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# KINETICS OF WATER AND CHLORIDE EXCHANGES DURING ADAPTATION OF THE EUROPEAN EEL TO SEA WATER

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Since Smith (1930) and Keys (1933) studied water and electrolyte exchanges in the eel much work has been done on osmoregulatory problems in teleosts, and the fundamental principles of osmoregulation in this group have now been established. The freshwater teleost drinks little and urinates abundantly to compensate for osmotic water uptake through the gill; the marine teleost drinks abundantly ('drinks like a fish' in fact) to make good water loss through the gill. The eel, euryhaline and migratory in varying salinities during its life-cycle, is an excellent choice for the study of osmoregulatory problems.

Transferring an eel from fresh water to sea water results in an increase of plasmatic electrolytes (Oide & Utida, 1968; Mayer & Nibelle, 1970; Kirsch, 1972). This increase could be caused by all or any of three factors – factors which it is difficult to dissociate experimentally or to estimate quantitatively. They are: (*a*) dehydration due to the reversal of the osmotic gradient between internal and external media; (*b*) an influx of electrolytes temporarily higher than the outflux; (*c*) a considerable uptake of water from the digestive tract.

The present work is concerned with the last of these phenomena, with regard to which previous data are contradictory. Oide & Utida (1968) report a gradually increasing ingestion of sea water *in vivo* with a maximum on the 5th day after transfer, followed by a reduction and stabilization. Kirsch (1972*b*) isolated the head region of the eel and observed a considerable ingestion of water at the moment of osmotic shock. We have therefore undertaken complete and detailed study of the kinetics of the drinking rate during sea-water adaptation, with a parallel study of the little-known chloride exchanges. Dehydration was also followed indirectly by measurements of body weight.

## MATERIAL AND METHODS

Silver eels, *Anguilla anguilla*, from the affluents of the Rhine were kept in running fresh water for at least 8 days before experimentation. The temperature was uncontrolled (14–18 °C) and the eels were not fed. They measured from 60 to 70 cm and had an average weight of $388{\cdot}3 \pm 9{\cdot}1$ g ($n = 95$).

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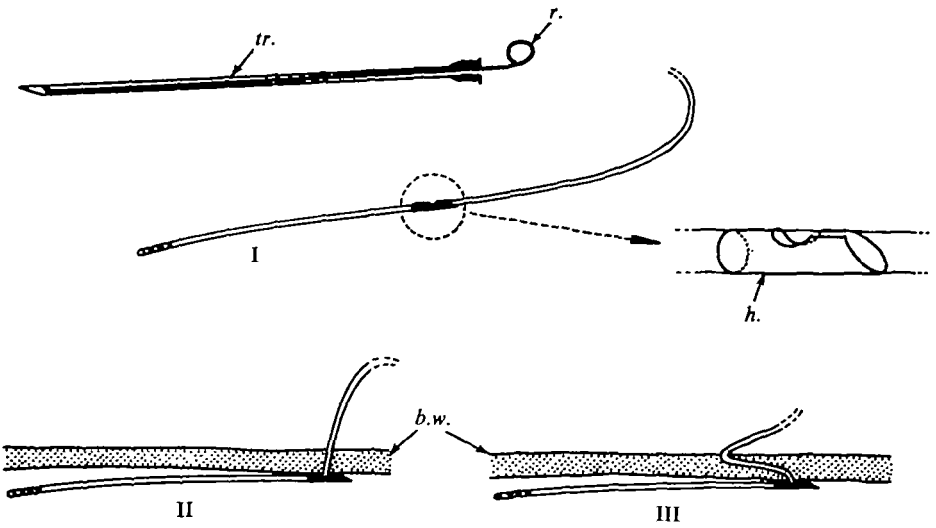


Fig. 1. Intraperitoneal catheter. *tr.*, Trocar; *r.*, rod; *h.*, hook; *b.w.*, body wall. I, catheter before setting; II, III, different steps of the setting.

### *Preparation of animals*

A special intraperitoneal catheter of thin polyvinyl tubing (0·6 mm internal diameter) was used (Fig. 1). A polyethylene hook (*h.*), 6 cm from the extremity, opened once the catheter was in place and prevented accidental displacement. The catheter was inserted through a 10 cm trocar, the eel being anaesthetized with a 1 ‰ solution of Ms 222 (methane tricaine sulphonate). The trocar was first completely inserted in a posterior–anterior direction into the body cavity 5 cm in front of the ano-genital papilla. During insertion the rod (*r.*) shielded the point of the trocar to protect the viscera, it was then withdrawn and the catheter inserted in its place. Then the trocar was removed and the hook was made to open against the muscular wall by pulling on the catheter. The external tube of the catheter was then threaded through a needle so that it could be pulled through the skin to a new exit about 2 cm away from the original hole made by the trocar, giving the final arrangement as in Fig. 1 (III). This S-bend reduced risks of leakage round the insertion point, especially in long-standing catheterization.

### *Experimental techniques*

Each weighed eel with its intraperitoneal catheter was placed in an individual sealed polyethylene bag in a restraining trough. Six of such eels were grouped in a common water-bath, isolating them from external disturbance. Each bag had a tube at each end for the water circulation (Fig. 2). The temperature was controlled (13 ± 1 °C).

During the pre-adaptation period the water irrigating the bags was in open circuit (A–B). The device for the circulation to be used during measurements was set up the day before experiments. It can work in open circuit A′–B′–B″ or in closed circuit. The closed circuit consisted of two tanks: an upper (*u.t.*) and a lower (*l.t.*). Water flowed from *u.t.*, over the eel and accumulated in *l.t.* (circulation C–B′). When the water level rose in *l.t.* the rising float switched on (microswitch, *mic.*) the pump (*p.*) which pumped the water (by E) back into *u.t.* The change from open circuit A′–B′–B″ to closed circuit

