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PROGRESSIVE PROCESSING OF INGESTED WATER IN THE GUT OF SEA-WATER TELEOSTS

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SUMMARY

The European eel's oesophagus, stomach and anterior and posterior intestine were perfused separately, *in vivo*. The oesophagus and anterior intestine play a major part in processing of ingested water. Serosal potential differences to mucosal measured *in vivo* were positive in all gut segments.

The Cl^- concentrations of luminal contents in different parts of the gut were measured in nine species of sea-water teleosts. The progressive decrease in Cl^- concentration resulted from local processing of the ingested sea water, and the beginnings of the oesophagus and of the intestine were the major processing sites.

INTRODUCTION

The gut of marine teleosts plays an essential part in compensating for the osmotic water loss through the gills. First, monovalent ions (Na^+, Cl^-) are absorbed in the oesophagus, down the concentration difference between the luminal solution and the blood (Kirsch & Laurent, 1975; Kirsch, Guinier & Meens, 1975; Hirano & Mayer-Gostan, 1976). Secondly, Na^+ and Cl^- ions are taken up in the intestine against a concentration difference (H. W. Smith, 1930; Skadhauge, 1969, 1974; Ando, 1975; Ando, Utida & Nagahama, 1975; Ando & Kobayashi, 1978). The stomach, where water and ion fluxes are small, complements oesophageal function (Hirano & Mayer-Gostan, 1976).

The energy-requiring part of the processing of ingested sea water occurs in the intestinal epithelium, where chloride ions are actively transported (Skadhauge, 1969, 1974; Ando *et al.* 1975; Ando, 1975; Field *et al.* 1978). In *in vitro* preparations of *Anguilla japonica*, the anterior part of the posterior intestine is the most active (Ando *et al.* 1975; Ando & Kobayashi, 1978).

These conclusions are based principally on experiments on isolated epithelia or on *in vivo* perfusions of very large gut segments, whole oesophagus or intestine. The present investigation was carried out on living animals, so that the sites and extent of water and chloride exchanges could be more accurately defined. In sea-water eels, four gut segments were simultaneously and separately perfused *in vivo*: oesophagus, stomach, and anterior and posterior intestine. In various marine and fresh-water teleosts, the luminal concentration of Cl^- was determined along the gut to localize regions of ion uptake.

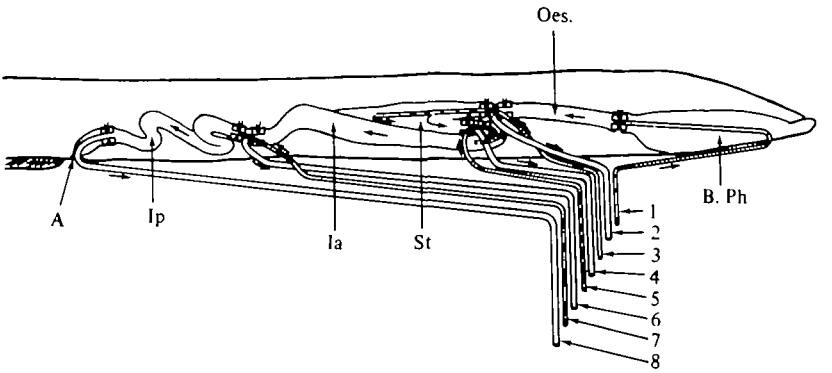

Fig. 1. Position of the cannulae in the gut of the eel. Arrows indicate the direction of perfusate circulation. A, Anus; BPh, bucco-pharyngeal cavity; Ia, anterior intestine (5 and 6); Ip, posterior intestine (7 and 8); Oes., oesophagus (1 and 2); St, stomach (3 and 4). Cannulae internal diameter: 0·87 mm for 1, 3, 5 and 7; 1·77 mm for 2, 4, 6 and 8.

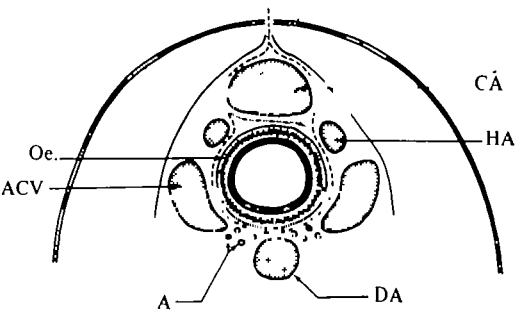

Fig. 2. Surgical ligation of the anterior oesophagus. A, Arterioles; ACV, anterior cardinal vein; CA, *conus arteriosus*; DA, dorsal aorta; HA, hypobranchial artery; Oes, oesophagus distended with rubber tube; S, skin. – – –, First step; –·–, second step.

MATERIALS AND METHODS

In vivo *perfusion of sea-water eel gut segments*

Silver eels (*Anguilla anguilla* L.) caught in Rhine River tributaries were kept in artificial sea water (SW), 520 m-equiv $Cl^-.l^{-1}$ and 440 m-equiv $Na^+.l^{-1}$ (Wiegandt GMBH, West Germany), for at least 3 weeks before experiments. Water temperature was maintained at 13 ± 0·5 °C. Eight eels, fasted for 24 h, were used in the perfusion experiments, performed in Strasbourg. They were anaesthetized with ethyl carbamate, 15 $g.l^{-1}$, for 20 min at 13 °C, then placed in ground ice to reduce oxygen consumption and allow up to 8 h for surgical preparation. The animals recovered in < 15 min when returned to running water at 13 °C. The gut was cannulated at eight levels with polyethylene catheters (Biotrol) (Fig. 1). To avoid ischaemia, ligatures were placed after isolation of the main blood vessels from the gut. The surgical procedure for ligation of the oesophagus inlet is shown in Fig. 2. A silicone catheter (Silastic 2, 0·30 mm internal diameter) was placed in the dorsal aorta via the pneumogastric artery. Catheters were passed individually through the body wall away from the surgical incisions.

