfish intestine were filled with the normal K-phosphate medium. No attempt was made to standardize the amount of medium placed in any one sac and this varied from 10–30 μl./cm. These sacs were shaken for 260 min at 25° C in the normal K-phosphate medium and the contents then expelled into a weighed container. The weight difference showed the amount of medium recovered and this, subtracted from the amount added, gave the net water loss. The expelled solution was then assayed for sodium, potassium and glucose. In other experiments, ouabain 50 μM was present in the lumen or in the medium surrounding the intestinal sac, throughout the period of incubation. Table 2 shows the net loss of sodium and water from the lumen, called the net luminal transfer,

TABLE 2. Sodium and water loss from the lumen of goldfish intestine

	Sodium (μ-mole/cm)	Difference from control		Water (μl./cm)	Difference from control	
		t	P		t	P
Control	$0{\cdot}90 \pm 0{\cdot}10$ (13)	—	—	$9{\cdot}3 \pm 0{\cdot}84$ (13)	—	—
Ouabain (lumen)	$1{\cdot}16 \pm 0{\cdot}19$ (6)	1·32	> 0·2	$7{\cdot}9 \pm 0{\cdot}86$ (8)	1·10	> 0·2
Ouabain (serosa)	$0{\cdot}79 \pm 0{\cdot}11$ (9)	0·74	> 0·4	$6{\cdot}6 \pm 0{\cdot}82$ (9)	2·21	< 0·05

Sacs were incubated for 260 min at 25° C in the normal K-phosphate medium, pH 7·1. The concentration of ouabain used was 50 μM.

measured under these various conditions. The control sac lost 9·3 μl. water and 0·9 μ-mole sodium/cm length and this represented not more than 75 % of the total water or sodium initially present. The total amount of sodium and water lost from the sac during incubation was not altered significantly by the quantity present at the start of incubation over the range studied (10–30 μl. water and 1·1–3·3 μ-mole sodium/cm intestine) and was not changed when ouabain was present in the lumen. The net luminal transfer of sodium was also unaffected by ouabain placed in contact with the serosal surface but in this case the transfer of water was significantly lower, 6·6 μl./cm; $P < 0{\cdot}05$.

Figure 4 shows the movements of potassium during incubation. Under control conditions the net loss of potassium was 25–65 % of the amount present at the start of incubation. The net luminal transfer was $26{\cdot}4 \pm 3{\cdot}3$ n-mole/cm (6). Ouabain in contact with the serosal surface during in-

cubation decreased the net luminal transfer of potassium and when the amount of medium initially present in the lumen exceeded 15 μl./cm the total amount of potassium within the lumen increased during incubation; the net flow of potassium had been reversed.

A negligible amount of glucose was left in the intestinal sac at the end of incubation under control conditions or when ouabain was present in the lumen. The quantity of glucose transferred was strictly dependent on the

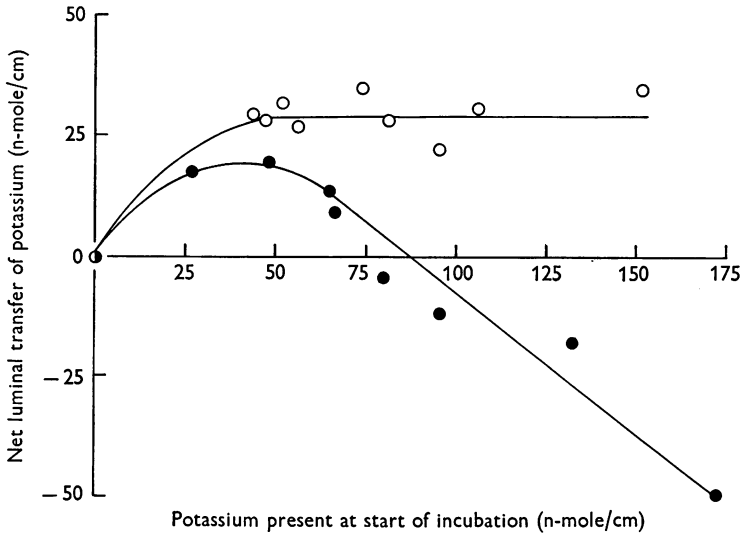

Fig. 4. The net luminal transfer of potassium in sacs of goldfish intestine measured after 260 min incubation at 25° C in normal K-phosphate medium. Open circles, net luminal transfer of potassium in sacs incubated in normal K-phosphate medium. Filled circles, net luminal transfer of potassium in sacs incubated in the same medium plus 50 μM ouabain.

