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COUPLING OF TRANSMURAL FLOWS OF NaCl AND WATER IN THE INTESTINE OF THE EEL (*ANGUILLA ANGUILLA*)

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INTRODUCTION

A major problem in intestinal physiology is how salt and water absorption are linked. A way of elucidating this problem is to measure the salt and water flows in an animal adapted to different physiological situations, and see how the transmural flows of salt and water are correlated. Marine teleosts are suitable experimental objects in this respect, since they drink the hyperosmotic sea water, absorb NaCl and water in the intestine, and excrete salt through the gills leaving water behind. When the salinity of the surrounding water is increased, the fish augment their drinking rate (Maetz & Skadhauge, 1968; Potts *et al.* 1967; Shehadeh & Gordon, 1969), their intestinal NaCl absorption rate (Hirano, 1967; Oide, 1967; Oide & Utida, 1967; Skadhauge, 1969) and their rate of salt turnover by the gills (Maetz & Skadhauge, 1968; Maetz, 1971). It was observed (Skadhauge, 1969) that the intestine of the European eel (*Anguilla anguilla*) could transport water from lumen to plasma even when the osmolality of luminal fluid was markedly higher than that of plasma, and it was found that the intestinal osmolality, which was large enough to stop the net water flow from lumen to plasma, seemed to vary with the net transmural NaCl flow. The apparent non-osmotic water flow is assumed to be caused by an osmotic flow into a hyperosmotic region in or between the epithelial cells (Curran, 1960; Diamond, 1964). In the former study (Skadhauge, 1969) the author suggested a double-flow model: the general osmotic water flow passed through a shunt pathway different from that taken by the solute-linked water flow. The present study was carried out in order to provide further experimental evidence for this model. Of particular interest were: (1) the amount of water following the NaCl absorption when the luminal osmolality and plasma osmolality were identical; (2) the osmolality at zero water flow as a function of the net NaCl absorption rate; and (3) the osmotic permeability coefficients.

The present measurements were carried out with an *in vivo* perfusion technique of the eel intestine with recycling perfusion fluids (Skadhauge, 1969). An open-circuit perfusion technique (Bindslev & Skadhauge, 1971) was also used in order to estimate the self-diffusional permeability coefficient of water, and the reflexion coefficient. The results will be interpreted in a following paper with the aid of a model of the transmural flow of salt and water along the length of the gut (Kristensen & Skadhauge, 1974).

MATERIAL AND METHODS

The present experiments were carried out at the Station Zoologique, Villefranche-sur-Mer, June–August 1969. The experimental procedure and analytical techniques were, except as outlined below, as reported previously (Skadhauge, 1969). Briefly, yellow European eels were adapted to fresh water (FW), sea water (SW) or $1\frac{1}{2}$ strength SW ($1\frac{1}{2}$ SW). Recycling perfusion experiments of the total intestine from the oral end of the anterior intestine to the anus were performed *in vivo*, and the transmural transport rates of water and NaCl were calculated from the initial volume and concentrations of Na^+ and Cl^- and a water marker, and the concentrations of the electrolytes and the water marker at the end of each 15 min experimental period. Phenol red served as a non-absorbable water marker. In this study ^{14}C polyethylene glycol (^{14}C-PEG) was also used (New England Nuclear Corp., Boston, lot no. 318–228). ^{14}C was analysed on the Nuclear Chicago Tricarb liquid scintillation spectrometer. 10 μl samples in 5 ml Bray solution were used; quenching correction was performed by use of an external standard and a standard curve relating counting efficiency for various degrees of quenching to the ratio of the numbers of counts of the samples, with and without external standard.

The average concentration change for the two water markers was used in the calculation. Diluted sea water and $MgSO_4$ solutions served as perfusion fluids. In a few experiments Na_2SO_4 or $MgCl_2$ solutions were used. In general four different recycling perfusion fluids were used on each experimental animal: a hyper- and a hypo-osmotic sea-water fluid, and a hyper- and a hypo-osmotic $MgSO_4$ fluid. In addition, a $MgSO_4$ solution slightly hypo-osmotic to plasma was infused into the oral end of the anterior intestine and collected from the anal catheter, and thus not recycled. In these last experiments HTO was added to the perfusion fluid, and ^{14}C-PEG omitted. The aim of these experiments was to measure the permeability to HTO in the absence of a net water movement.