Experimental procedure. The eel was placed in a restraining apparatus (Kirsch, 197

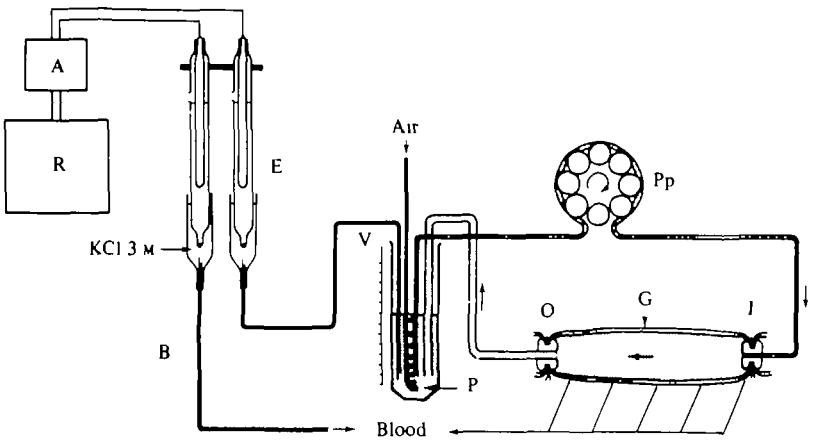

Fig. 3. Perfusion of an eel gut segment, with recording of the PDsm. A, Amplifier; Air, air bubbling; B, KCl-agar bridges; E, calomel electrodes; G, gut segment; I, inlet catheter; O, outlet catheter; P, perfusate; Pp, peristaltic pump; R, recorder; V, volume scale.

Each gut segment was perfused at the rate of 3 ml.min^{-1} by an external circuit (Fig. 3). The initial perfusates were full-strength SW for the oesophagus, SW $\frac{2}{3}$ (66 %) for the stomach, and SW $\frac{1}{2}$ (50%) for the intestinal segments. Perfusate was regularly sampled from each segment, and the volume and choride concentration, $[Cl^-]_e$, measured. When water absorption significantly reduced perfusate volume, SW diluted to adjust its $[Cl^-]$ to $[Cl^-]_e$ was added. Perfusion was continued until the $[Cl^-]_e$ of the dilute SW perfusate reached a stable value, designated $[Cl^-]_{e,f}$. Chloride influx, J_{ms,Cl^-}, of each segment was then calculated.

Measurements. Initial perfusate volume, 15–30 ml, was determined by adding polyethylene glycol ^{14}C or phenol red to the perfusate, and sampling after 10 min mixing, before progressive marker trapping by mucus would invalidate readings. Thereafter, volume was read directly on an external scale (Fig. 3). In order to calculate unidirectional chloride fluxes, perfusates prepared from SW containing 0·05–0·09 μCi.ml^{-1} of $^{36}Cl^-$ were sampled. Blood samples (100 μl) were collected, and the specific radioactivity and the chloride concentration, $[Cl^-]_l$, measured. $[Cl^-]$ was determined according to Sanderson (1952), except that the acetic acid was neutralized before sample addition.

The surface area of the digestive segments was measured at the end of the experiment by an indirect volumetric method. The organ was filled with water under a pressure of about 30 cm water. As the shape was roughly cylindrical, the water volume and length of the gut segment were used to calculate the epithelial area. This method gave results which were twice those obtained by the planimetric method, the use of which may involve contraction of the smooth muscles in the dissected organ.

Potential differences between blood and perfusate, PD_{sm}, were measured with calomel electrodes and KCl–agar bridges. PD_{sm} was measured only when perfusate $[Cl^-]_e$ had reached $[Cl^-]_{e,f}$, or when the SW perfusate had been replaced by Ringer's solution, of which the $[Cl^-]$ had been adjusted to $[Cl^-]_l$. The Ringer composition was: $Na_2HPO_4.12H_2O$, 21·8 mM; $NaH_2PO_4.H_2O$, 3·6 mM; NaCl, 143 mM; KCl, 3·3 mM; $(NH_4)_2SO_4$, 0·33 mM; KH_2PO_4, 0·36 mM; $CaCl_2$, 1.26 mM; $MgCl_2$, 4·0 mM; glucose, 1 g.l^{-1}; PVP (polyvinylpyrrolidone), 20 g.l^{-1}.

Table 1. *Symbols*

$[Cl^-]_l$	Chloride concentration in blood.
$[Cl^-]_e$	Chloride concentration in perfusate or lumen.
$[Cl^-]_{e,f}$	Stable perfusate or luminal $[Cl^-]$.
t	Time in *h*.
V	Perfusate volume in ml.
PD_{sm}	Potential difference between blood and perfusate.
$J_{x,y}$	Flux, where x represents direction and y, substance, either water ($ml.h^{-1}$) or chloride ($\mu equiv.h^{-1}$), expressed in relation to body weight or organ area. Thus:
J_{ms}	Influx, mucous to serous direction.
J_{sm}	Outflux, serous to mucous direction.
J_{net,H_2O} and J_{net,Cl^-}	Net flux of water or chloride, positive when there is a gain for the animal.

Calculations. For symbols, see Table 1.

When

V_t = perfusate volume in ml at time t in h,
$[Cl^-]_{e,t}$ = perfusate $[Cl^-]$ in $\mu equiv.ml^{-1}$ at t,
$[Cl^-]_{l,t}$ = plasma $[Cl^-]$ in $\mu equiv.ml^{-1}$ at t,
$[Cl^-]^*_{e,t}$ = perfusate $[^{36}Cl^-]$ in cpm at t,
$[Cl^-]^*_{l,t}$ = plasma $[^{36}Cl^-]$ in cpm at t,

the fluxes can be calculated according to the equations:

$$J_{net,H_2O} = \frac{V_{t_1} - V_{t_2}}{t_2 - t_1},$$

$$J_{net,Cl^-} = \frac{V_{t_1}[Cl^-]_{e,t_1} - V_{t_2}[Cl^-]_{e,t_2}}{t_2 - t_1},$$

$$J_{sm,Cl^-} = J_{ms,Cl^-} - J_{net,Cl^-},$$

$$J_{ms,Cl^-} = \frac{V_{t_1}[Cl^-]^*_{e,t_1} - V_{t_2}[Cl^-]^*_{e,t_2} - J_{net,Cl^-}([Cl^-]^*_l/[Cl^-]_l)}{(t_2 - t_1)([Cl^-]^*_e/[Cl^-]_e - [Cl^-]^*_l/[Cl^-]_l)},$$

where

$$[Cl^-]^*_e/[Cl^-]_e = \frac{1}{2}\left(\frac{[Cl^-]^*_{e,t_1}}{[Cl^-]_{e,t_1}} + \frac{[Cl^-]^*_{e,t_2}}{[Cl^-]_{e,t_2}}\right)$$

and

$$[Cl^-]^*_l/[Cl^-]_l = \frac{1}{2}\left(\frac{[Cl^-]^*_{l,t_1}}{[Cl^-]_{l,t_1}} + \frac{[Cl^-]^*_{l,t_2}}{[Cl^-]_{l,t_2}}\right)$$