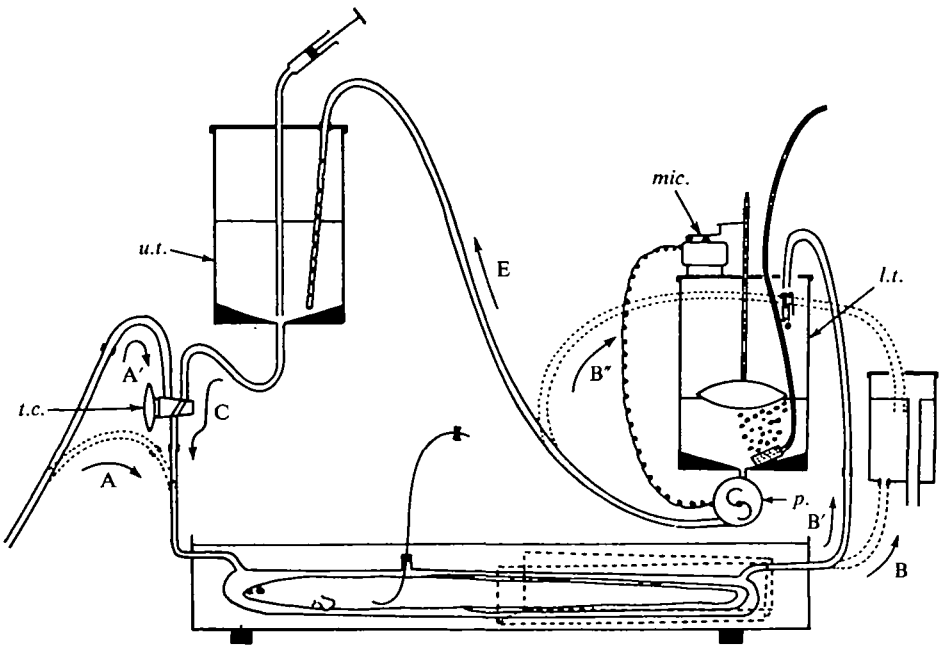


Fig. 2. Experimental device. *u.t.*, Upper tank; *l.t.*, lower tank; *t.c.*, three-way cock; *p.*, pump; *mic.*, microswitch. Water circulation: open circuit, A′, B′, B″; closed circuit, C, B′, E.

was made by reversing the three-way cock (*t.c.*) and changing the outflow of *u.t.* from position B″ to E.

All the eels were put into their individual bags at least 3 days before measurement in fresh water or transfer to sea water. Each group of 6 eels was pre-adapted to sea water for a different length of time. The water to which the eels were subjected could be changed during experiments without disturbing the animals by changing that in the tanks *u.t.* and *l.t.* in the closed circuit. This left a certain residual volume of water of about 500 ml (*v*) in the polyethylene bag and tubes. After the water change the mixing of all the water is very rapid (less than 15 min) and no account has been taken in our calculations of the initial slight disparity of the external medium. An experimental sequence consisted of the following phases:

(*a*) *Preparatory.* The eel was switched to a closed circuit with a volume of about 2 l + *v* for 30 min.

(*b*) *Homogenization of radio-chloride.* The water in tanks *u.t.* and *l.t.* was replaced by exactly 2 l of water. Eels were injected via catheter with 0·2–0·3 ml aqueous solution of $Na^{36}Cl$ at 100 m-equiv.$l^{-1}$ (10–15 $\mu$Ci). The animals were left 5 h in a closed circuit for good mixing of the tracer in their chloride spaces. Losses of $^{36}Cl$ into the external medium during this period were calculated from a final water sample.

(*c*) *Drinking rate measurements by Evans's* (1968) *technique and chloride outflux measurements.* Water in *u.t.* and *l.t.* was replaced by 2 l Millipore (0·22 $\mu$)-filtered water with PVP $^{125}I$ (polyvinyl pyrrolidone, 20 $\mu$Ci.$l^{-1}$) added. This phase lasted 1, 2 or 4 h (according to the adaptation programme), during which regularly spaced 5 ml water samples were taken, i.e. four samples for a 1 h phase, eight for a 2 or 4 h phase.

(*d*) *Rinsing.* The radio elements were washed out by 20 min rinsing in open circuit (A', B', B", Fig. 2).

(*e*) *Final measurements on eels.* Animals were anaesthetized and weighed. A 2 mm catheter was attached to a sampling tube placed in the rectum and a 15 mm diameter balloon placed in the oesophagus to retain all digestive liquids. A blood sample was taken from the aorta. The animals were killed, then the gut dissected and cut in five lengths between ligatures to avoid loss of gut contents. The pieces then immediately placed in counting tubes for a $\gamma$ well-counter.

## *Analysis of data*

### (A) *Drinking rate measurements*

A Mesco EMO 13 well-counter was used for estimating $^{125}I$ in the 2 ml water samples and in samples from the alimentary canal. All the samples from the gut were pooled since during the autopsy the intraluminal digestive liquid shifted considerably. The drinking rate (DR) was calculated as follows:

$$DR = \frac{{}^{*}I_{int} \times 100}{{}^{*}I_{em} \times \Delta_t \times P} \text{ (ml.h}^{-1}\text{.100 g}^{-1}\text{)}$$

where ${}^{*}I_{int}$ is the total radioactivity of the gut in dpm, ${}^{*}I_{em}$ the mean concentration of P.V.P. $^{125}I$ of the external medium in dpm.ml$^{-1}$, $\Delta_t$ the duration of the measurements in hours and $P$ the weight of the fish in grams.

The dilution of $^{125}I$ in each circuit in relation to the initial concentration (${}^{*}I_{ei}$ in dpm.ml$^{-1}$) enables the residual volume ($v$) to be calculated:

$$v = 2000 \text{ ml}\left(\frac{{}^{*}I_{ei} \text{ dpm.ml}^{-1}}{{}^{*}I_{em} \text{ dpm.ml}^{-1}} - 1\right)\text{(ml).}$$

### (B) *Measurements of plasma concentrations and turn-over rates of chloride*

Plasma sodium was measured with an Eppendorf flame-photometer and chlorides with an Aminco-Cotlove Chloridometer. $^{36}Cl$ was counted on a liquid scintillation spectrometer SL 40 with automatic correction for quenching, background and dead time. Samples were of 1 ml for the external water and 250 $\mu$l for the plasma to 10 ml Bray's solution. Plasma proteins were dissolved with 1 ml 'Soluene–100' Packard before adding the Bray's solution.