In the open-circuit experiments the net transmural flows of NaCl and water, when equilibrium had been attained, were calculated from the incoming flows and concentrations of Na^+ and Cl^- and the water marker and the outgoing concentrations of electrolytes and the water marker (Bindslev & Skadhauge, 1971). The perfusion speed was 22·5 ml/h.

Calculation of the self-diffusional permeability coefficients of water. From the HTO flow the apparent self-diffusional permeability coefficient of water in the mucosa-to-serosa direction was calculated from the open-circuit experiments in the following way. Since the samples of perfusion fluid from the three collection periods had approximately the same HTO concentration, a steady-state level of the plasma concentration was assumed to obtain. This means that the loss of activity from gut lumen to plasma equalled the loss from plasma to the surrounding streaming water. Taking the net water flow into account correction for the back-flow of isotope from plasma to gut lumen was made as in the previous experiments (Skadhauge, 1969) using the plasma concentration calculated at the end of the experiment. The HTO concentration of plasma was measured when plasma was obtained 10–20 min after the end of the experiment. An exponential loss to the surrounding water was assumed during this period with the rate constant as described for eels in FW and SW by Motais *et al.* (1969).

These authors found a rate constant of 42·4%/h for FW, 29·3%/h for SW. A value of 25%/h was assumed for 1½ SW. No further loss from lumen to plasma was assumed since the gut was emptied by air flushing immediately after the termination of the last collection period. The calculation of backflow of isotope was of minor importance since the calculated final plasma HTO concentration did not exceed 6% of the mean luminal concentration.

Blood samples were secured before and after the experiments by puncture of the tail vein. The analyses were carried out as reported previously (Skadhauge, 1969), HTO was analysed on the same instrument as ^{14}C with quenching correction. All analyses were carried out in duplicate.

CONTROL EXPERIMENTS

Concentration along the length of the gut in the natural state. In November 1970 control experiments were carried out at Danmarks Akvarium, Charlottenlund. Yellow European eels were adapted for 2 weeks to FW, SW and double-strength SW. After anaesthesia in the medium with 1·5% urethan for 10 min the eels were decapitated, blood was drawn by heart puncture, and the whole of the anterior and posterior intestines was removed. Both parts of the intestine were divided in an oral and an anal part, the contents of these parts were expelled into Beckman microtubes or directly sucked into glass capillaries. The samples were stored at 0 °C prior to analysis for osmolality and NaCl concentration of the supernatant after centrifugation.

Intestinal morphology. On yellow eels, treated as the above-mentioned control animals, fractions of anterior and posterior intestine were taken out and fixed in 4% formalin, embedded in paraffin, sectioned and stained with haematoxylin-eosin.

Concentration ratio of phenol red to ^{14}C-PEG. In order to compare the validity of the two water markers used, the ratio of the concentration at the start of the first experimental period to the concentration at the end of the last period in 44 recycling experiments was calculated for both of the water markers, and the ratio between the ratios for the two markers was calculated. If the behaviour of the two markers was identical this ratio should be unity. An average value of 1·028 with a coefficiency of variation of 3·5% was found. The conclusion is that the two markers yielded identical results, and thus seem equally valid.

RESULTS

A. *Recycling perfusion experiments*

Perfusion with dilute sea water

Six experiments in 1½ SW, nine in SW, and four in FW were carried out. In Fig. 1 a typical experiment in a SW-adapted eel is presented. The osmolality of the perfusion fluid which in the beginning is higher than that of plasma falls due to net water flow into the intestine and due to a net NaCl absorption. This is demonstrated by the fall in concentration of the water markers. When the water-marker concentration is minimum, the so-called 'turning point' is reached and the osmolality difference between perfusion fluid and plasma (TPΔOsm) is read from curve. Later the luminal osmolality falls below that of plasma and the water flow in the 15 min experimental period when that occurs is used to calculate the solute-linked water flow (J_{vs}). The net NaCl flow (J_{NaCl}) of that period is used to calculate the NaCl concentration of the absorbate at

