J. Exp. Biol. (1972), 57, 113–131
With 12 text-figures
Printed in Great Britain

PLASMA CHLORIDE AND SODIUM, AND CHLORIDE SPACE IN THE EUROPEAN EEL, *ANGUILLA ANGUILLA* L.

BY R. KIRSCH

Laboratoire de Zoologie et d'Embryologie expérimentales, Strasbourg (67)
et Groupe de Laboratoires de Strasbourg-Cronenbourg,
Laboratoire des Applications Biologiques, Strasbourg-3 (67) *France*

(*Received* 17 *December* 1971)

INTRODUCTION

Through a study of the plasma concentration (C) and the distribution space (E) of an ion, we can measure the quantity of exchangeable ions ($M = CE$), i.e. the size of the ion compartment, in a fish (Maetz, 1956). This parameter is a necessary point of reference for any study of the ion exchanges between a fish and the external medium.

It is well known that plasma chloride and plasma sodium increase in the eel during the transfer from fresh water (FW) to sea water (SW) (Duval, 1925; Keys, 1933; Sharratt, Chester Jones & Bellamy, 1964; Chan *et al.* 1967; Maetz, Motais & Mayer, 1969; Mayer & Nibelle, 1970). In the Japanese eel (Oide & Utida, 1967), this evolution includes a phase of initial hypermineralization of the internal medium followed by a deferred regulation. Mayer & Nibelle (1970) have observed that in *Anguilla anguilla* there is a progressive increase in the plasma concentrations from the fresh-water to the sea-water level, and an increase in the sodium space. However, Sharratt *et al.* (1964) noted very high concentrations in the sea-water *A. anguilla* (148–155 m-equiv $Cl^- l^{-1}$, 173–181 m-equiv $Na^+ l^{-1}$) which leads us to believe that a hypermineralization phase may also take place in this species.

Before this question can be studied it must be known whether the animal is perfectly adapted to its environment. This can only be determined through long-term studies. So we have elaborated new techniques which make it possible for us to perform experiments under good physiological conditions over a period of several consecutive weeks. While studying plasma chloride and sodium, we have measured the chloride space in the eel and shall compare our results with those of Mayer & Nibelle (1969) for the sodium space.

MATERIALS AND METHODS

Origin and maintenance of the animals

The eels were obtained in tributaries of the Rhine in northern Alsace. They were caught exclusively in eel-pots and kept for at least 15 days before experimentation in reserve tanks (well-aerated running tap water kept at a temperature of $13{\cdot}5 \pm 0{\cdot}5$ °C).

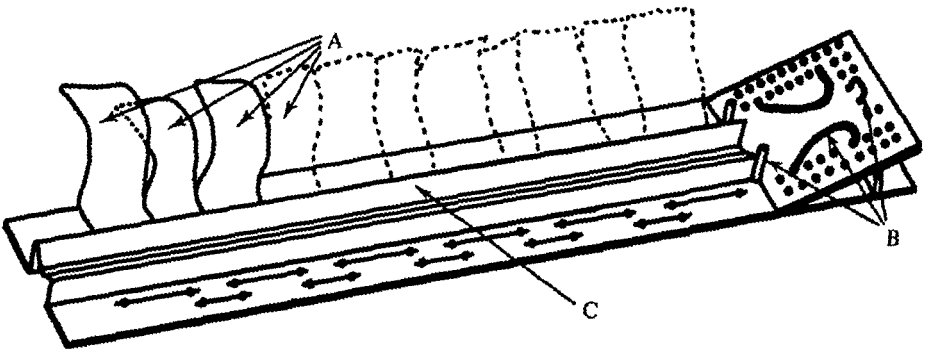

Fig. 1. Operating table: A, rubber retention bands; B, head retention apparatus; C, groove to retain body.

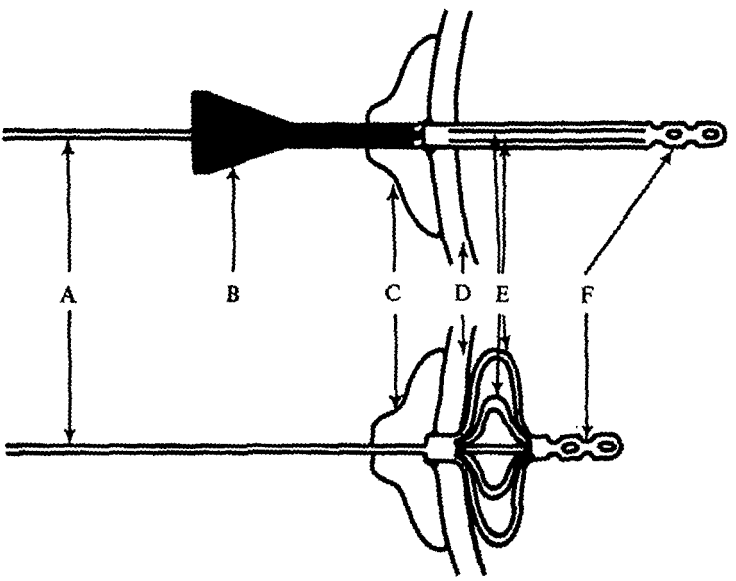

Fig. 2. Intra-bulbus cannula: above, before anchoring; below, cannula in position. A, Blood catheter; B, hypodermic needle; C, joint, D, wall of bulbus arteriosus; E, longitudinal lamellae; F, distal strainer.

Surgical techniques

After anaesthesia in a 30‰ ether bath for 15 min the eel was wrapped in dampened paper and placed on the operating table (Fig. 1). Anaesthesia was maintained during the operation by injecting a few ml of freshly prepared anaesthetic solution into the bucco-pharyngeal cavity.

The blood compartment of the eel was reached by using different types of cannulae extended outside the body by a flexible polyvinyl tube measuring 0·5 mm in diameter.

