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Physiology

Eel esophagus as an osmoregulatory organ

(ion and water permeabilities/dilution of sea water/seawater adaptation)

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ABSTRACT **Ion and water permeabilities were measured in the isolated esophagus of the eel (*Anguilla anguilla* and *A. japonica*), and compared with those in the stomach and the intestine. The freshwater eel esophagus was impermeable both to Na^+ and Cl^- ions and to water, whereas permeabilities to the ions increased selectively after seawater adaptation. The ion permeabilities of both the freshwater and the seawater eel stomach were lower than in the seawater eel esophagus, although water permeability was greater than in the esophagus. Sea water enclosed in the lumen was diluted three times more efficiently in the seawater eel esophagus than in the stomach. The intestinal permeabilities were greater than those of the esophagus and the stomach, and increased after seawater adaptation. In the eel, ingested sea water seems to be diluted mainly in the esophagus by passive diffusion of the ions into the blood without addition of water. After further but less important dilution in the stomach with salt removal and with water addition, the water is absorbed by the intestine, following active absorption of the ions. Thus the eel in sea water is able to replace water lost osmotically by drinking hypertonic sea water.**

It is well accepted that marine teleosts drink sea water to replace water lost osmotically across the body surface. The common view is that swallowed sea water, which is hyperosmotic to the body fluid, is diluted in the stomach and later in the intestine by osmotic influx of water from blood to lumen, and that the water is then absorbed from the intestine by some mechanism dependent on the active uptake of monovalent ions into the blood (1–3).

Unlike most of the teleost fishes, the eel has a gastrointestinal tract that is anatomically divided into three distinct regions: esophagus, stomach, and intestine (4). When the drinking rate of the seawater eel was measured by cannulating the esophagus, we noticed that the eel does not gulp water intermittently, but ingests it continually; the ingested water seems to move along the esophagus rather slowly (5). Recently, Kirsch and Laurent (6) found that swallowed sea water is already diluted to one-half by the time it reaches the posterior end of the eel esophagus. The present investigation was carried out in order to elucidate the role of the esophagus in the dilution of ingested sea water.

MATERIALS AND METHODS

Ten Japanese cultured eels, *Anguilla japonica*, weighing about 200 g each, were purchased from a commercial source and kept in a freshwater tank at 20° for at least 2 weeks before use. Five eels were then transferred to a seawater tank and kept there for 2 more weeks. Experiments were also done on the European eel, *A. anguilla*, weighing about 250 g. These eels were collected from the estuary of the river Rhône and kept in running fresh water (four eels) or sea water (four eels) at 17° for 3 weeks before use.

After decapitation, about two-thirds of the esophagus (2–3 cm in length) and the entire stomach and the intestine were isolated and washed by flushing with modified Krebs-Ringer bicarbonate (Ringer) solution with the following concentrations: 127 mM NaCl, 3.0 mM KCl, 2.5 mM $CaCl_2$, 3 mM $MgSO_4$, 1.25 mM KH_2PO_4, 25 mM $NaHCO_3$, gassed with 95% O_2–5% CO_2. The intestine was not used in *A. anguilla*. After equilibration for 30 min with Ringer solution, the gut regions were tied at one end with a cotton thread, filled with Ringer solution, and tied at the other end. Care was taken to avoid excess distension of the sac. The filled sac was lightly blotted on filter paper, weighed, and transferred to an incubation vessel containing 50 ml of Ringer solution. The vessel was gassed with 95% O_2–5% CO_2 and incubated with shaking at 20°. The final volume was obtained by weighing the sac before and after emptying. Each sac was then washed and filled with sea water (artificial sea water, Jamarin: 440 meq/liter of Na^+, 516 meq/liter of Cl^- or Villefranche Bay sea water: 540 meq/liter of Na^+, 600 meq/liter of Cl^-) and incubated in Ringer solution. The incubation time was 60 min for the esophagus and the stomach, while the intestine was incubated for 30 min to keep the change in concentration gradient minimal.