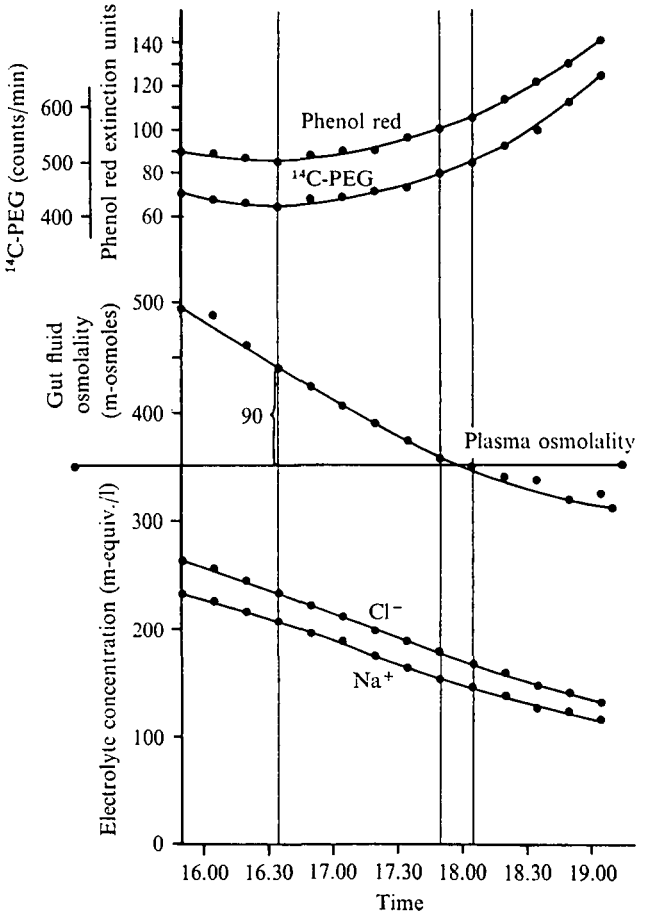

Fig. 1. Expt no. 3, 25. vi. 69, sea-water adapted eel, body weight 428 g. Recycling perfusion experiment with dilute sea water. The concentration of the water markers, the osmolality, and the concentrations of Na^+ and Cl^- are measured in samples taken every 15 min. Zero water flow occurs when the intestinal fluid is 90 mOsm hyperosmotic to plasma. The luminal osmolality falls eventually below plasma osmolality. The water flow in the experimental period when the luminal and plasma osmolality are identical is the solute-linked water flow.

plasma osmolality as J_{NaCl}/J_{vs}. This apparent NaCl concentration of the absorbate was observed to be higher than that of plasma in agreement with the observation that the luminal osmolality always fell below that of plasma in experiments of sufficiently long duration.

In Table 1 the net transmural flows of Na^+ and Cl^- (J_{Na} and J_{Cl}) calculated on the basis of the present experiments, are presented together with the osmolality and the plasma concentrations of Na^+ and Cl^-. The values do not differ significantly from those observed previously (Skadhauge, 1969). The results of the two studies were therefore lumped in the following calculations. It will also appear from Table 1 that J_{Na^+} was almost equal to J_{Cl^-} which makes it permissible to add the two flows to give a neutral salt flow for osmotic calculations. The relationship between body weight and the perfused area was slightly lower than in the previous experiments (400 g corresponding to 30–35 cm^2), presumably due to the use of larger fish.

Table 1. *Net intestinal NaCl absorption rates and plasma concentrations*

(The figures in parantheses denote S.D.)

	NaCl absorption rate		Plasma concentration			
	J_{Na}	J_{Cl}	Osm	Na	Cl	n
FW	222±98	276±120	303±36	121±14	101±28	4
SW	244±70	281±74	357±7	160±5	148±5	9
1½ SW	335±84	370±106	381±15	174±8	160±5	6
Units	μ-equiv./100 g.h		mOsm	m-equiv./l		