(*a*) *Intra-bulbus cannula*

The cannula was made up of a thin axial tube of polyethylene (Intramedic Pe 10) terminating in a distal strainer and surrounded with eight subterminal longitudinal lamellae (Fig. 2). To insert it, the base of the bulbus arteriosus was pierced with a

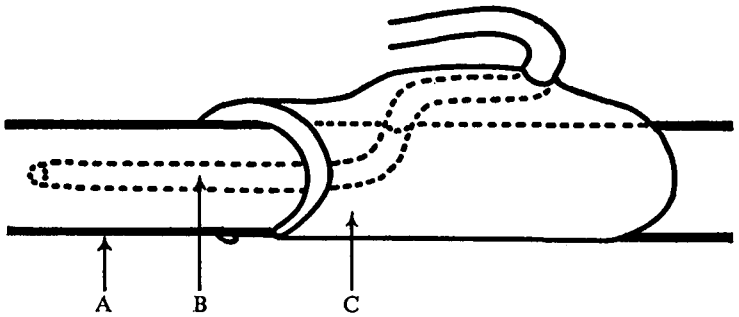

Fig. 3. Intra-aortic cannula: A, ventral aorta; B, polyvinyl tube; C, rubber jacket.

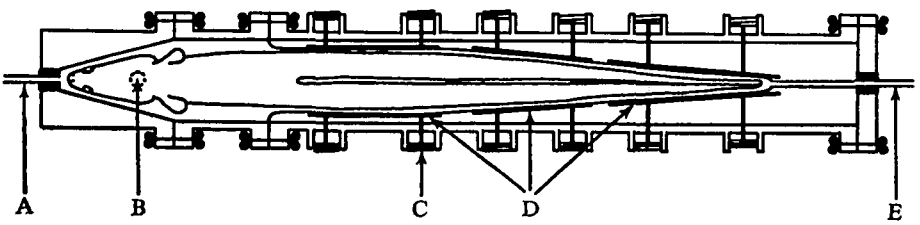

Fig. 4. Experimental tank seen from above: A, water entry; B, joint for exit of the extracorporal blood tube; C, regulating screw; D, retention apparatus; E, water exit.

needle 1 mm in diameter, and the cannula was passed through the orifice. Then a pull on the outer catheter above the needle made the lamellae unfold in the cavity and thus attached the cannula to the bulbus wall.

The intra-bulbus cannula can be used on an eel of any size, but it causes high post-operative mortality. Moreover, by opening the pericardium, the pericardial liquid is discharged and leads to troubles in the circulatory mechanism. This prevents any further experimentation in the early (2–3 weeks) post-operative phase.

Once the animal has recovered from the operation, it can be used for experiments for a long time; a test eel survived 2 years with a functional intra-bulbus cannula.

(*b*) *Intra-aortic cannula*

This cannula (Fig. 3) was composed of a thin polyvinyl tube which was introduced about 2 cm into the ventral aorta, in a posterior direction. It passed through a rubber jacket which covered the aorta and made the area of the surgical wound impermeable. The intra-aortic cannula can only be used on large eels (more than 400 g). The operation does not cause much shock and post-operative mortality is low; operated animals usually survive 3–4 months.

(*c*) *Cannulation of the pneumogastric artery and vein*

This procedure, described by Chester Jones *et al.* (1966) and Mayer & Nibelle (1970), has been used for experiments in extracorporal blood circulation. Unfortunately with this type of operation, only 2 weeks of experimentation were possible even in good conditions because vascular necroses set in after that period.

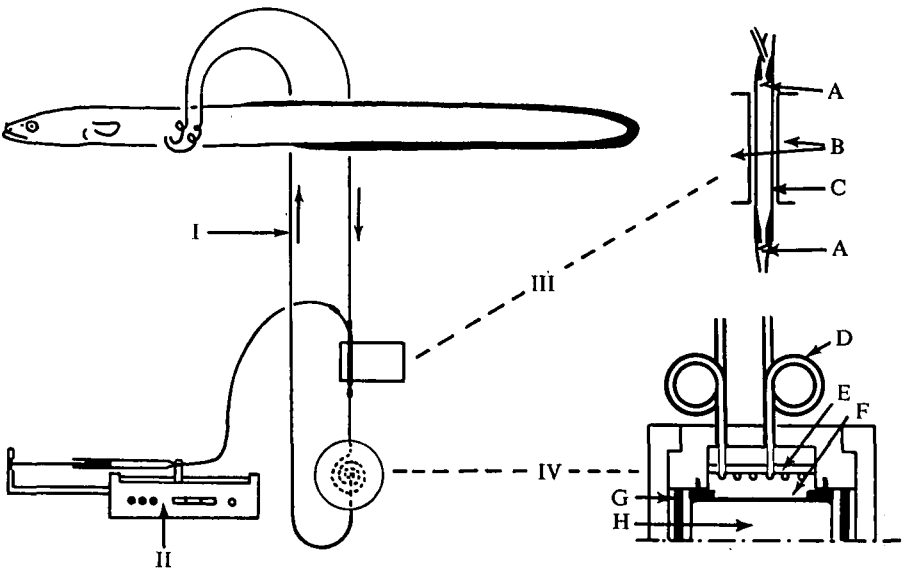

Fig. 5. Extra-corporal blood circuit: I, blood catheter (polyvinyl); II, perfusion syringe with heparinized solution; III, artificial heart; IV, counting device; A, unidirectional micro-valve; B, magnet; C, pulsing tube; D, opaque catheter; E, polyethylene joint; F, counting cell (SPF. La Radiotechnique); G, antimagnetic screen; H, Photo-multiplier tube.

The experimental tank

It is extremely important to reduce to the utmost the volume of the extra-corporal blood tube. All shocks must also be avoided. To ensure this, we used an experimental tank made of Plexiglas and consisting of several elements which took on approximately the form of the eel (Fig. 4). The rear part of the tank included a flexible polyethylene sack whose position could be adjusted to 2 or 3 mm from the body wall of the animal by means of a retaining apparatus surrounding it. This device avoided a too-constraining retention and thus made possible experiments lasting for several months without causing cutaneous lesions in the eel.

The experimental tanks were supplied with either well-aerated running tap water or with sea water in a closed circuit of 100 l, filtered and regenerated continuously.

Analysis and counting procedures

The blood samples (0·1 ml) were centrifuged for 5 min at 5000 r.p.m. to separate the plasma, in which the following were determined: the Na^+ concentration, with a flame photometer (1 % error); the Cl^- concentration, using the Sanderson method (1952) modified by the neutralization of the acetic acid before the sample was added (1 % error); the $^{36}Cl^-$ concentration, by counting 20 μl of plasma per dry sample with a low-background counter.