The surface area of the sac was measured after cutting the segment along its long axis and spreading on graph paper. Net movements of Na^+ and Cl^- ions were calculated for each incubation from the difference between the initial and the final concentrations of the ions. Sodium and chloride concentrations were estimated using a Hitachi 203 atomic absorption spectrophotometer or an Eppendorf flame photometer and Buchler-Cotlove chloridometer.

RESULTS

Since essentially the same results were obtained in *A. anguilla* and in *A. japonica*, the data from two species of the eels were combined in the following figures and table. When the organ was incubated on both sides with isotonic Ringer solution, no movement of either water or Na^+ and Cl^- ions was seen in the esophagus (Fig. 1). There was also no water and Na^+ movement in the stomach, whereas significant ($P < 0.01$) secretion of Cl^- ion into the lumen was observed. There was no significant difference in the Cl^- secretion rate between the seawater and the freshwater eel stomach. In contrast to the situation in the esophagus and the stomach, net absorption of water and the ions was seen in the intestine, and the rates of absorption were significantly greater in the seawater eel than in the freshwater eel.

Fig. 2 shows net movements of water and ions in the gut, filled with sea water and incubated in Ringer solution. In the freshwater eel esophagus, a small amount of water moved into the lumen and Na^+ and Cl^- ions moved out of the lumen, probably following the concentration gradient.

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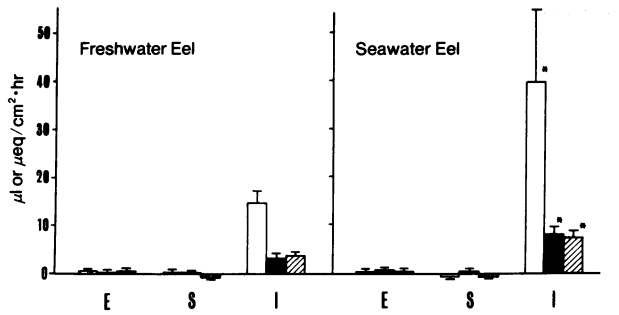

FIG. 1. Net movements of water and Na^+ and Cl^- ions in the isolated esophagus (E), stomach (S), and intestine (I) of the seawater and the freshwater eels, bathed on both sides with isotonic Ringer solution. Empty bars represent water movement, black bars Na^+ movement, and shaded bars Cl^- movement. Positive values indicate mucosa to serosa movement. Standard errors of the means (n = 9, 5 *A. japonica* and 4 *A. anguilla* for the esophagus and the stomach, and n = 5 *A. japonica* for the intestine) are indicated by vertical lines.

* Significantly different from the freshwater value at 5% level.

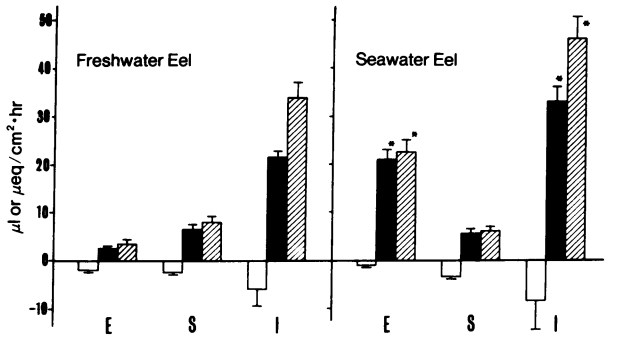

FIG. 2. Net movements of water and Na^+ and Cl^- ions in the isolated gut of the eel, filled with sea water and incubated in Ringer solution. Legend as in Fig. 1.

The ion movements increased greatly after seawater adaptation, without any change in water permeability. In the stomach, water movement was greater than in the esophagus, whereas the ion movements were greater than those in the freshwater eel esophagus but smaller than in the seawater eel esophagus. There was no difference in water and ion movements in the stomach between freshwater and seawater eels. The rates of ion movements in the freshwater eel intestine were as great as those in the seawater eel esophagus, and increased further after seawater adaptation. The water movement in the intestine was greater than in the esophagus and the stomach. However, it was highly variable, absorption of water being seen in some preparations, and no significant difference was detected between freshwater and seawater eels.