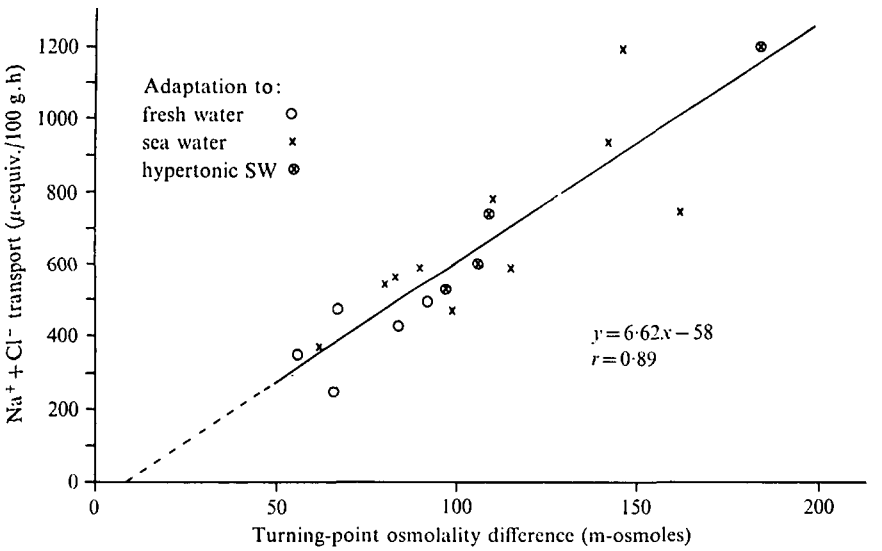

Fig. 2. The relation between the osmolality difference at zero water flow and the NaCl absorption rate. The sum of the Na^+ and Cl^- ions has been plotted against the 'turning-point osmolality difference' (see text). The findings are compatible with direct proportionality.

The turning-point osmolality difference as a function of the net transmural flow of NaCl

The results of 19 experiments are presented in Fig. 2. The TPΔOsm in each eel was compared to the average net flow of $Na^+ + Cl^-$ for the same eel, as the luminal concentration during these experiments stayed high enough to have near maximal transmural flow (Skadhauge, 1969). A linear relationship is apparent for the experimental values determined. They ranged from 50 to 200 mOsm and from 300 to 1300 μ-equiv./100 g.h respectively. An increase in J_{NaCl} of 100 μ-equiv./100 g.h brings about an increase in TPΔOsm of 15·2 mOsm. The regression coefficient, $b = 6{\cdot}62$, had a standard deviation (S_{byx}) of 0·83. When the linear regression line is extended to a zero NaCl flow the TPΔOsm also becomes close to zero. This may indicate that a non-osmotic water flow not linked to NaCl is of no quantitative importance for the water flow across the eel intestine. The FW-adapted fish had the lower NaCl absorption rates, the SW-adapted fish the higher, but there is some overlap.

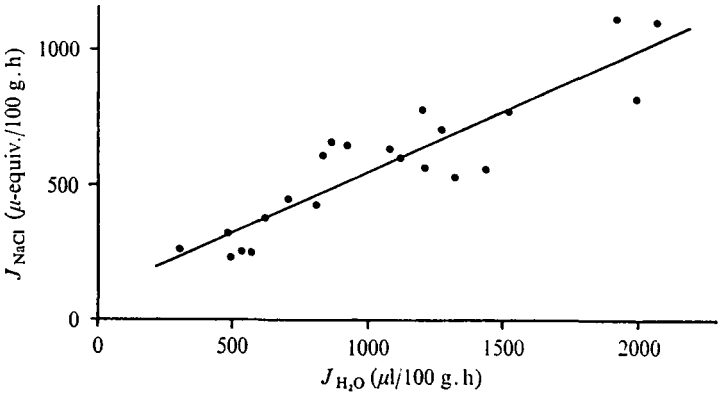

Fig. 3. The relation between net NaCl absorption rate and solute-linked water flow. The sum of the Na^+ and Cl^- flows for the 15 min experimental periods where luminal osmolality was equal to plasma osmolality have been plotted against the corresponding water flow. As in other epithelia proportionality was observed ($r = 0{\cdot}91$).

Table 2. *NaCl concentration of absorbate at zero lumen-to-plasma osmolality difference*

	$Na^+ + Cl^-$ concentration	S.D.	*n*
FW	524	165	7
SW	559	111	12
1½ SW	581	136	4
Unit	m-equiv./l		

The NaCl concentration of the solute-linked water flow

In a total of 23 experiments there was a 15 min experimental period in which the osmolality passed from above that of plasma to below. The net flow of $Na^+ + Cl^-$ for that period, divided by the corresponding water flow, was defined as the solute-linked water flow J_{vs}. This water flow was observed to be proportional to the NaCl absorption rate (Fig. 3) as also observed in other intestinal epithelia (Curran, 1960; Clarkson & Rothstein, 1960; Edmonds & Pilcher, 1972). When the solute-linked water flows were grouped according to the adaptation of the eels (Table 2) no significant difference was demonstrated. Thus the higher rate of transport of NaCl in the SW-adapted and the 1½ SW-adapted eel resulted in a similar increase in the water flow, so the hypertonicity of the absorbate was more or less independent of the adaptation of the animal. The average concentration of $Na^+ + Cl^-$ in the absorbate for all experiments was 554 μ-equiv./l with a S.E. of 27.