The continuous study of the blood radioactivity was made on an extra-corporal blood circuit which included a counting cell set up following the procedure described by Istin (1964) with certain modifications (Fig. 5). An artificial heart

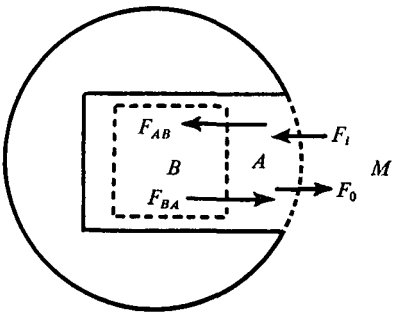

Fig. 6. Theoretical diagram of ion exchanges between a fish with two ion compartments (A and B) in the body in series with the external medium (M) considered as infinite: $F_{AB} = F_{BA}$ ion exchange between the compartments A and B; F_i = influx; F_o = outflux.

pumped the blood at a pulsed flux of 0·06 to 0·08 μl.min^{-1}. This pulsed circulation avoided the sedimentation of the blood cells despite the weakness of the flow required to maintain good physiological conditions. At the entry of the artificial heart a perfusion syringe injected a heparinized (200 mu.I.ml^{-1}) mineral solution isotonic with blood at a rate of 1 ml per day. The efficiency of the counting cell was determined during the experiment; a blood sample was taken, the plasma was separated and its radioactivity was determined in a dry-sample counter. Prior to the counting, the counter was calibrated with respect to the radioactive solution injected into the eel (10 μCi of $Na^{36}Cl$ in the form of 0·1 ml of aqueous solution at 100 m-equiv l^{-1}. Radiochemical Centre, Amersham).

Theoretical considerations

The volume of the distribution space (E) of an ion can be measured by diluting a radioisotope in the internal medium (Maetz, 1956; Mayer & Nibelle, 1969):

$$E = \frac{Q_0 - \Delta Q_t}{{}^*C_t};$$

Q_0 = quantity of the tracer injected into the animal at time 0,

ΔQ_t = tracer losses in the external medium at time t,

${}^*C_t$ = plasma concentration of the radioisotope at time t.

The experiment must last long enough to allow complete equilibration, i.e. saturation of all the body compartments, by the radioisotope. This procedure was used to measure E in fresh water when ΔQ_t is negligible in relation to Q_0. Conversely, in the sea-water eel, ΔQ_t is large and difficult to measure accurately because the tracer is considerably diluted in the external medium. In this case we have eliminated the parameter ΔQ_t by studying the time course of ${}^*C_t$ after the injection of the tracer. The graph showing the radioactive discharge cannot be easily interpreted unless the uni-directional ion fluxes are constant and the net flux is negligible. If this is the case, the following mathematical analysis can be used.

The eel is considered to be a system having two ion compartments A and B, set up in a series, with respective volumes V_A and V_B and having the same concentration C,

so that $E = V_A + V_B$ (Fig. 6). The external medium (M) is theoretically infinite and, from a practical point of view, so large that the radioactive backflux is negligible.

If F = the uni-directional ion flux or the quantity of ions transported per unit of time from one compartment to the other, $F_{AB} = F_{BA}$; F_{AM} = total outflux = F_0. At time o the radioactive tracer Q_0 is introduced into compartment A. Then if a = the number of radioactive ions in A at time t, b = the number of radioactive ions in B at time t, m = the number of radioactive ions in M at time t, the tracer exchanges can be summarized by the following equations:

$$-\frac{da}{dt} = \left(F_{AB}\frac{a}{V_A C}\right) - \left(F_{BA}\frac{b}{V_B C}\right) + \left(F_0\frac{a}{V_A C}\right), \tag{1}$$

$$-\frac{db}{dt} = \left(F_{BA}\frac{b}{V_B C}\right) - \left(F_{AB}\frac{a}{V_A C}\right), \tag{2}$$

$$\frac{dm}{dt} = F_0\frac{a}{V_A C}. \tag{3}$$

Considering C as constant during the whole time of the experiment the net flux being null, we can simply state

$$\frac{F_{AB}}{C} = \frac{F_{BA}}{C} = K_1 \quad \text{and} \quad \frac{F_0}{C} = K_2.$$

By combining all the preceding equations, we can calculate the number of radioactive ions in compartment A as a function of time and, therefore, the radioisotopic concentration ($^*C_A$) of A,

$$^*C_A = \frac{a}{V_A}.$$

The general equation is the sum of two exponential functions of the form (Motais, 1967)

$$^*C_A = Q_1\exp(\lambda_1 t) + Q_2\exp(\lambda_2 t), \tag{4}$$

$$Q_1 = \frac{Q_0}{2V_A\sqrt{S}}\left[\frac{K_1+K_2}{V_A} - \frac{K_1}{V_B} + \sqrt{S}\right],$$

$$S = \left[\frac{K_1+K_2}{V_A} - \frac{K_1}{V_B}\right]^2 + \frac{4K_1^2}{V_A V_B},$$

$$Q_2 = \frac{Q_0}{2V_A\sqrt{S}}\left[\frac{K_1}{V_B} - \frac{K_1+K_2}{V_A} + \sqrt{S}\right],$$

$$\lambda_1 = -\frac{1}{2}\left[\frac{K_1+K_2}{V_A} + \frac{K_1}{V_B} + \sqrt{S}\right],$$

$$\lambda_2 = -\frac{1}{2}\left[\frac{K_1+K_2}{V_A} + \frac{K_1}{V_B} - \sqrt{S}\right].$$

By using this equation, we can calculate V_A, V_B, K_1 and K_2 and therefore F_{AB} and F_0.

$$V_A = \frac{Q_0}{Q_1+Q_2}, \tag{5}$$

$$V_B = \frac{Q_0 Q_1 Q_2 (\lambda_1-\lambda_2)^2}{(\lambda_1 Q_2+\lambda_2 Q_1)^2 (Q_1+Q_2)}, \tag{6}$$

$$V_A+V_B = E = Q_0\frac{\lambda_1^2 Q_2+\lambda_2^2 Q_1}{(\lambda_1 Q_2+\lambda_2 Q_1)^2}, \tag{7}$$

$$F_{AB} = \frac{-Q_0 C Q_1 Q_2 (\lambda_1-\lambda_2)^2}{(\lambda_1 Q_2+\lambda_2 Q_1)(Q_1+Q_2)^2}, \tag{8}$$

$$F_0 = \frac{-Q_0 C \lambda_1 \lambda_2}{\lambda_1 Q_2+\lambda_2 Q_1}. \tag{9}$$

In the particular case of no exchange or slight exchanges between the animal and the external medium (the case of the fresh-water eel), the general equation (4) can be simplified.