J_{net,Cl^-} was determined from initial values of $[Cl^-]_e$ in perfusate up to $[Cl^-]_{e,f}$. The correlation between J_{net,Cl^-} and mean value of $[Cl^-]_e - [Cl^-]_l$ was calculated by integration of Cl^- concentrations in each experimental sequence according to the following equations:

$$[Cl^-]_e = \exp(-\tfrac{1}{2}(\log[Cl^-]_{e,t_1} + \log[Cl^-]_{e,t_2}),$$
$$[Cl^-]_l = \tfrac{1}{2}([Cl^-]_{l,t_1} + [Cl^-]_{l,t_2}).$$

In vivo $[Cl^-]$ *measurements along the gut lumen of teleosts*

Tenches (*Tinca tinca* L.) were caught in local ponds, and brown trout (*Salmo trutta morphofario* L.) and rainbow trout (*Salmo gairdneri* R.) purchased from local hatcheries

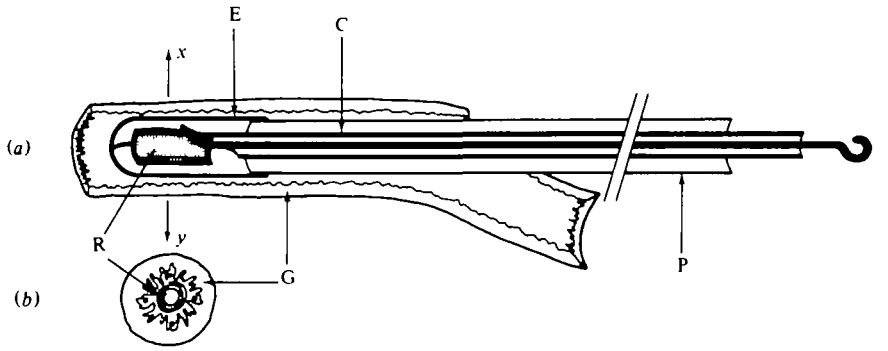

Fig. 4. Luminal medium sampling in the digestive tract. (*a*) Longitudinal section; (*b*) transverse section at *x*/*y*. C, Steel clamp; E, steel gut expander; G, gut; P, polyethylene tube; R, roll of filter paper.

They and locally caught eels were kept in running fresh water (FW), or in artificial SW for at least 3 weeks as described above for eels. SW species caught by trawling off the Shetland and Orkney Islands (oceanographic ship *Thalassa*, Institut Scientifique et Technique des Pêches Maritimes, expedition of April–May 1980) included dab (*Limanda limanda* L.), plaice (*Pleuronectes platessa* L.), megrim (*Lepidorhombus whiffiagonis* W.), whiting (*Merlangius merlangus* L.), cod (*Gadus morhua* L.) and mackerel (*Scomber scombrus* L.). Animals were fasted for at least 24 h before experiments.

Luminal fluid was sampled from several sites in the oesophagus, stomach and intestine, depending on the gut anatomy of the species, and the [Cl^-] determined. The fish was beheaded, the spinal cord destroyed to obviate movement during sampling, the pertinent part of the gut exposed, opened, and a steel expander put in place (Fig. 4). A roll of filter paper held in a polyethylene tube was brought into contact with the digestive epithelium, soaked in luminal fluid, and withdrawn without touching the incision wound. These samples were frozen and returned to Strasbourg for [Cl^-] measurements.

Sample weight was determined from the difference between the wet and original dry weight of the filter paper enclosed in a polyethylene tube to avoid evaporation. The wet filter paper was placed in neutralized acetic acid and the [Cl^-] determined according to Sanderson (1952). In order to verify that luminal solids picked up on the filter paper did not appreciably affect [Cl^-] determinations, wet control samples were dehydrated and weighed to evaluate the quantity of dry residues, derived essentially from mucus and representing very variable fractions of sample, up to 15 %.

RESULTS

In vivo *perfusion of SW eel gut segments*

Fig. 5 shows the results of a 70 h experiment on one eel, body weight 303 g. Perfusate volume was constant in the oesophagus, increased slightly in the stomach, and decreased rapidly in the anterior (AI) and posterior intestine (PI). The corresponding water fluxes, J_{net,H_2O}, which were large and positive in AI and PI, varied considerably probably because of large errors in volume determinations linked to spontaneous