The flows of NaCl and water flow in perfusion experiments with $MgCl_2$ and Na_2SO_4

Recycling perfusion experiments with hyperosmotic solutions of $MgCl_2$ and Na_2SO_4 were carried out in two SW-adapted eels. One is shown in Fig. 4. The average net flows of Na^+ and Cl^- were calculated for each perfusion fluid, and the results are recorded in Table 3. It will appear that the flows of both Na^+ and Cl^- were considerably reduced when the other ion was absent, as compared to the NaCl perfusion experiments (Table 1). The ion absent from the luminal fluid underwent a net secretion

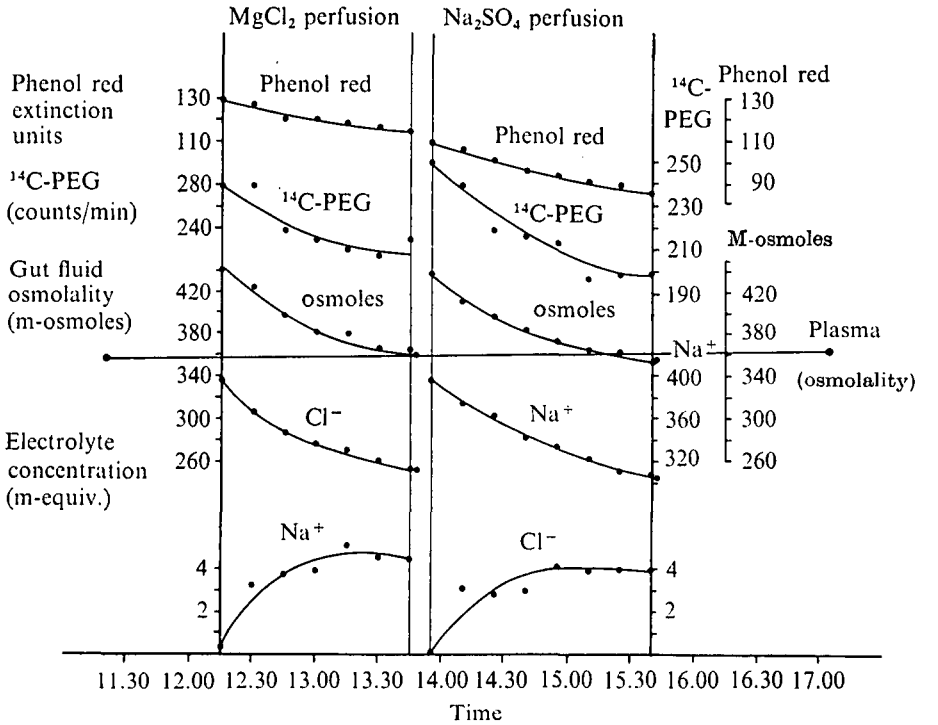

Fig. 4. Expt. no. 11, sea-water adapted eel, body weight 464 g. Recycling perfusion experiments with perfusion fluids of $MgCl_2$ or Na_2SO_4. These fluids do not result in solute-linked water flow. The water flow falls to zero, when the luminal osmolality reaches plasma osmolality. When either Na^+ or Cl^- ions are present and the other ion absent from the perfusion fluid the ion present is absorbed, whereas the other ion is secreted into the intestine.

Table 3. *Na^+ and Cl^- flows in $MgCl_2$, Na_2SO_4 and $MgSO_4$ perfusion experiments*

(Means ± S.D. of average values for each eel are reported.)

			J_{Na}	J_{Cl}	n
Recycling	$MgCl_2$	SW	−32	+27	2
	Na_2SO_4	SW	+46	−32	2
	$MgSO_4$	FW	−46 ± 16	−23 ± 8	6
	$MgSO_4$	SW + 1½ SW	−74 ± 24	−62 ± 21	10
Open circuit	$MgSO_4$	FW	−34 ± 11	−15 ± 6	3
	$MgSO_4$	SW + 1½ SW	−58 ± 37	−39 ± 32	5
	Unit		μ-equiv./100 g.h		

of the same magnitude as in the $MgSO_4$ perfusion experiments (Table 3). In both eels the experiments were continued until the luminal osmolality approached that of plasma, at which point the water flow (which was still from plasma to lumen) vanished (Fig. 4). Thus a solute-linked water flow was not observed if Na^+ and Cl^- were not both absorbed.