#### *Calculations of chloride turn-over rate* ($\lambda$)

Given that:

${}^{*}C$ is the $^{36}Cl$ concentration in dpm.ml$^{-1}$ of the external medium during the experimental measurements:

${}^{*}C_h$ is the $^{36}Cl$ concentration in dpm.ml$^{-1}$ at the end of the 5 h homogenization period;

${}^{*}C_{eq}$ is the concentration of $^{36}Cl$ in dpm.ml$^{-1}$ at equilibrium between internal and external Cl compartments;

$C_{pl}$ is the plasma chloride concentration in m-equiv.l$^{-1}$;

$C_{ext}$ is the chloride concentration of the water in m-equiv.l$^{-1}$;

$P$ is the body weight of the fish in g;

$Q$ is the quantity of $^{36}Cl$ injected in dpm;

and that the duration of the measurements is sufficiently short for it to be assumed that the chloride fluxes are constant, then in a system with two compartments $^*C$ increases progressively according to the equation (Motais, 1967):

$$^*C = {}^*C_{eq}(1-e^{-\lambda t}). \quad (1)$$

Since the residual volume $v$ is present at the beginning of the flux measurement $^*C_0$ is not zero, so under our experimental conditions

$$^*C = {}^*C_{eq}(1-e^{-\lambda t})+C_0 \quad \text{with} \quad {}^*C_0 = \frac{{}^*C_h \times v}{2000+v}.$$

Our measurements were too short for the equilibrium $C_{eq}$ to be reached. This value was calculated from the following equation:

$$^*C_{eq} = \frac{Q-(^*C_h \times 2000)}{\frac{C_{pl} \times 0{\cdot}24 \times P}{C_{ext}}+2000+v}.$$

For this calculation the chloride space ($E$) was taken to be 24% of the body weight. The inaccuracy introduced by this assumption is negligible since the chloride compartment of the eel is very small compared with that of the sea water.

The above equations provide all the data necessary for calculating by equation (1) the chloride turn-over rate ($\lambda$) from the experimental values of $^*C$.

*Calculation of chloride outflux*

$$F_{out} = E \times \lambda \times C_{pl} \ (\mu\text{-equiv.h}^{-1}.100\,\text{g}^{-1}),$$

with $E$, the chloride space expressed in ml.100 $g^{-1}$, $\lambda$ expressed in % $.h^{-1}$, $C_{pl}$ expressed in $\mu$-equiv.$ml^{-1}$. To calculate the chloride space it is necessary to know the total amount of chloride lost by the eel into the external medium ($\Delta_Q$).

$$\Delta_Q = {}^*C_t\,(2000+v)+2000 \times {}^*C_h \ (\text{dpm.}).$$

$^*C_t$ is obtained by extrapolation from the two last experimental measurements of $^*C$ since it cannot be measured directly because of the rinsing procedure. The chloride space, $E$, is then

$$E = \frac{Q-\Delta Q}{^*C_{pl}} \ (\text{ml}),$$

with $^*C_{pl}$, the plasma radio-chloride, in dpm.$ml^{-1}$.

## EXPERIMENTAL RESULTS

### *Time course of changes in body weight*

The ratio between final body weight (at the moment of killing) and initial body weight (when placing the intraperitoneal catheter) was calculated for each eel. The averages of these ratios in all the experimental series are given in Fig. 3(*a*, *b*). Their changes with time are a result of the action on the body weight of two tendencies: one

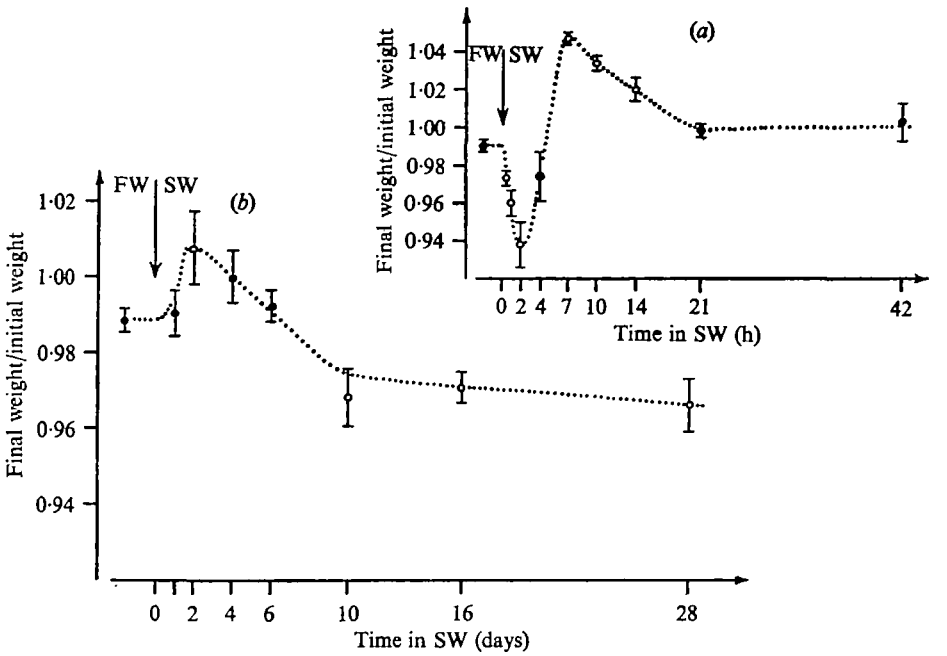


Fig. 3. Kinetics of the ratio between final body weight and initial body weight. ●, Differences between SW and FW values non significant. ○, Differences between SW and FW values significant at $P < 0{\cdot}02$. (*a*) With time in days after FW–SW transfer. (*b*) With time in hours after FW–SW transfer.

to increase weight by accumulation of water drunk, the other to diminish weight by urinary, faecal and osmotic losses.

Fig. 3(*a*) shows that the eels lost up to 5% of the initial weight during the first 2 days after transfer to sea water; then, after a phase of hypercompensation at its maximum on the 7th day, they returned to the initial weight by three weeks and maintained it subsequently. Water influx thus compensates water losses. Actually, after 3 weeks, metabolic losses should have resulted in a reduction of weight; that the weight is the same as at the beginning of the experiments must indicate either some water retention or an increase of the intraluminal digestive volume.

A study of the weight ratio during the first day (Fig. 3*b*) reveals that the eels first gain weight, the weight loss only becoming apparent after 4–6 h. If water loss starts immediately after transfer, it would seem to be masked at first by overcompensatory water entry.