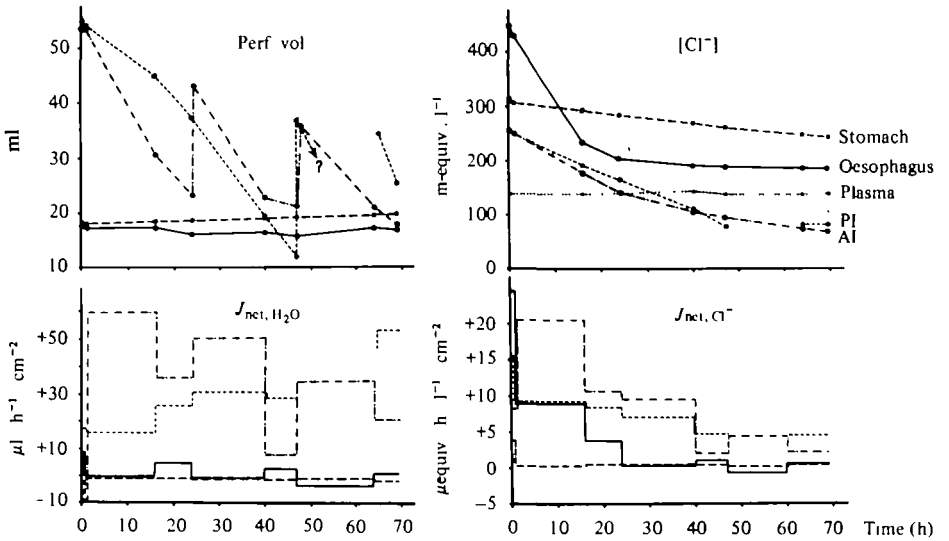

Fig. 5. SW eel, body weight 303 g, perfused for 70 h. Perf. vol., perfusate volume; $[Cl^-]$; J_{net,Cl^-}; J_{net,H_2O}; *v.* time in h. ●—●, Oesophagus; ○ - - - ○, stomach; ● –·– ●, AI, anterior intestine; ○----○, PI, posterior intestine; ✱.....✱, plasma. Step-changes for AI and PI in perf. vol.: perfusate added to make up volume of depleted intraluminal solution. Between 48 and 65 h, PI had absorbed more water than was available along volume scale, rendering readings impossible in this interval.

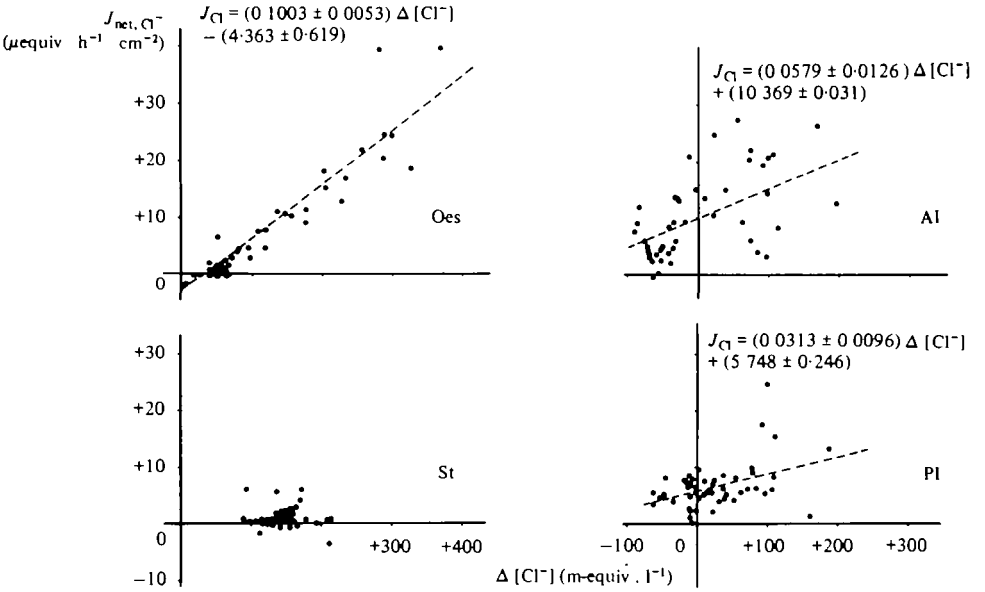

Fig. 6. J_{net,Cl^-} (µequiv.h^{-1}. cm^{-2}) as a function of the transepithelial chloride concentration difference $\Delta[Cl^-]$ in m-equiv.l^{-1} in all experiments. AI, Anterior intestine; Oes, oesophagus; PI, posterior intestine; St, stomach.

Table 2. In vivo *net chloride and water fluxes across gut segments of SW eels at the end of the perfusion period* (*means* ± S.E.M.)

	n	Area ($cm.kg^{-1}$)	$[Cl^-]_{e,f}$ (m-equiv. l^{-1})	$[Cl^-]_{l,f}$ (m-equiv. l^{-1})	J_{net,H_2O} ($\mu l.h^{-1}.cm^{-2}$)	J_{net,Cl^-} (μequiv. $h^{-1}.cm^{-2}$)	J_{net,Cl^-} (μequiv. $h^{-1}.kg^{-1}$)
Oesophagus	7	72·7 ±6·7	179 ±4	135 ±3	+0·36 ±0·26 (NS)	+0·31 ±0·15 (NS)	+20·43 ±10·74 (NS)
Stomach	7	87·5 ±14·5	252 ±7	135 ±3	−1·7 ±0·8 (NS)	+0·20 ±0·14 (NS)	+18·27 ±12·50 (NS)
Anterior intestine	7	63·4 ±5·6	86 ±18	135 ±3	+70·4 ±12·0 **	+5·94 ±0·94 ***	+364·04 ±62·09 ***
Posterior intestine	8	86·0 ±6·9	108 ±10	135 ±3	+37·0 ±6·0 ***	+4·38 ±0·59 ***	+373·23 ±55·85 ***

Comparison of experimental results to zero: NS, no significant difference; **, significant for $P < 0.01$; ***, significant for $P < 0.001$. Area of the digestive segment in the perfusion experiments; $[Cl^-]_{e,f}$, perfusate chloride concentration and $[Cl^-]_{l,f}$ plasma chloride concentration at the end of experiment.

motility of the gut. Plasma $[Cl^-]$ was stable, showing that the animal was in steady state conditions. Oesophageal $[Cl^-]_e$ decreased rapidly by a large positive J_{net,Cl^-}. In the stomach, $[Cl^-]_e$ decreased so slowly that a constant value, $[Cl^-]_{e,f}$, was never attained, and J_{net,Cl^-} was correspondingly low. In AI and PI, $[Cl^-]_e$ decreased and J_{net,Cl^-} was high and positive, as for the oesophagus.