In fact $F_0 = 0$ thus $K_2 = 0$.

Therefore

$$^*C'_A = Q_1 \exp(\lambda_1 t)+Q_2 \tag{10}$$

and

$$V_A+V_B = E = Q_0/Q_2.$$

Q_2 represents here the plasma radioactivity at equilibrium after the equilibration phase.

Accuracy of the results

The handling of the data of $^*C_A$ as a function of t was made by computer and included χ^2 tests. Based on these tests, the preceding mathematical analysis was not statistically incompatible with the numerical results obtained.

λ_2 and Q_2 were always determined with a very slight experimental error, but the error was rather large in the case of λ_1 and Q_1.

λ_1 was always very large in relation to λ_2, which means that the following can be deduced from equations (7) and (9):

$$E \simeq \frac{Q_0}{Q_2} \quad F_0 \simeq -\frac{Q_0 C \lambda_2}{Q_2} = -EC\lambda_2.$$

As a result, the relatively large experimental error of λ_1 and Q_1 had almost no repercussions on the calculation of E and F_0. On the other hand, V_A, V_B and F_{AB} (equations (5), (6), (9)) contained a large irreducible error whose effect had to be limited by statistical calculations based on several experiments.

EXPERIMENTAL RESULTS

Plasma chloride and plasma sodium

I. Fresh-water eels

Fig. 7 shows the pairs of measurements of plasma concentrations in sodium (C_{Na}) and chloride (C_{Cl}) for each of the 99 eels used. The blood samples were taken before

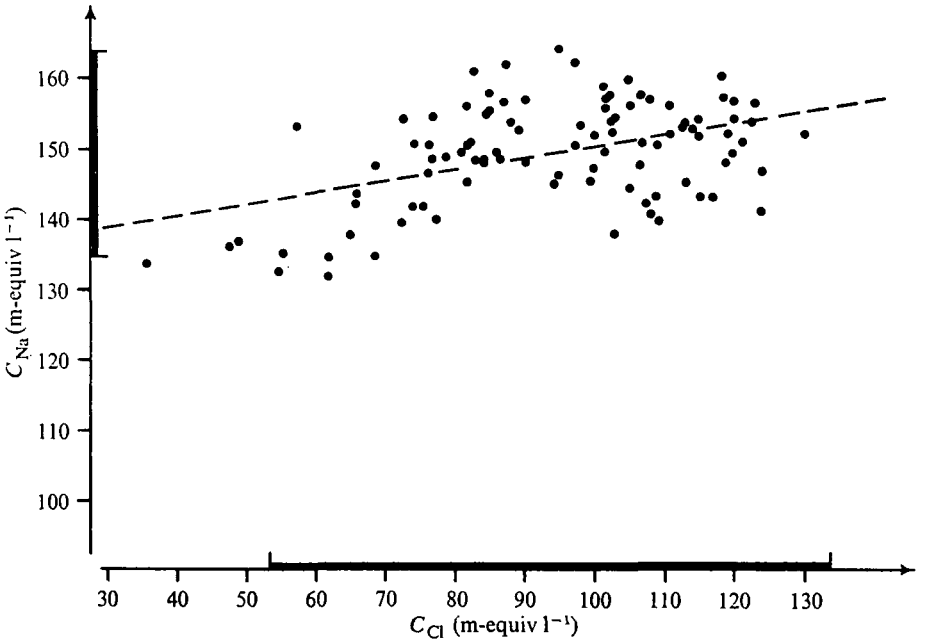

Fig. 7. Correlative study of plasma concentrations of sodium (C_{Na}) and chloride (C_{Cl}) in fresh-water eels. Axes are thickened at the interval of statistical distribution of 95 % of the experimental data. Body weight of eels, $567{\cdot}5 \pm 16{\cdot}3$ g ($\bar{x} \pm$ S.E.).

and during different experiments (study of the urinary excretion, gill ion flux, chloride space).

C_{Na} and C_{Cl} vary little over a short period of time in fresh-water eels, and the mean of the measurements taken from each animal has been used in our study of the linear regression between C_{Na} and C_{Cl}:

$$C_{Na} = (0{\cdot}16 \pm 0{\cdot}033)\, C_{Cl} + (134{\cdot}1 \pm 3{\cdot}09)$$

in brackets, mean $\pm$ standard deviation.

The correlation between C_{Na} and C_{Cl} is significant ($P < 0{\cdot}001$), but the slope of the linear regression curve is slight. C_{Na} varies little as against the very large variations of C_{Cl}, as is proven by the coefficient of variation CV:

$$CV = 100 \text{ standard deviations/mean of the variable,}$$

$$\text{mean} \pm \text{S.E.} \quad C_{Na} = 149{\cdot}2 \pm 0{\cdot}73, \quad CV_{Na} = 4{\cdot}92,$$

$$\text{mean} \pm \text{S.E.} \quad C_{Cl} = 93{\cdot}6 \pm 2{\cdot}03, \quad CV_{Cl} = 21{\cdot}61.$$

The fresh-water eel's plasma sodium is therefore relatively stable and fully independent of the plasma chloride. Our statistical calculations have shown, moreover, that there is no significant correlation between C_{Na} and C_{Cl} in eels whose C_{Cl} is larger than 80 m-equiv l^{-1}.