amount added over the range studied (90–250 n-mole/cm) and represented 95 % of the total. The luminal transfer of glucose was much reduced when ouabain bathed the serosal surface. In this case the mean transfer was 108 n-mole/cm (6) and this was independent of the amount present at the start of incubation. An incubation period of 260 min had been chosen as a time which would give a substantial loss of water, sodium and potassium from the lumen but it was obviously too long to give quantitative data on the maximal control luminal transfer of glucose. Transfers were therefore determined over a 60 min incubation period. In these experiments the empty sacs were incubated at room temperature for 30 min in medium, with or without ouabain, before the determination of glucose transfer to allow equilibration with the external medium. The results are shown in

Fig. 5. When the normal K-phosphate medium was used, 98% of the available glucose was transferred by the end of incubation. It seemed unwise to use a shorter incubation period and so the glucose concentration of the medium was increased two-, four- and eight-fold, inside and outside the sacs, and the experiments repeated. At the higher glucose concentrations the control rate of transfer was no longer dependent on the initial

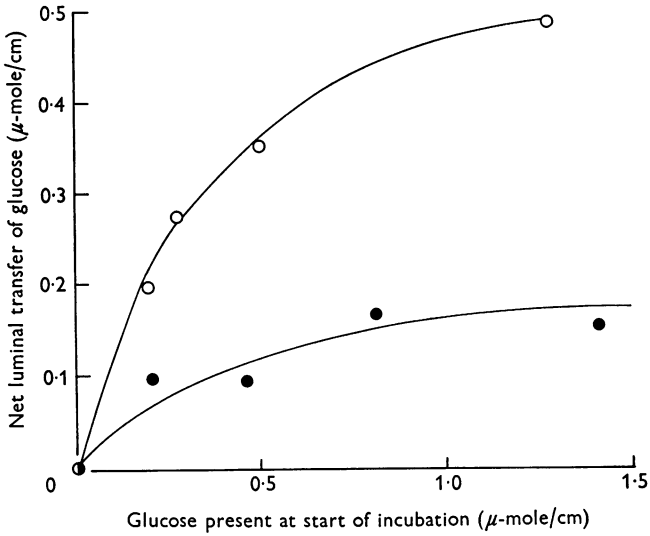

Fig. 5. The net luminal transfer of glucose in sacs of goldfish intestine incubated for 60 min at 25° C in normal K-phosphate medium. Open circles, sacs bathed in normal K-phosphate medium. Filled circles, sacs bathed in normal K-phosphate medium plus 50 μM ouabain.

glucose concentration; the net luminal transfer was 483 n-mole/cm/hr. When ouabain was in contact with the serosa an increase in the concentration of glucose caused only a small increase in the net loss of glucose from the lumen (89–150 n-mole/cm/hr).

DISCUSSION

If it is assumed that ouabain is a specific inhibitor of cell membrane ATPase then many cells within the goldfish intestine must depend on this enzyme to maintain their ionic equilibrium with the external medium. Gross changes of the tissue potassium and sodium occur in the presence of ouabain, respiration falls and the water content increases. These effects are not seen in smooth muscle prepared by scraping away the villi of the intestine but this does not mean that they are specific for the mucosa.

There are many different types of cell within the lamina propria including some red blood cells and these at least might be expected to control their ionic composition through a mechanism sensitive to ouabain (Dunham & Glynn, 1961).