Kinetic studies on eight SW eels (body weights 300–760 g) gave similar qualitative results. However, the rates of absorption of water and chloride varied greatly among individuals. Fig. 6 shows J_{net,Cl^-} as a function of the transepithelial concentration difference ($\Delta[Cl^-]$) in the four gut segments. In the oesophagus, J_{net,Cl^-} was almost proportional to the transepithelial $\Delta[Cl^-]$ (linear regression significant for $P < 0.00001$; $n = 49$) and fell to zero when the luminal chloride concentration still exceeded that of plasma. The maximal value of J_{net,Cl^-} calculated from the linear regression was for the oesophagus perfused with SW. Using a mean value for plasma $[Cl^-]$ of 135 m-equiv.l^{-1} (Table 2), the maximum J_{net,Cl^-} equalled 34·2 μequiv.$h^{-1}.cm^{-2}$. Taking the mean area of the oesophagus as 72·7 $cm^2.kg^{-1}$ (Table 2), the maximum J_{net,Cl^-} for the whole animal was 2·486 μequiv.$h^{-1}.kg^{-1}$. In the stomach, J_{net,Cl^-} was low and not correlated with the transepithelial $\Delta[Cl^-]$. There was a significant correlation between J_{net,Cl^-} and $\Delta[Cl^-]$ in AI ($P < 0.0001$; $n = 45$) and PI ($P < 0.013$; $n = 51$) despite considerable individual variations

Fig. 7 shows J_{net,H_2O} in AI and PI as a function of the transepithelial chloride concentration difference, $\Delta[Cl^-]$, the only gut segments where the correlation between these two factors differed significantly from zero, $P < 0.00001$ for AI ($n = 45$) and PI ($n = 51$). At isoconcentration between luminal medium and plasma ($\Delta[Cl^-] = 0$), J_{net,H_2O} was much higher in AI than in PI. J_{net,H_2O} shifted from positive to negative values for $\Delta[Cl^-] = 111$ m-equiv l^{-1} in AI, and 93 m-equiv.l^{-1} in PI, corresponding respectively to $[Cl^-] = 246$ and 228 m-equiv.l^{-1} in the intestinal lumen.

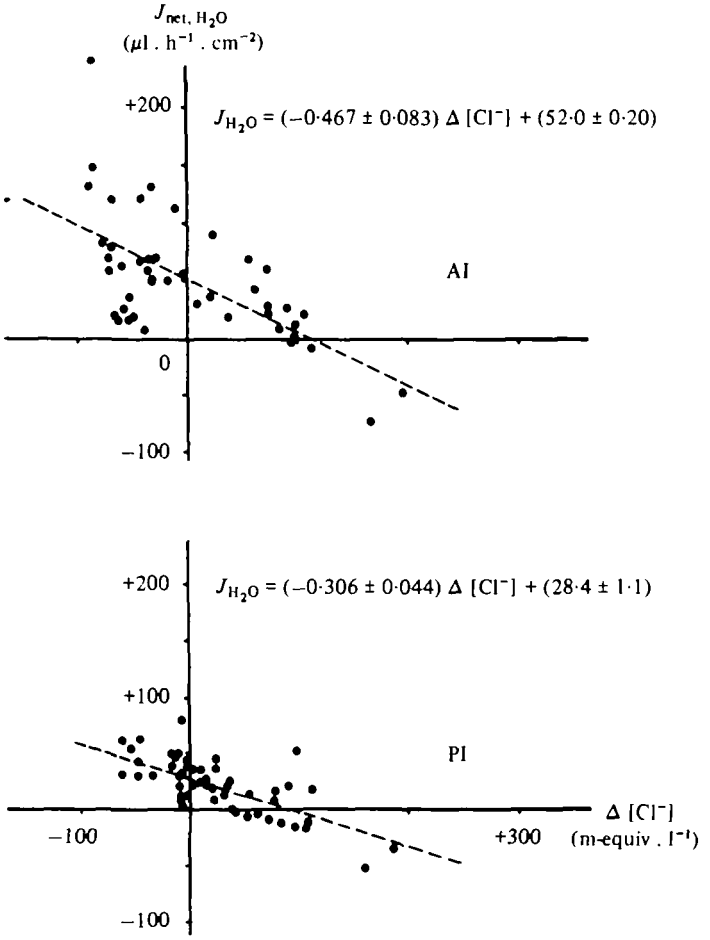

Fig. 7. J_{net,H_2O} (μl . h^{-1} . cm^{-2}) as a function of transepithelial chloride concentration difference Δ[Cl$^-$] in m-equiv . l^{-1}. AI, Anterior intestine; PI, posterior intestine.

Table 2 gives the mean values of J_{Cl^-} and J_{H_2O} at the end of the experiments when the luminal concentrations had attained constant values, $[Cl^-]_{e,f}$, except in the stomach where $[Cl^-]_{e,f}$ was not reached. The net water fluxes were not significantly different from zero in the oesophagus, and in the stomach were slightly negative and at the limit of significance. Values of J_{H_2O} were very large and positive in the intestine. The AI absorbed twice as much water as the PI. At $[Cl^-]_{e,f}$, J_{net,Cl^-} was very low in the oesophagus and stomach, and high in AI and PI. The total chloride absorption at $[Cl^-]_{e,f}$ in the intestine was 737 μequiv Cl$^-$. h^{-1} . kg^{-1}.

Table 3 presents values of unidirectional chloride fluxes, J_{ms,Cl^-} and J_{sm,Cl^-}, and of potential differences, PD, across the four gut segments at the end of the perfusion periods. Few measurements were obtained because of the difficulty of continuing *in vivo* perfusion to stable perfusate [Cl$^-$]. Cl$^-$ influxes and outfluxes were very high in all four gut segments, despite their extremely different J_{net,Cl^-} values (Table 2). This was unexpected for the oesophagus and stomach, where J_{net} values were near zero at the

Table 3. *Unidirectional chloride fluxes and potential differences across the gut of the SW eel during segmentary perfusions* in vivo