The osmotic permeability coefficient of water ($MgSO_4$ perfusion experiments)

The osmotic permeability coefficient of water (P_{os}) was calculated for each eel from experiments with $MgSO_4$ containing perfusion fluid as μl/100 g.hr.mOsm assuming

a reflexion coefficient of unity. Perfusion fluids that were hyper- and hypo-osmotic to plasma were used. Three experiments in FW-adapted eels, three in SW-adapted eels and two in 1½ SW-adapted eels were performed.

The results were variable and the P_{os} did not appear to depend upon the salinity. Rectification of the water flow could not be demonstrated because of the variability. The average value (mean ± S.D.) for the flow for all eels in the present study in the serosa-to-mucosa (s-m) direction was $7{\cdot}5 \pm 3{\cdot}6$ µl/100 g.h.mOsm, and $3{\cdot}0 \pm 1{\cdot}6$ µl/100 g.h.mOsm in the mucosa-to-serosa (m-s) direction. Since 100 g corresponds to 10·0 cm^2 intestinal surface area the mean value of these permeability coefficients corresponds to $4{\cdot}7 . 10^{-3}$ cm/sec, taking the partial molar volume for water to be 55·5 moles/l.

The values for flow in the s-m direction were comparable to those obtained in the previous study (Skadhauge, 1969), but those in the m-s direction were only one-third of the previous values. A possible explanation for the varying results will be given in the discussion.

The NaCl mobility

The net transmural flow of Na^+ and Cl^- from plasma to lumen was observed in the recycling $MgSO_4$ experiments. Since the NaCl flow in the other direction must be zero due to the low luminal NaCl concentration, the measured flow may be taken to indicate the passive flow, and is thus a measure of the passive permeability of the intestinal wall to NaCl. As no difference could be observed between SW-adapted and 1½ SW-adapted animals these groups were combined and compared to findings in FW-adapted animals (Table 3). Although the transport was larger, the permeability (assuming a zero transmural potential difference) comes out almost identical for the two groups due to the higher plasma concentration of NaCl in the SW-adapted and 1½ SW-adapted animals. The average Na^+ permeability was $1{\cdot}1 \times 10^{-5}$ cm/sec for FW-adapted eels, $1{\cdot}3 \times 10^{-5}$ cm/sec for SW-adapted eels. The average Cl^- permeability was $0{\cdot}7 \times 10^{-5}$ cm/sec for FW-adapted eels, $1{\cdot}2 \times 10^{-5}$ cm/sec for SW-adapted eels. The concentration difference was calculated from the plasma concentration (Table 1), and the average luminal concentrations. These were: FW Na^+, 6 m-equiv./l; FW Cl^-, 3 m-equiv./l; SW Na^+, 9 m-equiv./l; and SW Cl^-, 6 m-equiv./l.

B. *Open-circuit experiments*

With this technique three experiments were carried out in FW-adapted animals, four in SW-adapted and two in 1½ SW-adapted animals. In preliminary experiments it was observed that up to 30 min might pass before the outcoming fluid had a stable phenol red concentration and a constant osmolality. Consequently this time was allowed to elapse after the start of the perfusion before collection of samples of the outgoing fluid was begun. The outgoing concentration was determined as the average of three 5 min collection periods after the equilibrium time had passed.

The self-diffusional permeability coefficient of water

The apparent self-diffusional permeability coefficient of water (P_d) was calculated for each experimental animal from perfusion experiments with $MgSO_4$ at an osmolality

Table 4. *Osmolality and NaCl concentrations of luminal contents of the intestine in SW-adapted and 2 SW-adapted eels*

(Mean ± S.E. No. of experiments in parantheses.)