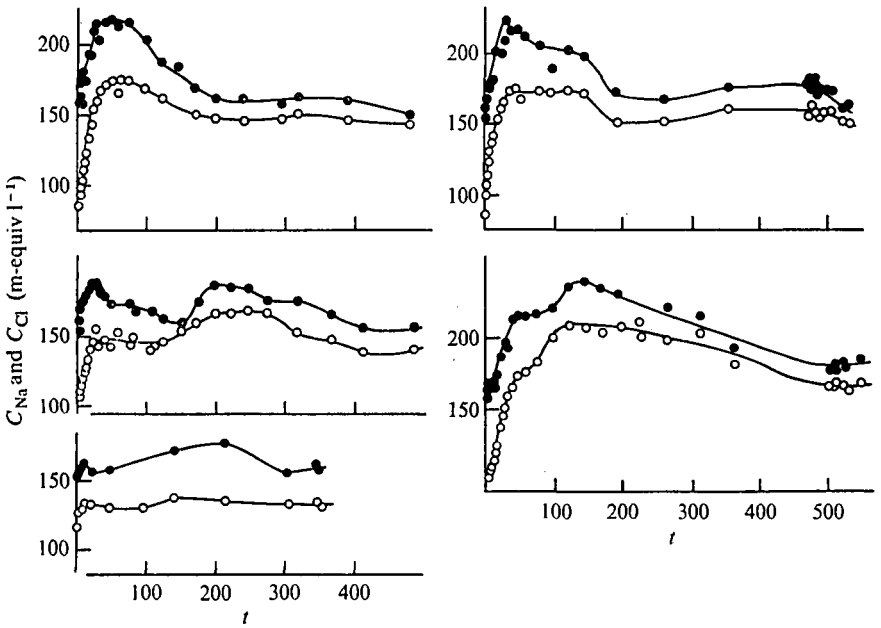

Fig. 8. Time course of plasma concentrations of sodium (●) and chloride (○) during adaptation of fresh-water eels to sea water. t in hours after the fresh-water to sea-water transfer.

II. Eels transferred from fresh water to sea water

During the fresh-water to sea-water adaptation period, the changes of C_{Na} and C_{Cl} vary greatly from one eel to another. For this reason, individual results have been reported in Fig. 8. The following observations are based on these graphs:

(i) C_{Na} and C_{Cl} have an almost parallel progression in each eel.

(ii) The initial adaptation phase is characterized by an extremely rapid increase in C_{Na} and C_{Cl}. This phase can last up to 48 h after the FW–SW transfer.

(iii) The highest levels of concentration reached vary greatly from eel to eel. After the maximum plasma ionic load has been reached, C_{Na} and C_{Cl} diminish slowly and all the eels arrive at the same level of equilibrium.

We call the period during which C_{Cl} and C_{Na} are higher than the levels of equilibrium the 'hypermineralization phase'. This phase is practically non-existent in certain eels that reach their level of equilibrium in less than 48 h. In others, several successive hypermineralization phases can be noticed.

The diversity in the deferred regulation contrasts sharply with the parallelism that can be observed in the time course of C_{Na} and C_{Cl} in all the animals during the initial phase of ionic loading of the internal medium. The correlation between C_{Na} and C_{Cl} is very significant ($P < 0{\cdot}001$) during this period and the linear regression $C_{Na} = aC_{Cl} + b$ does not differ significantly from one eel to another. The common linear regression curve (Fig. 9) has thus been calculated to be:

$$C_{Na} = (0{\cdot}62 \pm 0{\cdot}02)\, C_{Cl} + (101{\cdot}1 \pm 2{\cdot}84) \quad n = 58$$

in brackets, mean ± standard deviation.

During the initial phase of ion loading the eel maintained the electrical balance of its internal medium by retaining sodium and chloride in proportionally equal amounts.

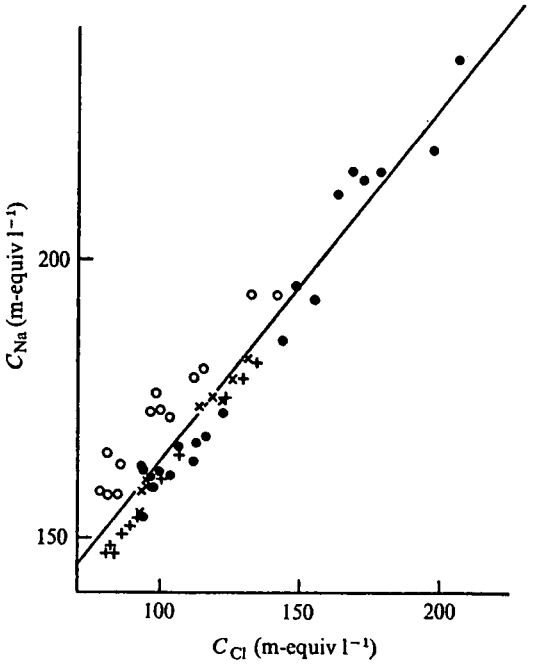

Fig. 9. Correlation of plasma concentrations of sodium (C_{Na}) and chloride (C_{Cl}) in eels during initial phase of sea-water adaptation. Each symbol corresponds to one eel.

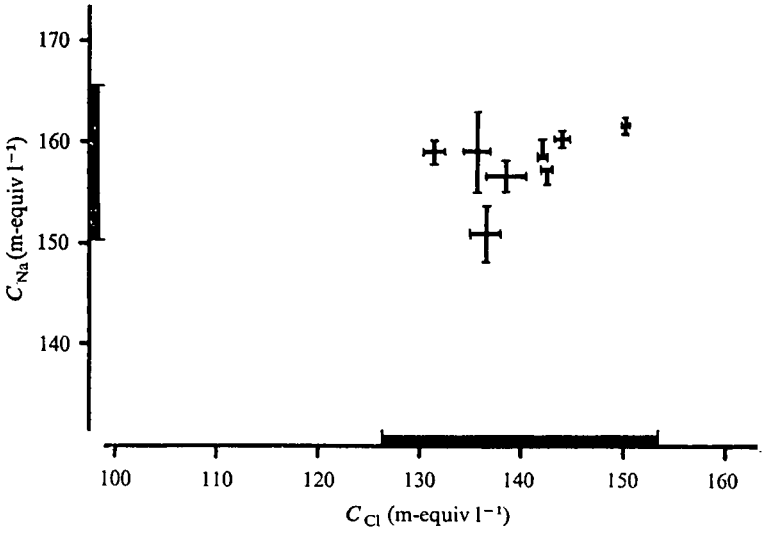

Fig. 10. Mean and S.E. of plasma concentrations of sodium (C_{Na}) and chloride (C_{Cl}) in eight sea-water eels. Body weight of eels, $541 \pm 52{\cdot}3$ g ($\bar{x} \pm$ S.E.).