*Time course of drinking rate*

Fig. 4(*a*) gives the general evolution of this parameter as measured over 4 h periods. The drinking rate fluctuates in much the same way as the body weight, its variations preceding the corresponding weight variations. It falls during the first 12–16 h after transfer and then rises to a maximum about the 7th day (360 $\mu l.h^{-1}.100\ g^{-1}$), after which a gradual reduction leads to a steady value of $137{\cdot}2 \pm 18{\cdot}5\ \mu l.h^{-1}.100\ g^{-1}$ ($n = 10$) at 3 weeks. This value is not significantly different from that of freshwater-adapted animals but is significantly lower than the maximum on the 7th day ($P < 0{\cdot}01$).

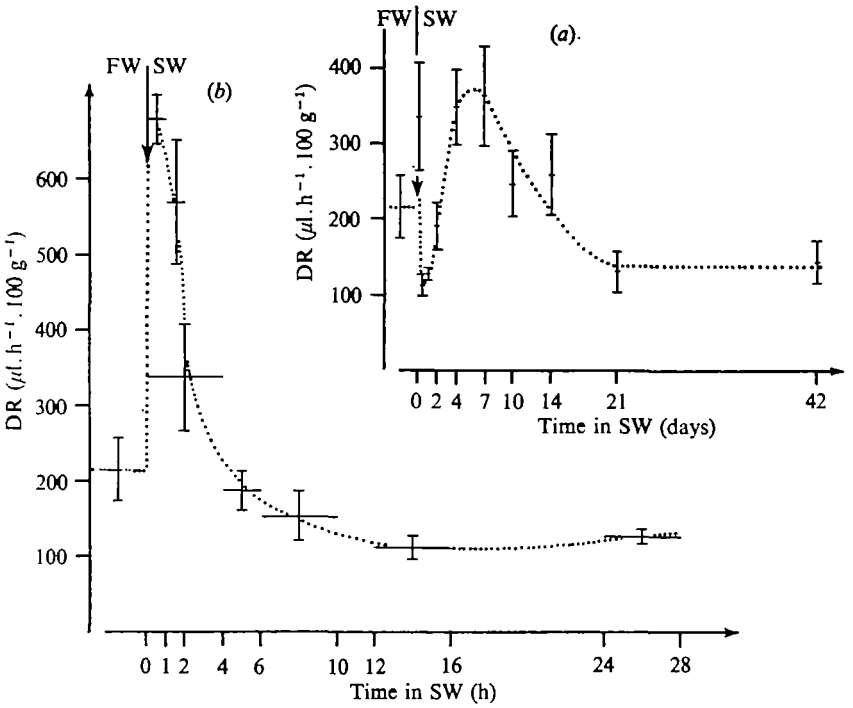


Fig. 4. Changes with time of the drinking rate (DR). Horizontal bars show the duration of the experimental measurements.

Measurements made over 4 h periods give average values which may mask any rapid changes of drinking rate. For this reason, the drinking rates during the first 24 h of sea-water adaptation were measured at more frequent intervals and for shorter experimental durations (Fig. 4*b*). From this curve it can be seen that the osmotic shock causes a highly significant threefold increase of the drinking rate during the first hour ($P < 0{\cdot}01$). The rate then falls rapidly; after 2 h adaptation it is still higher than the freshwater level ($P < 0{\cdot}01$) but not subsequently. It continues to drop to a minimum at 12–16 h.

The incorporation of a considerable volume of water during the first 6 h after transfer to sea water, and the relatively small variation in body weight during this period, will be discussed below in relation to the animals' dehydration on changing media.

*Time course of plasma composition and exchangeable chloride*

Fig. 5 illustrates the kinetics of the plasma sodium and chloride concentrations. It can be seen that the time courses of these are dissimilar. During the first 7 days a hypermineralization of sodium occurs, which is maximal on the second day. Subsequently the plasma sodium remains stable at approximately the freshwater level. Plasma chloride also increases during the first 7 days but here the peak is less important and reached on the first day; only the 24 h and 4-day values are significantly higher than those of the fully seawater-adapted animal. During the second day a transitory fall of the plasma chloride concentration brings it to a value comparable to that of

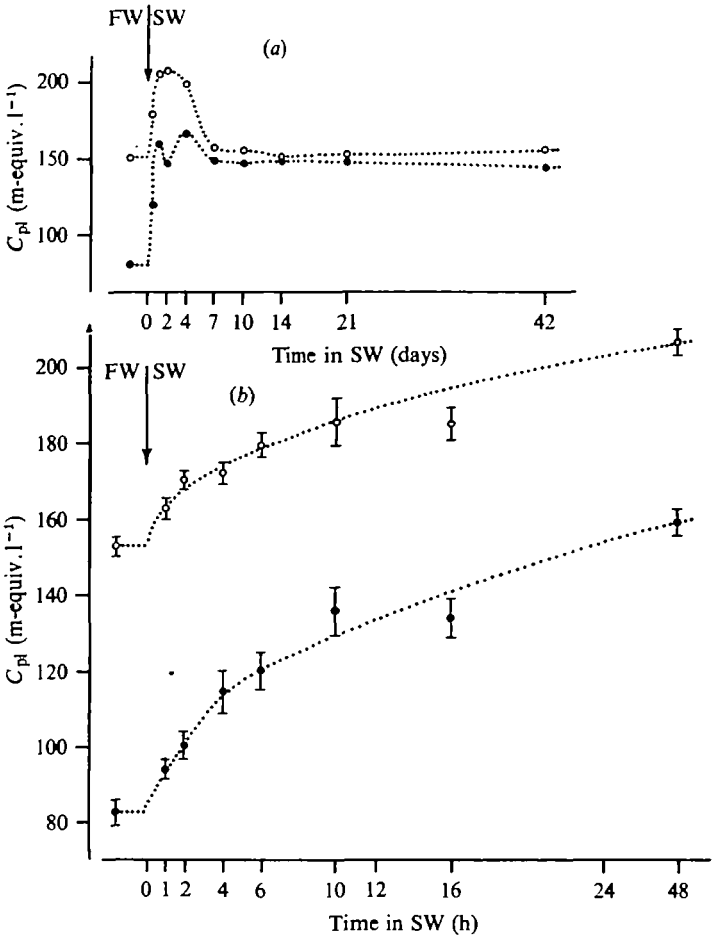


Fig. 5. Changes with time of plasma sodium and chloride concentrations ($C_{pl}$). ○, Plasma sodium concentrations. ●, Plasma chloride concentrations.

seawater-adapted animals. On and after the seventh day the chloride concentration has stabilized at a level very significantly higher than that of the freshwater animal.