	Anterior intestine		Posterior intestine		
	Upper	Lower	Upper	Lower	Units
Na^+	102 ± 13 (8)	94 ± 13 (9)	77 ± 13 (9)	40 ± 14 (5)	m-equiv./l
Cl^-	99 ± 19 (8)	109 ± 22 (7)	95 ± 15 (9)	83 ± 19 (4)	
Osm	458 (2)	491 ± 28 (5)	385 ± 34 (5)	349 ± 18 (4)	mOsm

which caused a negligible net water flow. No difference was apparent between the groups, and the average value for nine animals (three in FW, SW, and 1½ SW) was calculated to be 2·49 ± 1·25 ml/100 g.h, corresponding to $(6{\cdot}7 \pm 3{\cdot}2) \times 10^{-5}$ cm/sec (mean ± S.D.). The apparent P_{os} is thus 70 times larger than the apparent P_d. The rate of loss of HTO from the luminal perfusion fluid was measured in three SW-adapted eels and one FW-adapted eel. For these four animals the permeability coefficient was calculated to $(19 \pm 3) \times 10^{-5}$ cm/sec. The calculation was based on the concentration fall in the first 2 h as reported previously (Skadhauge, 1967).

The reflexion coefficient

Assuming $MgSO_4$ to be totally impermeant the reflexion coefficient of the solutes of plasma should appear as the ratio between the osmolality of impermeant solutes and plasma osmolality at zero net transmural water flow (Katchalsky & Curran, 1965). The perfusion was accordingly carried out with fluids slightly hypo-osmotic to the expected osmolality of plasma with a perfusion rate slow enough to result in detectable changes in phenol red concentration if they occurred and large enough to result in small differences of the osmolality between the incoming and the outgoing fluids. The net volume flow has been calculated as a function of the difference between the arithmetric mean of the osmolality of incoming and recovered fluid and the osmolality of plasma. The net water flow (-216 ± 225 µl/100 g.h) was not significantly different from zero, and the average of the mean osmolalities was $+17 \pm 27$ mOsm. This only permits the estimate of the reflexion coefficient to be within 0·9–1·1.

Osmolality and concentrations of Na^+ and Cl^- in the intestinal lumen

The samples were collected from both the upper and the lower parts of the anterior and the posterior intestine. This made possible measurements on four fractions along the length of the gut. Three eels adapted to double-strength SW, seven to SW and four to FW were used. It was not always possible to extract enough fluid from each segment to make analysis possible. In FW-adapted eels it was in most cases impossible to analyse the samples due to a high content of mucus. These samples have therefore been excluded from the following analysis. In the SW-adapted and two SW-adapted eels the intestine contained more fluid, and white precipitates (presumably $CaCO_3$) were found in the posterior intestine. No difference was apparent between SW-adapted and two SW-adapted eels, and the values have been combined and presented in Table 4. The main picture is that the contents of the anterior end of the anterior intestine correspond to approximately half-strength sea water, whereas at the lower end of the

gut NaCl concentrations are around 40–80 m-equiv./l, but the osmolality remains close to that of plasma.

Intestinal morphology

Sections of upper and lower parts of anterior and posterior intestine were examined in samples from one eel adapted to FW, two to SW, and two to 2 SW. At the light microscopy level no difference was found between the states of adaptation with regard to height of the epithelial cells, number of goblet cells, gross morphology and general development of the villi and cristae, or the thickness of the connective tissue in the core of the villus.

DISCUSSION

The coupling of salt and water flows

In this study a linear correlation between the net transmural flow of NaCl and the osmolality against which the intestine could transport water was observed (Fig. 2).