Efficiency of seawater dilution was compared in the seawater eel gut filled with sea water by expressing water and ion movements in water gain or Na^+ loss per ml of fluid (Table 1). The seawater eel esophagus gained little water but lost a large amount of Na^+ (and also Cl^-) ions; sea water is diluted in the esophagus to two-thirds in 1 hr, mainly by removal of the ions. Sea water seems to be diluted in the stomach by passive diffusion of the ions (less than in the esophagus) and also by an osmotic influx of water (more than in the esophagus). However, 1 ml of sea water enclosed in the stomach was diluted only by 10% in an hour; dilution of sea water in the esophagus was about three times more efficient than in the stomach. On the other hand, dilution of sea water enclosed in the intestine was twice as fast as in the esophagus; Na^+ concentration decreased to two-thirds 30 min after incubation in the intestine and after 60 min in the esophagus.

DISCUSSION

Smith (1) proposed that marine teleosts drink sea water to replace water lost osmotically across the body surface. Based on changes in ion composition of gastrointestinal fluid, he suggested that swallowed sea water is diluted first in the stomach and later in the intestine with an osmotic influx of water from the blood; subsequent absorption of water takes place in the intestine following active absorption of monovalent ions. Various authors tested this hypothesis on *in vitro* preparations from several teleost species by introducing sea water into the gut lumen. They observed that intestinal sacs filled with sea water and incubated in Ringer solution rapidly gained weight, followed by a weight loss (absorption) after some time (7–9). In *Anguilla japonica*, the reversal of water flow occurred earlier in the seawater eel intestine than in the freshwater eel (9). A similar observation was also made *in vivo* by Skadhauge (10) in the perfused intestine of *A. anguilla*, and the osmolality of the perfusion fluid which corresponds to zero net flow of water (the turning point osmolality) was higher than plasma osmolality by 126 milliosmoles/kg in seawater eels and by 73 milliosmoles/kg in freshwater eels. This indicates that the seawater eel intestine is able to absorb water from hypertonic salt solution (about half-strength sea water).

However, our results show that ingested sea water is diluted before it reaches the intestine and even before it reaches the stomach. The esophagus thus plays an important role in dilution of ingested sea water. We have shown that the esophagus of the seawater eel is highly permeable to Na^+ and Cl^- ions but not to water: sea water is diluted in the esophagus mainly by salt removal without water addition. *Dilution* is therefore a misleading term; *desalting* is more

Table 1. Movements of water and Na^+ ion in the isolated gut of the seawater eel (*A. japonica*), filled with sea water and incubated in Ringer solution

	Incubation time (min)	Water gain/ml initial fluid (μl)	Na^+ loss/ml initial fluid (μeq)	Na^+ concentration (meq/liter)	
				Initial	Final
Esophagus	60	7.3 ± 4.2	135.9 ± 13.9	443 ± 3.1	310 ± 8.7
Stomach	60	26.4 ± 7.4	31.4 ± 8.4	443 ± 3.1	401 ± 8.5
Intestine	30	47.9 ± 13.5	105.0 ± 15.4	442 ± 3.7	323 ± 17.2

* Mean ± SEM (n = 5).

accurate. A similar conclusion was obtained *in vivo* by perfusing the esophagus of *A. anguilla* with sea water (6).

The sequence of events in replacing water lost osmotically in the seawater eel may be as follows. In the steady state, eels in sea water drink water continually, the drinking rate being modified probably by sphincter action at the beginning of the esophagus. During the slow passage along the esophagus, Na^+ and Cl^- ions diffuse passively into the blood; these ions are quickly excreted from the gills, thus keeping the elevation of plasma ion concentration minimal. The seawater eel in the steady state drinks water at the rate of about 0.35–0.4 ml/100 g·hr (11). Since the eels used in the present study weighed about 200 g, 0.7–0.8 ml of sea water would move along the esophagus in an hour. When 1 ml of sea water was enclosed in the seawater eel esophagus, 135 μeq of Na^+ ion were removed from the lumen in an hour, resulting in two-thirds dilution. Considering that ion movement in the isolated preparation would be less than the movement *in vivo* with intact blood circulation, and also that about two-thirds of the esophagus was used in the present study, dilution of ingested sea water in the esophagus would be even more efficient *in vivo*.