From the simultaneous measurements of chloride space and plasma chloride the exchangeable chloride can be calculated, i.e. $M_{Cl} = E_{Cl} \times P_{Cl}$. The time course of $M_{Cl}$ (Fig. 6) presents a rapid increase during the first 24 h in sea water followed by a significant drop at 48 h to level of that of fully seawater-adapted (42 days) animals. This decrease is the result of simultaneous diminutions of the chloride space and of plasma chloride. After 48 h a second increase occurs and the curve follows with a certain lag that of the drinking rate. In the seawater-adapted eel the exchangeable chloride is about 3200 $\mu$-equiv. 100 $g^{-1}$. This is 80% higher than that of the freshwater-adapted eel.

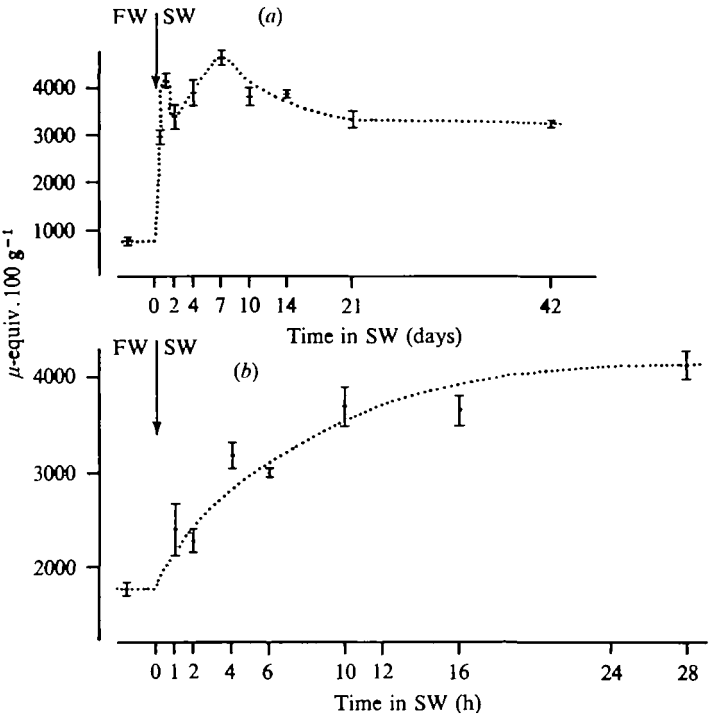


Fig. 6. Changes with time of the exchangeable chloride.

*Time course of chloride exchanges*

The total chloride outfluxes are represented in Fig. 7. In fresh water the outflux is very small; it increases 40-fold on transfer of the eel to sea water. Even so, chloride loss is very low (about 8·2 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$) during the first 6 h. Subsequently there is a progressive increase to a maximum on the 4th day (500 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$). This is followed by a reduction and stabilization of the flux after 14 days at a level of 230·3 ± 28·2 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$ ($n$ = 14). It should be noted that after 48 h the flux (384·0 ± 60·9 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$) is already significantly higher than that of fully seawater-adapted animals at a time when a low drinking rate only allows for a chloride intake from the ingested sea water of 110 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$. This difference of uptake and outflux values probably accounts for the drop of plasma chloride and exchangeable chloride measured at 48 h. Thus the early establishment of this high outflux permits the elimination of the excess salt absorbed during the second phase of intense drinking.

The net chloride fluxes ($F_{net}$) were calculated from the data of exchangeable chloride and ingested chloride (from the drinking rate knowing that sea water contains 570 $\mu$-equiv.$l^{-1}$ chloride). $F_{net}$, ingested chloride ($En_D$) and outflux ($F_{out}$) are given in Fig. 8.

During the first 12 h of adaptation the outflux is very low, consequently the influx is roughly equivalent to the net flux. In this initial phase, therefore, it is the ingestion of sea water which may be the main factor responsible for the increase of chloride in the organism. Nevertheless $En_D$ is always lower than $F_{net}$ and also the intestinal

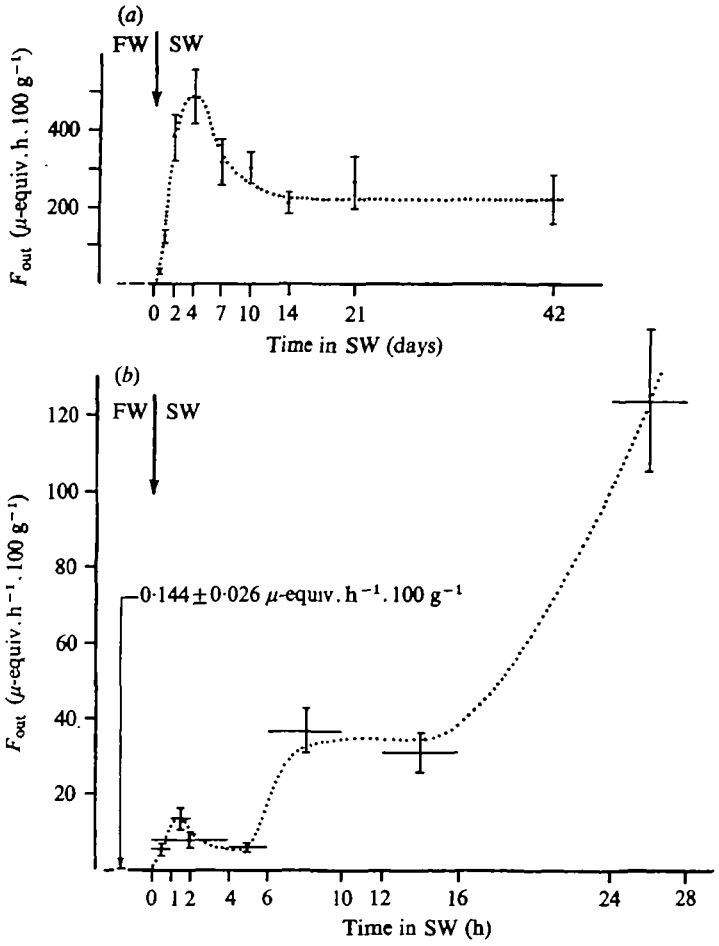


Fig. 7. Changes with time of the chloride outflux, $F_{out}$.

absorption of the chlorides is not instantaneous. There are two possible hypotheses to account for the difference between $En_D$ and $F_{net}$: either the establishment of a branchial influx immediately after transfer to sea water or a false over-evaluation of the $F_{net}$ as a result of the present indirect method of estimation.