			Perf		Ringer		Gill
	J_{ms,Cl^-}	J_{sm,Cl^-}	PD_{mes}	PD_{eq}	PD_{mes}	PD_{eq}	PD_{mes}
Oesophagus	33·30	32·93	—	—	+18·0	−0·3	+19·7
	±6·63	±6·62	+9·5	−6·7	+13·0	+0·2	+10·2
	(n = 6)	(n = 6)	+11·5	−6·4	+18·7	+0·1	+15·5
Stomach	23·41	23·05	+15·5	−13·5	+23·5	+0·4	+10·2
	±3·66	±3·57	+26·0	—	—	—	+15·5
	(n = 3)	(n = 3)					
Anterior intestine	23·42	19·11	—	—	+17·5	+7·0	+19·7
	±6·21	±5·12	+8·5	+20·0	+9·75	+3·3	+10·2
	(n = 3)	(n = 3)					
Posterior intestine	12·29	7·07	—	—	+25·5	+37·9	+19·7
	±2·33	±2·39	+3·5	+36·4	+8·5	+23·6	+10·2
	(n = 6)	(n = 6)	+24·0	+24·0	+40·5	+7·5	+15·5

Owing to technical difficulties only a small number of PD were obtained, and individual values are given. J_{ms,Cl^-}, unidirectional chloride flux in the muco-serous direction (μequiv.h^{-1}.cm^{-2}); J_{sm,Cl^-}, unidirectional chloride flux in the sero-mucous direction (μequiv.h^{-1}.cm^{-2}); Perf, end of perfusion experiments; Ringer, luminal perfusion with Ringer solution adjusted to plasma Cl^- concentration; PD_{mes}, measured potential difference (mV); PD_{eq}, potential difference for Cl^- passive equilibrium distribution according to Ussing's flux ratio equation (mV).

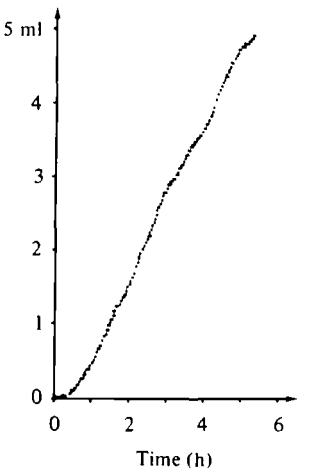

Fig. 8. Drinking rate in the eel: water accumulation (ml) at the outlet of the oesophagus.

end of the perfusion periods. PD across the digestive epithelium was electropositive on the serosal side of all segments, whether the luminal liquid was diluted SW at the end of the perfusion period, or a Ringer's solution perfusate.

In vivo [Cl^-] *measurements along the gut lumen of teleosts*

The eel, unlike higher vertebrates, drinks continuously (Fig. 8). No free luminal water could be removed with a syringe from the dissected eel gut, except from PI and the

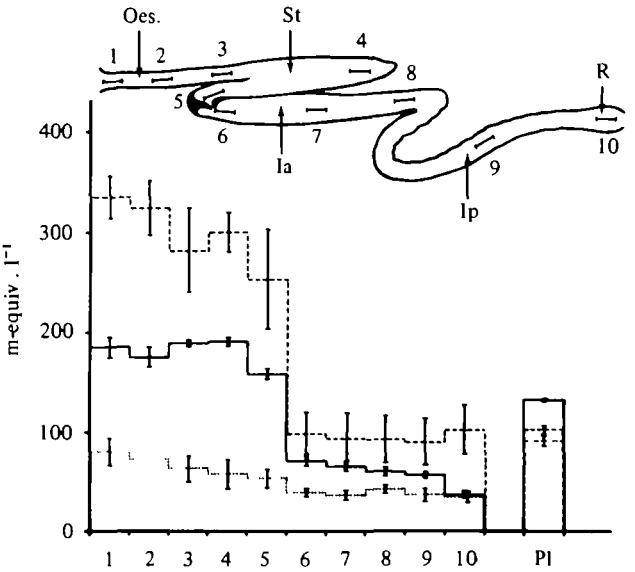

Fig. 9. Average luminal chloride concentrations along the gut of the eels. ——, adapted to SW for at least 3 weeks (n = 8); ....., FW-adapted (n = 7); – – –, 1-day SW-adapted (n = 6); vertical bars representing the SEM; Ia, anterior intestine; Ip, posterior intestine; Oes, oesophagus; Pl, plasma; R, rectum; St, stomach; 1–10, sampling sites.

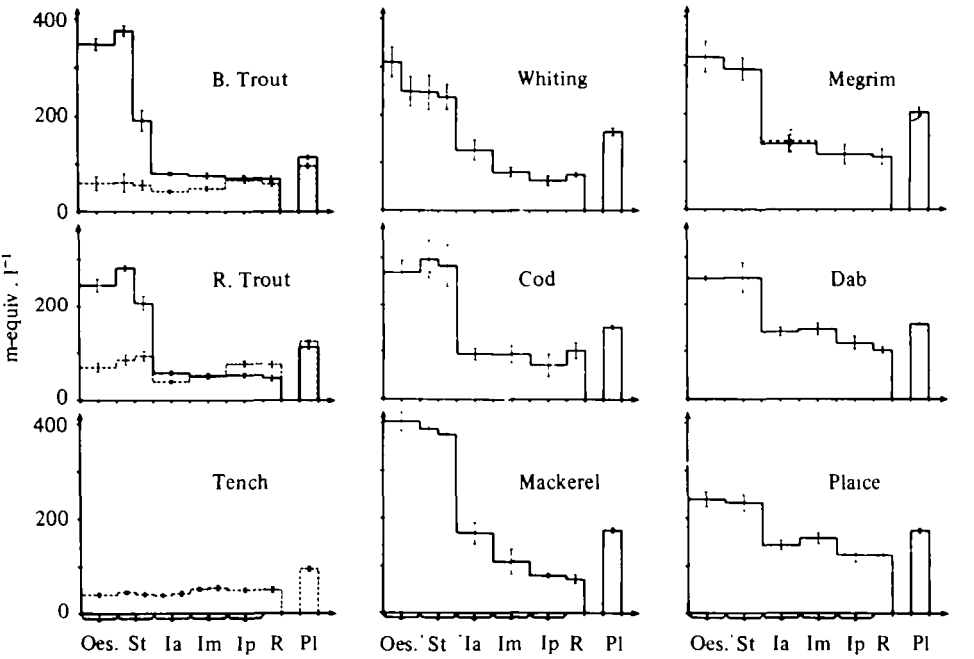

Fig. 10. Chloride concentration along the gut of nine species of teleosts. ——, SW teleosts; – – –, FW teleosts; –·–, chloride concentration in the pyloric caeca of the SW megrim.