Thus, stomach receives partly "desalted" sea water, and further dilution will take place by an osmotic influx of water and passive loss of ions following concentration gradient. In the goosefish, *Lophius piscatorius*, Smith (1) found that concentrations of Mg^{2+} ions in the gastric fluid are lower than in sea water, and ascribed this to dilution with gastric juice and to an osmotic influx of water from the blood. In the eel, however, dilution with gastric juice may not be so important, since no appreciable water movement was seen when the stomach was incubated on both sides with identical Ringer solution. Moreover, the stomach was less permeable to ions than the seawater eel esophagus, and dilution of the enclosed sea water in the esophagus was about three times more efficient than in the stomach. Considering the fact that the stomach receives already "desalted" sea water, dilution in the stomach may not be important, at least in the eel. In this respect, it is interesting to find that Na^+ concentration and osmolality of the gastric fluid are always greater than those of the body fluid in the goosefish (1), the eel (7), and the flounder (12). According to Skadhauge (13), the osmolality of the luminal fluid taken from the upper end of the anterior intestine of *A. anguilla* is about 460 milliosmoles/kg, corresponding to about half-strength sea water. Considering the "turning-point osmolality" or the capacity of the seawater eel intestine to absorb water from hypertonic salt solution (10), the seawater eel seems to be able to absorb water from the intestine without losing water from the body.

In contrast to the esophagus of the seawater eel, the freshwater eel esophagus was almost impermeable to both water and the ions. The physiological importance of the impermeability of esophagus to water and ions is not clear, since the eel in fresh water is known to drink little water (1, 5, 11). Since the water permeability of the intestine was still greater than that of the esophagus and the stomach, the small amount of fresh water the eels ingest will be absorbed partly in the stomach and completely in the intestine. At any rate, the change in ion permeability observed in the eel esophagus seems to be part of the adaptation to different environmental salinities. In the present study, an increase in ion and water permeability was also seen in the intestine following seawater adaptation. This increase in absorptive capacity of the intestine has been repeatedly observed in the eel, and is known to be inhibited by prolactin in the freshwater eel and facilitated by cortisol in the seawater eel (3, 14). It seems highly probable that the ion permeability of the eel esophagus is also under hormonal control. The involvement of hormones, permeability characteristics, and morphological changes associated with the functional changes in the eel esophagus invite further study.

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1. Smith, H. W. (1930) *Am. J. Physiol.* **93**, 480–505.
2. Maetz, J. (1974) in *Biochemical and Biophysical Perspectives in Marine Biology*, eds. Malins, D. C. & Sergent, J. R. (Academic Press, London, New York, San Francisco), pp. 1–167.
3. Hirano, T., Morisawa, M., Ando, M. & Utida, S. (1975) in *Intestinal Ion Transport*, ed. Robinson, J. W. L. (Medical & Technical Pub. Co., Lancaster, England), pp. 300–317.
4. Suehiro, Y. (1942) *Jpn. J. Zool.* **10**, 1–303.
5. Hirano, T. (1974) *J. Exp. Biol.* **61**, 737–747.
6. Kirsch, R. & Laurent, P. (1975) *C. R. Acad. Sci. Paris* **280**, 2013–2015.
7. Sharratt, B. M., Bellamy, D. & Chester Jones, I. (1964) *Comp. Biochem. Physiol.* **11**, 19–30.
8. House, C. R. & Green, K. (1965) *J. Exp. Biol.* **42**, 177–186.
9. Utida, S., Isono, N. & Hirano, T. (1967) *Zool. Mag.* **76**, 203–204.
10. Skadhauge, E. (1969) *J. Physiol. (London)* **204**, 135–158.
11. Oide, H. & Utida, S. (1968) *Mar. Biol.* **1**, 172–177.
12. Hickman, C. P., Jr. (1968) *Can. J. Zool.* **46**, 457–466.
13. Skadhauge, E. (1974) *J. Exp. Biol.* **61**, 737–747.
14. Utida, S., Hirano, T., Oide, H., Ando, M., Johnson, D. W. & Bern, H. A. (1972) *Gen. Comp. Endocrinol. Suppl.* **3**, 317–327.