After 2 days in sea water the netflux becomes negligible; the outflux is thus capable of eliminating the chlorides absorbed by the intestine or entering by the gills.

After the 7th day the $F_{out}-En_D$ difference is relatively constant at about 140 μ-equiv.h$^{-1}$.100 g$^{-1}$. This flux can be taken to be the branchial influx. Between the second and seventh day, however, the $F_{out}-En_D$ difference is considerably bigger which signifies either that the branchial influx is much higher than in the seawater-adapted animal, or that owing to a lag in intestinal absorption, the amount of chloride absorbed is greater than the amount drunk in the period of experimental measurements. This is possible in view of the time taken for the water to move down the gut and for the change of the absorptive capacity of the gut. In any case the two possibilities are not mutually exclusive.

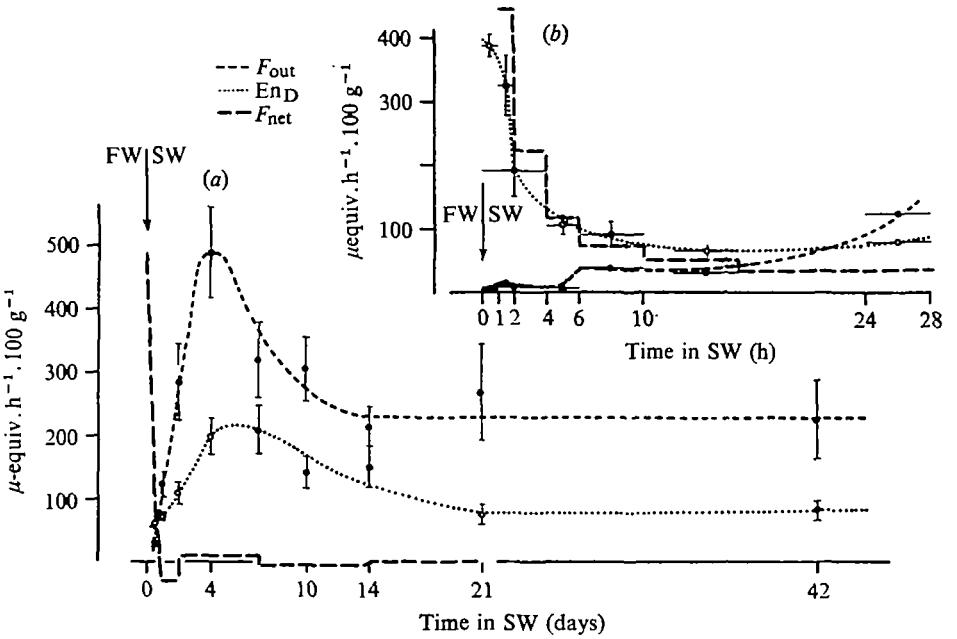


Fig. 8. Changes with time of the chloride fluxes. $F_{out}$, outflux; $En_D$, digestive entry; $F_{net}$, net flux.

## DISCUSSION

### *Water balance*

#### *Adapted animals*

A drinking rate of 143·7 $\mu$l.h$^{-1}$.100 g$^{-1}$ was found in our seawater-adapted eels at 13 ± 1 °C. This value agrees with those made at similar temperatures by Maetz (1970), namely 167 $\mu$l.h$^{-1}$.100 g$^{-1}$ at 15 °C, and by Gaitskell & Chester Jones (1970), namely 149 $\mu$l.h$^{-1}$.100 g$^{-1}$ at 12 °C. Like Motais & Isaia (1972) we found no significant differences between this value and that of our freshwater-adapted animals. Gaitskell & Chester Jones do not give values for normal freshwater-adapted silver eels but their data for control animals in hypophysectomy experiments at 12 °C are not significantly different ($P > 0{\cdot}05$) from those of their seawater-adapted animals. Maetz & Skadhauge (1968) found a drinking rate of 135 $\mu$l.h$^{-1}$.100 g$^{-1}$ and a sea-water drinking rate of 325 $\mu$l.h$^{-1}$.100 g$^{-1}$ at 20 °C.

In the majority of cases, therefore, no correlation between adaptation to sea water and increased drinking occurs.

Nevertheless, even if the quantity of water drunk is the same in both milieux, the conditions for absorption are not equivalent since the ionic and osmolar concentrations of the two media are very different. Fresh water entering the intestinal lumen produces rapid general hydration of the fish whereas when sea water enters the lumen it causes an initial net flux of water into the lumen, a flux which is negative in relation to the rest of the organism. Hence the intraluminal sea water is first diluted and subsequent reabsorption of water follows as a consequence of ionic absorption

(Utida *et al.* 1967; Skadhauge, 1968). Utida, Hirano & Kamiya, 1966, 1969) have also shown *in vitro* that the capacity of the intestine for water absorption is greater in seawater-adapted than freshwater-adapted animals, and similarly greater in silver eels than in yellow eels. Water exchanges through the intestinal walls also show seasonal variations, and are increased during catadromic migration (Utida *et al.* 1969). Our material was silver eels studied in autumn and it may be assumed that in seawater urinary water loss was very low (Chester Jones, Chan & Rankin, 1969) and the skin impermeable (Kirsch, 1972*b*). Under these circumstances a water intake of 144 $\mu$l.h$^{-1}$.100 g$^{-1}$ would compensate adequately for osmotic loss due to branchial permeability.