Table 1. *Chloride space measurements by different techniques in fresh-water eels*

W (g)	M	E %	$100\Delta E/\bar{E}$
474	*IV*	27·4 ± 0·5	1·84
	IV	26·9 ± 0·9	
693	*IV*	28·7 ± 0·3	−4·09
	IV	29·9 ± 0·7	
490	*IV*	22·3 ± 0·4	−1·78
	IP	22·7 ± 0·7	
594	*IV*	24·6 ± 0·6	−1·61
	IP	25·0 ± 1·0	
396	*IA*	26·8 ± 0·6	−0·74
	IV	27·0 ± 0·9	
702	*IA*	23·5 ± 0·4	−0·85
	IV	23·7 ± 0·7	
*558 ± 51 g		*25·8 ± 1·05	*−1·2 ± 0·78

*: $\bar{x} \pm$ S.E.

W, Body weight; M, infusion method of $Na^{36}Cl$.; *IV*, intra-vascular; *IP*, intra-peritoneal; *IA*, intestinal absorption.

E %, Chloride space/100 g ± error due to measuring techniques.

III. Sea-water adapted eels

The stability of C_{Na} and C_{Cl} during several consecutive days was chosen as a test of the sea-water adaptation of the eels. For each of the eight eels studied the mean and standard error are given in Fig. 10. The distribution of C_{Cl} as well as that of C_{Na} showed a low coefficient of variation:

$$\text{mean} \pm \text{S.E.} \quad C_{Na} = 157{\cdot}9 \pm 1{\cdot}13, \quad CV_{Na} = 2{\cdot}04,$$

$$\text{mean} \pm \text{S.E.} \quad C_{Cl} = 139{\cdot}9 \pm 2{\cdot}02, \quad CV_{Cl} = 4{\cdot}10.$$

Sea-water eels, as opposed to fresh-water eels, showed a strict homeostasis for both plasma sodium and chloride.

Chloride space

I. Fresh-water eels

(*a*) *Reliability of measurements.* $^{36}Cl^-$ can be infused in eels intra-peritoneally, intra-vascularly, or even by intra-gastric injection (by buccal-oesophageal catheter). In the latter case the radioisotope reaches the internal medium through intestinal absorption. Preliminary experiments showed that complete equilibration of the tracer requires as much as 18 h after an intra-vascular or an intra-peritoneal injection and 4 days after an intra-gastric injection. Our measurements have taken these delays into account. Table 1 shows the results obtained by pairing the different marking procedures in six eels.

Chloride space varies greatly from one eel to another, but the measurements remain nearly the same. The difference between any two corresponding measurements is statistically less than 2·3 % of the mean value ($P = 0{\cdot}05$).

(*b*) *Effect of chloride outflux on the measurement of chloride space.* The chloride space in one eel was studied at regular intervals over a period of six consecutive months. During this time the experimental conditions remained the same (running tap water

Table 2. *Apparent increase in chloride space as a function of time in a fresh-water eel*

(Weight, 693 g before experiment, 622 g after experiment.)

				Linear regression $\log E = at + \log E_0$		
	t	n	Correlation probability	a†	$\log E_0$‡	E_0‡
Injection 1	0– 866 h	17	$P < 0{\cdot}01$	$(155 \pm 42)\ 10^{-6}$	$5{\cdot}3428 \pm 0{\cdot}0106$	$209{\cdot}1 \pm 2{\cdot}6$
Injection 2	0–790 h	8	$P < 0{\cdot}05$	$(189 \pm 66)\ 10^{-6}$	$5{\cdot}2880 \pm 0{\cdot}0141$	$197{\cdot}9 \pm 3{\cdot}5$
	935–3650 h	11	$P < 0{\cdot}005$	$(89 \pm 19)\ 10^{-6}$	$5{\cdot}3125 \pm 0{\cdot}0427$	$202{\cdot}9 \pm 3{\cdot}2$

t, Time between injection and measurement of E; n, number of measurements; E, apparent chloride space (in ml); E_0, apparent chloride space at t_0.

† Mean ± standard deviation.

‡ Mean ± S.E. reduced by linear regression.

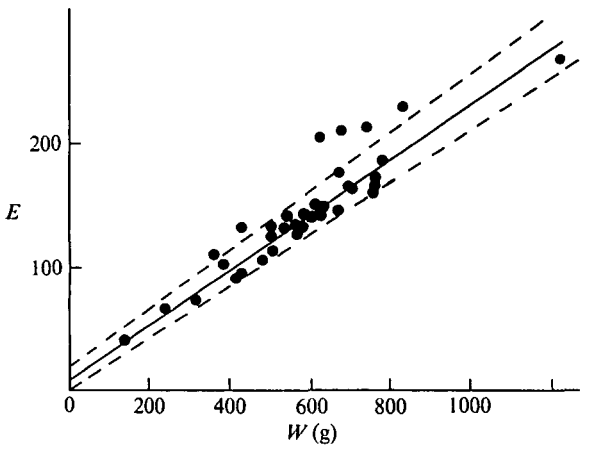

Fig. 11. Correlation of total chloride space (E) and body weight (W) in fresh-water eels.

saturated with air, 0·6 m-equiv $Cl^-\ l^{-1}$; 0·4 m-equiv $Na^+\ l^{-1}$; temperature 13·5 °C). The details of the measurement sequences after two injections of $^{36}Cl^-$ are given in Table 2. For each sequence, it is assumed that the outflux is constant. The apparent chloride space, calculated by the formula $E = Q_0/{}^*C_t$ increases, approximately according to an exponential function of t.

$$E = E_0 \exp(at) \quad \text{or} \quad \log E = at + \log E_0.$$

The results obtained show a maximum increase in apparent chloride space of 0·04 %/h. This corroborates the low losses of chloride in fresh-water eels and shows that these losses can be neglected even in the case of a several days' delay between the tracer injection and the measuring of plasma radioactivity and chloride space.