Table 4. *Species used for* [Cl^-] *determination in the gut fluids*

Species	Water		
	SW	SW1	FW
	Body weight in g (mean ± S.E.M.) (*n*, number of fish)		
Eel, *Anguilla anguilla* L.	356 ± 31 (8)	390 ± 57 (6)	272 ± 43 (7)
Rainbow trout, *Salmo gairdneri* R.	246 ± 14 (6)	—	280 ± 15 (6)
Brown trout, *Salmo trutta morpho fario* L.	273 ± 9 (6)	—	310 ± 13 (7)
Tench, *Tinca tinca* L.	—	—	450 ± 80 (6)
Megrim, *Lepidorhombus whiffiagonis* W.	592 ± 106 (6)	—	—
Dab, *Limanda limanda* L.	221 ± 39 (6)	—	—
Cod, *Gadus morhua* L.	1675 ± 110 (6)	—	—
Mackerel, *Scomber scombrus* L.	498 ± 37 (6)	—	—
Whiting, *Merlangius merlangus* L.	657 ± 160 (6)	—	—
Plaice, *Pleuronectes platessa* L.	330 ± 59 (5)	—	—

SW, Animals caught in sea water or adapted for at least 3 weeks to sea water; SW1, eels adapted for 1 day to sea water; FW, animals caught in fresh water.

rectum. This implies that there may be a laminar flow of ingested water at least along the anterior gut. The comparison of successive local chloride concentrations (Fig. 9) thus characterizes the extent of local chloride exchanges.

Chloride concentrations along the gut lumen were measured in 10 teleost species. Fig. 9 shows [Cl^-] at ten sites and in the plasma of eels adapted to SW, to FW, or after 1 day in SW, SW1. Fig. 10 gives luminal [Cl^-] at several sites and in the plasma of nine other teleosts. Mean body weights, number of fish, and water of origin or adaptation appear in Table 4.

In SW animals, luminal [Cl^-] in the first few millimetres of the oesophagus was very low compared to the [Cl^-] of SW, 520 m-equiv.Cl^-.l^{-1}. The range was 185–348 m-equiv.l^{-1}, 36–67 % SW, except in the mackerel, 403 m-equiv.l^{-1}. [Cl^-] changed very little then up to the stomach. In species with a U-shaped stomach – trout and eel – [Cl^-] decreased substantially in the well-differentiated tubular posterior ascending section, but [Cl^-] in the luminal liquid was still higher than in plasma. In all SW animals, there was a large decrease of [Cl^-] along the first few millimetres of the intestine, to values lower than plasma values. Luminal [Cl^-] changed little along the remainder of the intestine, except in the mackerel.

In FW animals, intraluminal [Cl^-] was low and practically constant along the whole gut. In euryhaline species, the PI [Cl^-] did not differ significantly between SW- and

FW-adapted specimens. When FW eels were transferred to SW for one day, the average [Cl^-] values were higher than those of SW animals, and the individual values more scattered.

DISCUSSION

Osmoregulation in the eel

Oesophagus. The SW perfusion experiments confirm previously published results. There are large influxes of Cl^- from SW to plasma down the concentration difference across the water-impermeable oesophageal epithelium. Our values of J_{net,Cl^-} for the total oesophagus perfused with SW are comparable to those obtained on isolated oesophageal sacs by Hirano & Mayer-Gostan (1976) (i.e. 34·2 and 24·0 μequiv.h^{-1}.cm^{-2} respectively), but are slightly larger, probably because physiological conditions, especially the blood supply, are better in the *in vivo* preparation. The real difference may be even larger, for the planimetric measurement used by Hirano & Mayer-Gostan probably underestimates oesophagus area by a factor of 2.

Unlike the perfused animal, normal eels drink water continuously and SW does not reach the end of oesophagus (Figs. 8 and 9). J_{net,Cl^-} in the normal animal can be calculated, from the mean drinking rate of SW-adapted eels, 1·437 ml.h^{-1}.kg^{-1} (Kirsch & Mayer-Gostan, 1973), and the [Cl^-] of SW and luminal water (present work) at the end of oesophagus, 520 and 189 m-equiv.l^{-1} respectively, to be 475 μequiv.h^{-1}.kg^{-1}. This is about one-fifth of the value of J_{net,Cl^-} for SW perfusion, 2.486 μequiv.h^{-1}.kg^{-1}. Thus only the very anterior oesophagus is necessary to decrease the [Cl^-] to a threshold below which the oesophagus is unable to transfer Cl^- from lumen to blood. Similar values for this threshold were obtained in perfusion experiments, 179 m-equiv.l^{-1} (Table 2), and *in situ*, 189 m-equiv.l^{-1} (Fig. 9). For a Cl^- exchange equally distributed in the oesophageal epithelium, and a J_{net,Cl^-} linearly related to transepitheal $\Delta(Cl^-)$ (Fig. 6), about two-fifths of oesophageal length would be necessary to decrease the Cl^- concentration from that of ingested SW to the threshold. But the most anterior Cl^- concentration measured *in situ*, 186 m-equiv.l^{-1}, was not significantly different from the threshold, and localized well to the front of the first two-fifths of the oesophagus. The first few millimetres of the oesophagus are thus much more efficient in SW processing than the remainder. A detailed functional and structural analysis of the initial oesophagus segment is in progress.

Extensive modifications of the oesophageal structure occurring during the eel's adaptation from FW to SW (Laurent & Kirsch, 1975; Yamamoto & Hirano, 1978) take about 7 days and are related to oesophageal Cl^- exchange efficiency. The present observations (Fig. 9) show that after only 24 h of SW adaptation the whole oesophagus participates in Cl^- exchanges, and that the threshold characteristic of the SW animal is not reached at the end of the oesophagus.