*Animals transferred from fresh water to sea water*

The day-to-day changes of body weight recorded are similar to those observed by Keys (1933) in the european eel (physiological state not given) and Oide & Utida (1968) in the cultivated Japanese eel. Between 12 and 48 h after transfer the drinking rate is reduced, resulting in a 5% body weight loss by dehydration, a phenomenon also recorded by Sharatt, Bellamy & Chester Jones (1964). Our curves agree those of Oide & Utida (1968) in showing a rising drinking rate reaching a maximum between the 4th and 7th days and followed by a decrease and stabilization. This stabilization occurred after the 14th day in the eels of Oide & Utida (1968) but only after 3 weeks in ours. A more detailed study of the kinetics of these parameters during the first hours of adaptation to sea water has only been reported by Kirsch (1972*b*) for drinking rate as measured by a totally different technique.

A study of the body weight curves in relation to the total amount of water drunk permits certain conclusions.

During the first hour after transfer the eel drinks about 680 $\mu$l.h$^{-1}$.100 g$^{-1}$ and should therefore show a weight gain of 680 mg, but in fact only gains 140 mg; a loss of about 540 $\mu$l.100 g$^{-1}$ thus occurs during this period. A rapid reduction of weight loss is characteristic of adaptation to the new medium; thus, during the first 6 h the animal drinks 1720 $\mu$l for a weight increase of 300 mg at the end of this period or an average loss for the whole period of 240 $\mu$l.h$^{-1}$.100 g$^{-1}$. This limitation of weight loss is probably largely due to a reduction of urinary water loss, which is known to be very low after the transfer to sea water of the eel (Chester Jones *et al.* 1969) and the flounder (Lahlou, 1967). It may also be partly due to a reduction of the osmotic permeability of the gill as suggested by Utida *et al.* (1967). Nevertheless the change of medium results in a disturbance of the water balance which, in spite of the animal's capacity to reduce losses, rapidly becomes negative, as shown by the significant weight loss after 10 h in sea water. It is only after 3 weeks in this medium that the water balance reaches a steady state, with an intake of 140 $\mu$l.h$^{-1}$.100 g$^{-1}$ of which, according to Oide & Utida (1968), 75% is absorbed.

An important problem to be resolved is what determines the ingestion of water. Why, for instance, does the animal not drink between 24 and 48 h after transfer, a period when it is in a state of considerable water deficiency; and why, on the contrary, does it drink as soon as it is placed in sea water even though it cannot yet be suffering from lack of water? Water deficiency seems to be a logical stimulus for drinking and appears to be the factor provoking the second intense drinking phase, implying that the water drunk is absorbed. Oide & Utida (1967) found that absorption *in vitro*

showed a maximum on the 5th day, after which it decreased. The situation is different during the first 48 h, however. Utida *et al.* (1967) introduced sea water into the intestine of a freshwater-adapted animal and found a water movement in the opposite direction. Skadhauge (1969) also showed that sea water in the intestine was first diluted before being reabsorbed. Thus, during the first hours after transfer to sea water, the sea water drunk acts as a factor limiting further drinking partly because of its own volume and also because it draws water from the internal medium and thus fills the intestinal lumen. Further drinking must thus depend on absorption; there is a correlation between drinking rate and intestinal absorption. So, after a delay when the gut's absorption is no longer a limiting factor, dehydration of the internal medium would be the stimulatory factor, and stimulation would be proportional to water loss, particularly osmotic loss through the gills.

Can water deficiency also be responsible for the drinking at the time of osmotic shock? One tends to think of dehydration as a progressive, slow phenomenon, but it is conceivable that there are changes of water distribution inside the body, and a local dehydration at the branchial filaments or even elsewhere in the vascular system could well be rapid and initiate a drinking reflex. At the moment of osmotic shock when the gill is plunged into a steep osmotic gradient there must be immediate water loss at this site. Our measurements, cumulative over a whole hour, could not show such a phenomenon. It could also be that the animal becomes dehydrated during the first 20 or 30 min and then drinks during the second part of the hour. Again, the final estimate at the end of the hour would not reveal this. We therefore carried out the following experiment: eels from the group discussed above were each placed in a small polyethylene weighing scoop and left in running fresh water overnight. They were then weighed every 5 min for 30 min in fresh water and then for 60 min in sea water. Each weighing meant 1 min out of water but still within the plastic container. In both fresh water and sea water there was a progressive loss of weight, probably due to a discharge of mucus which occurred during the weighing periods and which was subsequently washed away. After the first 5 min in sea water, however, there was a significant weight increase ($+0{\cdot}97 \pm 0{\cdot}29\,\%$, $n = 5$). Thus the eel drinks a considerable quantity of water at the moment of transfer as a result of a rapid reflex independent of the over-all dehydration of the animal. This reaction, however, can only be observed with animals pre-adapted to their little polyethylene containers; animals which are handled before the series of weighings drink large amounts of fresh water and then drink little when transferred to sea water.

At the moment of osmotic shock it is therefore either a local dehydration or a sensory stimulus which triggers off the drinking reflex.

### *Chloride balance*

The time courses of the sodium and chloride concentrations in the plasma were similar to those already described (Mayer & Nibelle, 1970; Kirsch, 1972*a*). The two curves are not parallel; the readjustment, as a function of time, of the sodium exchanges is different from that of the chloride exchanges.

*Measurements of chloride outflux*

*Outflux in adapted eels.* Kirsch (1972*a*) had already studied chloride outflux from changes of plasma concentration and had found the following values for adapted eels: freshwater-adapted, 0·28 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$; seawater-adapted, 247·4 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$. The fluxes found in the present study are similar, i.e. fresh water 0·14 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$; sea water 230·3 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$. That the freshwater level is extremely low in the present series is without physiological significance: it was derived from 4 h of measurements whereas Kirsch's measurements were made over 3 months. The sea-water values are the same and significantly lower than those of Motais (1967) and Maetz (1971), owing very probably to the fact that our animals were larger and that shock phenomena were reduced to a minimum in our experiments.

After 3 weeks in sea water the chloride fluxes and drinking rate are in a steady state, and a chloride balance sheet can be drawn up. If one assumes that chloride absorption is totally efficient in the gut, chloride uptake by the gut is 78 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$ (this is lower than the maximal transport capacity of the intestine which Skadhauge & Maetz (1967) found to be 438 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$). After the initial phase of adaptation chloride lost through the rectum is negligable (Kirsch, 1972*b*). Chloride entry through the gill is thus 230 − 78 = 152 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$, in other words the branchial influx represents two-thirds and the branchial net flux one-third of the branchial outflux.