The osmolality of the solute-linked water flow remained constant when J_{NaCl} increased due to adaptation to waters of higher salinity. This means that the solute-linked water flow was proportional to J_{NaCl} (Fig. 3) as observed in other epithelia when J_{NaCl} was varied as a result of changing NaCl concentration (Curran, 1960; Clarkson & Rothstein, 1960; Edmonds & Pilcher, 1972). It was observed that the osmotic permeability was not correlated with the adaptation of the animals, although the order of magnitude was constant around 10 μl/100 g.h.mOsm. These findings are in agreement with the model proposed to account for the relationship between salt and water flows (Skadhauge, 1969). This assumed the water flow which occurs in the absence of an osmotic difference across the epithelium to be proportional both to the net NaCl absorption rate and to the osmotic permeability coefficient of water. If this flow proceeded through the lateral intercellular spaces more or less independently of the osmolality of the luminal fluid, this hypertonicity might create an osmotic water flow through the cells from plasma to lumen proportional to the osmolality difference and the osmotic permeability of the cell. That model predicts direct proportionality between J_{NaCl} and osmolality difference at zero water flow, if P_{os} is constant. If, for example, the NaCl transport is doubled this would drag double the amount of water from lumen to plasma (if as observed the tonicity of this flow is constant), resulting in a doubling of the osmolality difference against which the epithelium could transport water. The relatively constant tonicity of the solute-linked water flow in the absence of a transepithelial osmotic difference may indicate a relatively weak dependence on the P_{os} as is possible according to the mathematical treatment of Diamond & Bossert (1967). If the osmotic permeability coefficient in the serosa-to-mucosa direction – as found in these experiments – is relatively constant, proportionality between J_{NaCl} and TPΔOsm will be the result. The P_{os} predicted by this hypothesis is of the magnitude observed experimentally. 1000 μ-equiv. NaCl/100 g.h equals 1800 μl solute-linked water and predicts a TPΔOsm of 152 mOs. The P_{os} resulting in 1800 μl water flow in the other direction would be 11·8 μl/100 g.h.mOsm. Accepting a P_{os} of this magnitude also permits the conclusion that the solute-linked water flow only declines slowly as a function of increasing luminal osmolality.

The general conclusion from the present experiments is that they are compatible

with the hypothesis of the 'solute-linked' water flow being secondary to NaCl transport (possibly into lateral intercellular spaces) with the general osmotic flow occurring through a shunt pathway (possibly through the cells). The tight junctions may be permeable to water and ions (Frömter & Diamond, 1972). The large variation in P_{os} unfortunately makes it impossible to include variations in this parameter in the model to explain the changes in water flow induced by adaptation to waters of higher salinity; only an average value can be used, and only the influence of changes beyond one order of magnitude can be assessed.

The osmotic permeability coefficient

The large variation in P_{os} which was much beyond that of other parameters and thus presumably not due to experimental errors makes two questions relevant: (i) Are there different eel populations with different osmotic permeabilities ? (ii) Is the osmotic permeability a parameter which is not regulated when the eel is adapted to waters of different salinities ? If, for example, the net intestinal water absorption were fairly insensitive to the actual value of P_{os} it is understandable that P_{os} is observed as a varying parameter. Both questions raised here may – at least tentatively – be answered in the affirmative. The eels used were definitely yellow eels. But since there is no way of knowing whether they will turn silver later in the year or remain yellow it cannot be excluded that the experimental animals differed in certain endocrinological respects. Certain physiological parameters such as osmotic permeability coefficients may change before the external appearance has changed.

Intestinal transport parameters change in the Japanese eel when it turns silver before it leaves the river. Oide & Utida (1967) and Utida *et al.* (1967) observed in isolated intestine from silver eels from the river a larger sodium absorption associated with a lower osmolality of the transportate than in yellow eels. The values were close to those of silver eels from the sea. Seasonal variations in these parameters were also observed (Utida, Hirano & Kamiya, 1969). The second question has been answered by computer calculations (Kristensen & Skadhauge, 1974). The result is that the P_{os} seems to have little influence upon the net transmural water absorption. This was also found to be the case for the coprodeum and large intestine of dehydrated birds (Skadhauge & Kristensen, 1972) which transport salt and water similarly to the teleost intestine.

SUMMARY

1. An *in vivo* perfusion of the intestine of the yellow European eel (*Anguilla anguilla*) was used to measure the net absorption of NaCl and water, the osmotic permeability coefficient, the solute-linked water flow, and the osmolality difference against which the intestine could transport water as functions of the salinity of the surrounding water. The eels were adapted to fresh water, to sea water, and to 1½ strength sea water.

2. The osmolality difference against which the intestine could transport water was observed to be linearly related to the net transmural flow of NaCl; the solute-linked water flow had a constant hypertonicity in spite of differing net flows of NaCl. The findings are in agreement with the hypothesis of uphill water movement being caused by local osmosis due to the salt flow and with a shunt leak proportional to the transmural osmotic difference.

3. An important part of adaptation to waters of higher salinity is a pronounced increase in the intestinal absorption of NaCl.

4. The osmotic permeability coefficient varied from experiment to experiment without relation to the state of adaptation. An explanation for this finding may be that the osmotic permeability of the intestinal epithelium is of little importance for the total intestinal transfer of water.

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