Stomach. In perfusion experiments, there was no significant Cl^- net flux through the stomach wall of fasted animals (Table 2); in contrast, Hirano & Mayer-Gostan (1976) observed a J_{net,Cl^-} of about 5 μequiv.h^{-1}.cm^{-2} in isolated stomachs containing SW. A small water loss in the sero-mucosal direction, described by Hirano & Mayer-Gostan, appeared possible. However, in our perfusion experiments the final luminal Cl^- con-

centration, 252 m-equiv.l^{-1}, was well above the *in situ* value, 190 m-equiv.l^{-1}, which was the same as the posterior oesophageal Cl^- concentration, 189 m-equiv.l^{-1}. Thus probably no dilution by sero-mucous water flux occurs in the stomachal sac *in vivo*.

In the posterior ascending part of stomach, excluded in perfusion experiments, luminal [Cl^-] *in situ* decreased to 158 m-equiv.l^{-1}. Consequently it is not known whether this 17 % decrease is linked to net flux of solute or water.

Intestine. As previously shown (Skadhauge, 1969, 1974; Ando, 1975; Ando *et al.* 1975; Ando & Kobayashi, 1978; Hirano & Mayer-Gostan, 1976), the anterior and posterior intestine rapidly absorb Cl^- ions and water. Skadhauge (1969, 1974) reported zero net water flux in the eel intestine when osmolality was 126 m-osmol higher in the lumen than in the plasma. This corresponds approximately to our observed difference in Cl^- concentrations at zero net water flux of 111 m-equiv.l^{-1} in AI and 93 m-equiv.l^{-1} in PI (Fig. 7). At isoconcentration between plasma and lumen, Hirano & Mayer-Gostan found J_{net,Cl^-} of about 7·6 μequiv.h^{-1}.cm^{-2} for the whole intetine, while we observed 10·4 (AI) and 5·7 μequiv.h^{-1}.cm^{-2} (PI) (Fig. 6). Ando *et al.* (1975), however, described an efficiency for Cl^- transport greater in the posterior than in the anterior intestine of *Anguilla japonica*. Ando & Kobayashi (1978) reported net water fluxes at isoconcentration for the anterior and posterior part of stripped posterior intestine of 61·6 and 35·2 μl.h^{-1}.cm^{-2} respectively. Our *in vivo* perfusions gave 52·0 for anterior intestine and 28·4 μl.h^{-1}.cm^{-2} for posterior intestine (Fig. 7); thus, in European eels, the anterior intestine also absorbs water more efficiently than the posterior intestine. Cl^- concentrations in the intestinal lumen *in situ* in SW eels ran from 70 ± 5 at the proximal end to 56 ± 3 m-equiv.l^{-1} at the distal end, and 36 ± 4 m-equiv.l^{-1} in the rectum (Fig. 9). These values are lower than those reported by Skadhauge (1974) in eels in which free water could be collected in the intestinal lumen, and also lower than final concentrations reached in our perfusion experiments, 86 in AI and 108 m-equiv.l^{-1} in PI.

The limiting luminal concentration of Cl^- for transepithelial absorption of this anion is thus probably not reached as long as free-flowing water is available in the intestine. In steady-state, SW-adapted eels, the luminal [Cl^-] is very low from the beginning of the intestine, indicating that a very short stretch of the anterior intestine absorbs most of the Cl^-. Cl^- concentration decreases in a few millimetres, from 158 m-equiv.l^{-1} in the stomach to 70 m-equiv.l^{-1} in the intestine. The functional and structural characteristics of the initial part of the intestine are now being investigated.

Unidirectional Cl^- fluxes and transepithelial potential differences

The gut epithelium is very permeable to Cl^- ions, and significant unidirectional fluxes were observed even in conditions where J_{net,Cl^-} was close to zero, at the end of the perfusion experiments (Table 3), as in the oesophagus and stomach. This may represent a capacity for SW processing by these epithelia which are immediately efficient when the eel gulps its prey and SW enters the posterior oesophagus and stomach.

The oesophagus and stomach are seropositive relative to the lumen, as is the rabbit's oesophagus (Powell, Morris & Boyd, 1975), and Cl^- ions are not passively distributed according to the theoretical potential calculated by Ussing's flux ratio equation (Ussing,

1958) (Table 3). Previous data on unidirectional Cl^- fluxes and potential differences across the intestine of teleosts are all derived from *in vitro* preparations (Lahlou, Smith & Ellory, 1974; Smith, Ellory & Lahou, 1975; Ando, 1975; Ando *et al.* 1975; Ando, 1978; Field *et al.* 1978; Duffey *et al.* 1979; Albus, Groot & Siegenbeek Van Heukelom, 1979; Huang & Chen, 1971; Utida *et al.* 1972) and all give seronegative values, indicating an active Cl^- transport (Ando *et al.* 1975; Ando, 1975; Ando & Kobayashi, 1978; Field *et al.* 1978). In marked contrast, we always observed seropositive potential differences, as reported for the rat intestine (Hardcastle, Hardcastle & Redfern, 1980). Thus the main part of the Cl^- exchanges could be passive, according to the theoretical potential difference calculated by Ussing's flux ratio equation. Our results are too scanty to allow detailed quantitative analysis, but are reliable as to the direction of the potential difference, as the gill potentials measured simultaneously were seropositive (Table 3) as reported previously for *in vivo* preparations by House & Maetz (1974).

Gut and osmoregulation in different teleost species

Oesophageal osmoregulatory function has been previously demonstrated on isolated preparations of the whole oesophagus of several SW teleost species (Kirsch, 1978). The present determinations of local luminal Cl^- concentrations show that in all the SW teleosts investigated, two regions are essential for Cl^- absorption: the first few millimetres of the oesophagus and of the intestine. In the intestine, a relatively stable luminal fluid composition may be physiologically important, since Holstein (1979*a*, *b*) has shown in the cod that gastric acid secretion is inhibited when the intestine is perfused with SW or 66 % SW, but that the secretion is normal when the intestine is perfused with 50 % or 33 % SW. Thus the ability to osmoregulate in the most anterior parts of the gut may be necessary for normal digestive function.

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