Sodium and potassium were both lost from the lumen of the goldfish intestine during incubation under control conditions. The potassium loss was less than that of sodium or water so that, at the end of incubation, the concentration of potassium in the fluid remaining within the sac had increased but that of sodium remained constant within the error of the estimations. The osmolarity of the luminal fluid may also have been changed by removal of glucose and it is not possible under these conditions to postulate the mechanisms involved in the transfer of the ions. The tissue concentration of sodium doubled during incubation with ouabain but there was no significant effect on the net luminal transfer of this ion. It is possible that sodium loss under these conditions was due to passive equilibration with cells affected by ouabain. Ouabain is known to slow the net transfer of sodium from the mucosa to serosa of the rabbit ileum (Schultz & Zalusky, 1964). The net flux of sodium from the intestine of the marine teleost *Cottus scorpius* used by House & Green (1963) was several times larger than that of the goldfish. This might be a reflexion of experimental techniques used but it does agree with the fact that marine teleosts have to absorb large amounts of sodium to obtain water, the unwanted sodium then being extruded from the gills.

The total potassium in the luminal fluid increased when ouabain bathed the serosal surface of the intestine. At the same time the tissue content of potassium was halved. This represented a loss of 155 μg of potassium from 100 mg of goldfish intestine and most of this loss must have been to the medium surrounding the intestine. The potassium which leaked back into the sac showed that the membranes of the mucosal cells were permeable to potassium and therefore probably affected by ouabain.

The luminal transfer of water was reduced when ouabain bathed the serosal surface of the goldfish intestine. The scatter of results was quite large but the difference was significant at the 5% level of probability. Smyth & Taylor (1957) reviewed and extended the evidence for an active transport of water by the intestinal mucosa of the rat and it was later suggested by Newey, Sanford & Smyth (1963) that glucose supplied the energy for the transfer of water. It is therefore particularly interesting that ouabain added to the serosal surface of the goldfish intestine should inhibit the luminal transfer of glucose. Probably the fall in fluid transfer is secondary to the effect on glucose transfer.

McDougal, Little & Crane (1960), with strips of hamster intestine, found that D-galactose is actively transported at or near the luminal

border of the mucosal cells since the highest concentration of D-galactose, 1 min after the start of incubation, is 3 times that in the surrounding fluid or the lamina propria. While it is difficult to criticize this work Newey *et al.* (1963) were able to distinguish between the site of entry of glucose and the site at which it is actively transported. The luminal transfer of glucose measured in the goldfish intestine and in the presence of ouabain, was approximately 110 n-mole/cm whether the time of incubation was 60 *or* 260 min. A glucose loss which is independent of time suggests a saturation phenomenon; the entry of glucose to the mucosa is not changed but the second stage transfer of the sugar is blocked. Of course the ouabain would take some time to penetrate muscle and reach the mucosa and some loss of glucose could occur before inhibition was established but the 60 min incubation period was preceeded by a 30 min equilibration of the empty sac in a solution of ouabain and yet the final result was the same.

The immediate entry of some glucose into the mucosa when the cell membrane ATPase is inhibited by ouabain places the site of inhibition behind the luminal border of the mucosal cell and this is in agreement with the fact that ouabain in the lumen of the intestinal sac, in intimate contact with the brush border microvilli, is without effect on glucose or water transfer. At the base of the mucosa is a substrate-specific ATPase (Hollands & Smith, 1964) and an assumption that this is one site for active transport of glucose in the goldfish intestine would agree with the known findings. However, the quick changes in potential obtained by removal or addition of glucose to the medium bathing the mucosa (Smith, 1964) imply that the luminal border is also a site for active-transport mechanisms involving glucose.

SUMMARY

1. Goldfish intestines were incubated in a saline solution medium and their sodium, potassium and water contents and oxygen uptakes measured.

2. The water content was raised and the oxygen uptake lowered when ouabain was present. These effects were dependent on the concentration of ouabain over the range 1–100 μM.

3. When oubain bathed the serosa of sacs of goldfish intestine the tissue potassium was halved, the tissue sodium was doubled and the oxygen uptake was inhibited. These changes probably did not occur in the smooth muscle layers and were not seen when ouabain was confined to the lumen of the sac.

4. The net luminal transfers of sodium, potassium, water and glucose from sacs of goldfish intestine were measured.

5. The transfer of glucose and water, but not of sodium, was slowed when ouabain was in contact with the intestinal serosa and the net flux of

potassium was reversed. None of these changes took place when ouabain was present only in the lumen of the sac.

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