(*c*) *Correlation between chloride space and body weight.* Fig. 11 presents the chloride space (E) of 39 eels as a function of body weight (W). The correlation between these variables is significant ($P < 0{\cdot}001$) and linear regression is calculated to be

$$E = (0{\cdot}221 \pm 0{\cdot}014)\,P + (12{\cdot}16 \pm 8{\cdot}82)$$

in brackets, mean ± standard deviation.

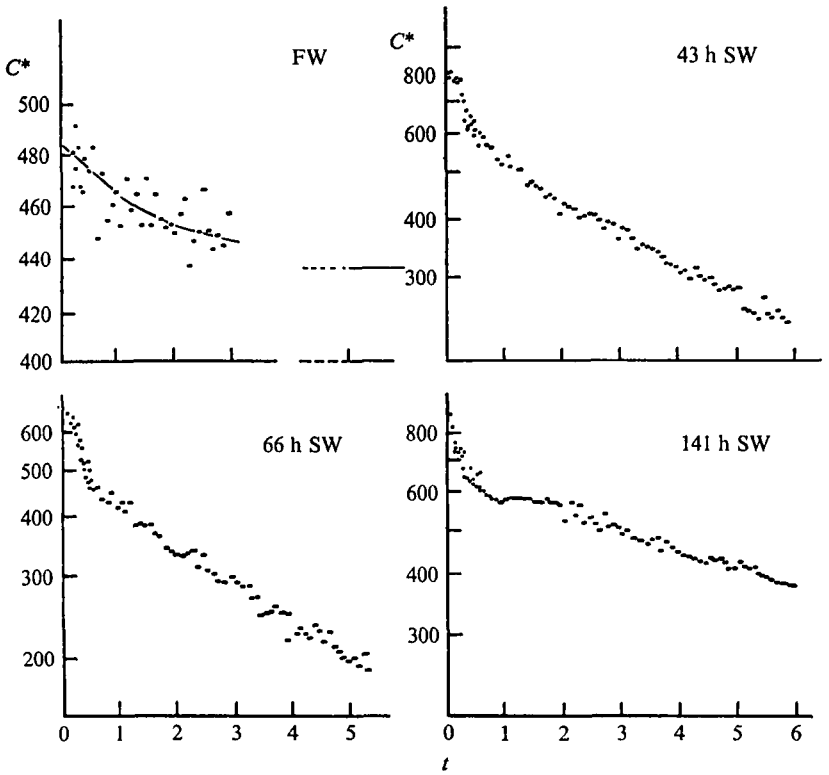

Fig. 12. Curves of loss of radioactivity from the blood of one eel (735 g) after intra-vascular injection of $^{36}Cl^-$. C^*, plasma concentration of $^{36}Cl^-$ (log scale 6 times larger in FW than in SW). t, time in hours after injection of $^{36}-Cl^-$.

The large error in the ordinate at the origin indicates that chloride space is not uniquely a function of body weight but undergoes fluctuations due to secondary factors. For this reason, we also tested the correlation between plasma chloride and chloride space/100 g body wt (E %):

$$E\,\% = (0{\cdot}043 \pm 0{\cdot}020)\, C_{Cl} + (20{\cdot}53 \pm 1{\cdot}81),$$

$$\text{mean} \pm \text{S.E.} \quad C_{Cl} = 88{\cdot}5 \pm 3{\cdot}5,$$

$$\text{mean} \pm \text{S.E.} \quad E\,\% = 24{\cdot}38 \pm 0{\cdot}44.$$

The correlation between C_{Cl} and E % is not very significant ($P < 0{\cdot}05$). However, the fraction of chloride space independent of plasma chloride (20·53 ± 1·81 %) is not statistically different from the fraction depending on body weight (22·1 ± 1·4 %). Fresh-water eels have, therefore, a basic chloride space of 20·5 to 22 ml/100 g body wt and an additional component as a function of the physiological state of the animal.

II. Eels transferred from fresh water to sea water

The temporary nature of the hypermineralization of the internal medium in these eels made it possible for plasma chloride to be considered as constant only in very short experiments of 5–10 h and only after the initial phase of the ion loading of the

Table 3. *Example of kinetic study of loss of radioactivity from the plasma in fresh water and during adaptation to sea water*

W	M	C	$\frac{V_A}{(V_A+V_B)}$	F_{AB}	$\lambda_2 \times 10^{-2}$	F_o	E %	E_{SW}/E_{FW}
736 g	FW	125·1	0·90	1,442	≃ 0	≃ 0	24·3	1
—	43 h SW	145·6	0·68	25,744	14·7 ± 0·24	4,415	—	0·831
—	66 h SW	140·2	0·66	50,171	18·0 ± 0·34	5,661	—	0·907
—	141 h SW	137·7	0·64	79,980	9·0 ± 0·14	2,454	—	0·806

W, Body weight; M, external medium; FW, fresh water; h SW, time of adaptation to sea water; C, plasma concentration of chloride (in m-equiv l^{-1}); V_A, V_B, ion compartments of body (blood belongs to A); F_{AB}, chloride flux between compartments A and B (in μ-equiv h^{-1} kg^{-1}; λ_2, chloride turnover rate; F_o chloride outflux (in μ-equiv h^{-1} kg^{-1}); E_{SW}/E_{FW}, ration between SW and FW chloride spaces (in ml).

Table 4. *Chloride space in eels transferred from fresh water to sea water*

W	n	M	C	$V_A/(V_A+V_B)$	F_{AB}	$\lambda_2 \times 10^{-2}$	F_o	E %	E_{SW}/E_{FW}
617 ± 83	5	FW	105·9 ± 9·1	0·77 ± 0·04	5,715 ± 1,499	≃ 0	≃ 0	22·7 ± 1·4	1
619 ± 73	5	FW	114·8 ± 6·4	0·76 ± 0·05	5,764 ± 2,171	≃ 0	≃ 0	23·3 ± 0·9	1
		SW	144·8 ± 2·4	0·52 ± 0·09*	35,011 ± 9,279	13·7 ± 1·77**	3,946 ± 380		0·840 ± 0·009***

Same captions as in Table 3. n, Number of eels. The statistical comparison of the FW and SW results is significant. *$P < 0·05$; **$P < 0·02$; ***$P < 0·001$.