*Chloride outflux during adaptation to sea water.* The chloride outflux increases as soon as the change of medium is made, but the increase is irregular for the first 16 h owing to irregular faecal loss during this initial phase of adaptation (Kirsch, 1972*b*). The outflux value between 0 and 16 h as measured by the radioisotope technique is probably lower than the true total chloride loss because the chlorides in the intestinal lumen become tagged from the internal medium, and as this is not an instantaneous process their specific activity is at first lower than that of the internal chlorides, and the chloride faecal loss is thus underestimated.

After 24 h the outflux is higher than that in seawater-adapted animals. Between 24 and 48 h branchial chloride excretion is sufficient to bring about a transitory fall of the plasma chloride concentration during this period when the eel is drinking little. The outflux increases up to the fourth day; the excess chlorides resulting from the second intense drinking phase can thus be eliminated, and the plasma chloride level is stabilized by the 7th day even though the drinking rate is high.

*Exchangeable chloride*

*In adapted eels.* Freshwater-adapted animals have an exchangeable chloride of 1780 $\mu$-equiv.100 $g^{-1}$; seawater-adapted, 3200 $\mu$-equiv.100 $g^{-1}$. The freshwater eels were kept unfed in a water containing very little chloride and so must have been in a particularly depleted state. Freshwater eels recently captured during a period when they feed have a plasma chloride concentration of 130 m-equiv. $l^{-1}$ which, with a chloride space of 24%, gives an exchangeable chloride of 3000 $\mu$-equiv.100 $g^{-1}$.

Kirsch (1972*a*) found the average chloride content of 46 freshwater eels after variable periods of fasting to be 2242 $\mu$-equiv.100 $g^{-1}$, with five eels having higher

values than 3000 $\mu$-equiv. 100 $g^{-1}$. The freshwater eel, although not efficient at pumping chloride with the gills, can increase the exchangeable chloride mass when it feeds. This may be a temporary capacity and the resulting chloride content has a different physiological significance from the constant high chloride content involved in the regulation of water metabolism, which occurs in seawater-adapted eels. It is also possible that the chloride compartments are the same in freshwater and seawater animals but unsaturated in starving eels.

In the case of sodium Mayer & Nibelle (1969) found a significant increase of the exchangeable fraction, both concentration and space increasing. In the present study, however, we found no increase of the chloride space (FW: 21·8% ± 0·7, $n = 11$; SW: 22·8 ± 1·1, $n = 5$). Neither did we find a 10% diminution of the chloride space in sea water as had Kirsch (1972*a*), but this is in part explained by the fact that the present values incorporate the intraluminal digestive chlorides whereas Kirsch studied the plasma of animals first in freshwater then during sea-water adaptation.

*Exchangeable chloride during sea-water adaptation.* The exchangeable chloride is calculated from the chloride space and the plasma chloride concentration. The degree of accuracy in the estimation of these parameters is thus of importance. The chloride space is defined as the apparent volume within the fish throughout which the ion is dispersed uniformly at the same concentration as that of the plasma. Immediately after transfer to sea water, however, the eel drinks a large quantity of sea water and the presence of this in the intestine renders the chloride compartment heterogeneous. Furthermore Kirsch (1972*a*) showed that transfer triggered off a redistribution of internal chlorides and an increase in their exchange rate. In view of these changes the above definition of the chloride space can only have a reduced precision during the initial phase of sea-water adaptation and it would be imprudent to draw quantitative conclusions concerning the time course of changes in the exchangeable chloride during this period.

Plasma chloride concentration, on the other hand, is measured directly. An increase of the plasma concentration would seem to be an essential factor for initiating active ionic excretion by the gills, as the studies of Mayer & Nibelle (1970) and Kirsch (1972*b*) have also indicated. The drop in chloride concentration at 48 h indicates that the chloride excretory mechanisms are functioning and adequate for the removal of the supplementary chloride being brought in by drinking.

In conclusion, the sequence of events occurring after the eels' transfer to sea water is as follows.

Ths osmotic shock on transfer causes the eel to drink a considerable quantity of sea water. This water cannot be immediately used by the organism, however, because the absorptive capacity of the intestine is as yet insufficient, and thus the intestine becomes full and the drinking rate falls rapidly in spite of the fact that the animal continues to suffer dehydration from osmotic loss. This dehydration, together with intestinal absorption, leads to an increase of the plasma chloride concentration and to a stimulation of the branchial outflux which, after 48 h, is sufficiently rapid to eliminate the excess chlorides absorbed from the intestine. At same time the capacity of the intestine for absorption increases, thus reducing the volume within the lumen and permitting an increased drinking rate which in turn compensates from the maximal dehydration at 48 h.

The eel reaches a state of equilibrium after 3 weeks in sea water. The secondary reduction of the drinking rate between 1 and 3 weeks is due to a decreased need for water which is reflected in a reduction of the branchial permeability to water.


## SUMMARY

Using isotopic procedures, the drinking rate and chloride exchanges were studied in the eel *Anguilla anguilla* during transfer from fresh water to sea water.

1. Following transfer to sea water there is a threefold increase of the drinking rate (lasting about 1 h). Then it falls to a minimum after 12–16 h and rises again to a maximum level about the seventh day after the transfer. Then a gradual reduction leads to a steady value which is not significantly different from the one observed in fresh water.

2. The changes with time of the plasma sodium and chloride concentrations are given. Their kinetics are not completely alike.

3. The chloride outflux increases 40-fold on transfer of the eel to sea water, but even so it is very low. After the sixth hour in sea water there is a progressive increase in the flux, so that on the fourth day it is higher (500 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$) than in the seawater-adapted animals (230 $\mu$-equiv.$h^{-1}$.100 $g^{-1}$).

4. Drinking rate values in adapted animals are discussed in relation to the external medium. The kinetics of the drinking rate together with variations in body weights after freshwater–seawater transfer are discussed in relation to the possible stimulus of the drinking reflex.

5. Chloride fluxes (outflux, net flux, digestive entry) are compared and lead one to assume that in seawater-adapted fish one-third of the chloride influx enters via the gut and two-thirds via the gills.


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