The calculations for sea-water eels are based on the mean of the measurements made during the first 8 days of adaptation.

internal medium. The chloride space was studied here through the kinetics of loss of radioactivity from the plasma after an intravascular injection of $Na^{36}Cl$.

Fig. 12 shows the successive curves obtained from one eel in fresh water and in sea water. The mathematical interpretation of these data led to the results recorded as an example in Table 3.

Since the eel could not be weighed during the experiment, chloride space was based on 100 g for eels only in fresh water, while in sea water the relation between the sea-water total chloride space and the fresh-water total chloride space was studied (E_{SW}/E_{FW}). From this example it appears that the chloride space diminishes during the FW–SW transfer and that not only the total outflux of chloride (F_o) increases considerably but also the intra-corporal ion exchanges (F_{AB}).

The time course of chloride turnover and, therefore, of F_0, varies from one eel to another as a function of the importance of the hypermineralization phase. This will be discussed in a later study. Conversely, the gradual increase in F_{AB} in sea water was not noticed in the other eels studied, and F_{AB} passed directly to a high value after the FW–SW transfer.

Table 4 shows the statistical means of our measurements. The two series of fresh-water eels prove the good reliability of our measurements despite the large errors in $V_A/(V_A+V_B)$ and in F_{AB}. The eels transferred from fresh water to sea water confirm the following observations made on the example:

(i) a reduction in chloride space; the loss of body weight was only 1–2/10,000/h of experiment, and could not explain this reduction;

Table 5. *Chloride space in the same eels adapted to fresh water or to sea water*

W	n	M	C	λ_2	F_0	E %	E_{SW}/E_{FW}
387 ± 26 g	4	FW	86·6 ± 4·0	≃ 0	≃ 0	26·2 ± 1·7	1
—	—	SW	149·9 ± 5·1	4·74 ± 1·088	1988 ± 372	—	0·94 ± 0·004

Same captions as in Table 3.

(ii) a modification in the internal distribution of chloride;

(iii) a very important increase not only in the peripheral ion exchanges, but also in the intra-corporal ion exchanges.

III. Sea-water adapted eels

Chloride space was measured in four fresh-water eels, then again after 21 days of adaptation to sea water when the equilibrium of the ion composition of the internal medium had been reached. The loss of radioactivity from the plasma was much slower in the eel completely adapted to sea water than during the initial phase of adaptation. We therefore studied the kinetics of loss of radioactivity by successive samplings based on the following time-table: 1, 3, 5, 7, 10, 14, 22, 30, 46, 72, 96 h after the injection of $Na^{36}Cl$. The results obtained (Table 5) from the experimental curves proved that the chloride space was smaller in sea-water eels than in fresh-water eels.

IV. Eels transferred from sea water to fresh water

The experiment was made on four eels, using an intra-aortic cannula. The eels were operated on after they had spent 3 weeks in sea water. As in the preceding experiments the chloride space was measured twice in sea water, at 8-day intervals, then again after the re-adaptation of the animals to fresh water. The mean of the ratio was: $E_{SW}/E_{FW} = 0{\cdot}90 \pm 0{\cdot}3$. This ratio is significantly less than 1 and shows that the reduction of the chloride space observed in sea water is reversible.

DISCUSSION

I. Plasma concentrations of chloride and sodium

Fresh-water eels show a spread of plasma chloride concentrations four times greater than that of plasma sodium. Callamand (1943) showed that in spite of chloride variation the blood pH remains invariable in fresh-water eels. The relative stability of plasma sodium indicates, therefore, that the drop in chloride is compensated for by the retention of another anion in eels during the stage of demineralization. The bicarbonate ion probably compensates for this drop. It has already been shown by Maetz & Garcia-Romeu (1964) that *Carassius* brings about a Cl^- external for HCO_3^- internal exchange in the gills. Dejours (1969) established that in the same species the outflux of HCO_3^- was cancelled out if the fish was transferred from an external medium with chloride (10 m-equiv $Cl^-\ l^{-1}$) to one without it, and that the fish even absorbed external CO_2 immediately after this transfer. *Carassius* therefore accumulates HCO_3^- ions in its internal medium to compensate for its Cl^- losses, and the return to a chloride medium is accompanied by a compensatory increased excretion of CO_2. The retention of bicarbonates may be a mechanism compensating for the

functional inability of eel gills to absorb chloride in fresh water whereas they absorb sodium easily. This problem still remains to be studied.

After the FW–SW transfer the eel shows a rapid ion loading of its internal medium and retains more chloride than sodium ($Na^+/Cl^- = 0{\cdot}62$). But Boucher-Firly (1935) noted that, under these conditions, the total CO_2 of plasma is reduced very fast. The preferential retention of chlorides is, therefore, also accompanied by an increased excretion of bicarbonates.

The ion loading of the internal medium generally results in a temporary hypermineralization similar to the one observed in *Anguilla japonica* by Oide & Utida (1967) and by Hirano & Utida (1968). The rapid adaptation without hypermineralization, observed by Mayer & Nibelle (1970) is exceptional in the eels we have studied. The diversity in intensity and duration of the hypermineralization phases of individual eels reflects their very different potentialities for sea-water adaptation. Utida *et al.* (1966) discovered a very different pre-adaptation of the osmoregulation effectors (intestine and gills) used for transport of water and electrolyte in yellow and silver eels. It can be seen from the diversity of hypermineralization phases shown by our silver eels that this difference in potentialities persists even after skin changes.

The very large range of variation in plasma concentration of Cl^- and Na^+ indicates that eels possess a double euryhalinity: one for the body, where slow mechanisms of adaptation modify the physiology of the osmoregulation effectors so as to bring the plasma ion concentrations to the same equilibrium levels in all the eels studied; and one for the cells, enabling eels to support without any damage large concentrations of plasma chloride and sodium for several days

(205 m-equiv $Cl^{-1}\,l^{-1}$, 235 m-equiv $Na^+\,l^{-1}$).

Owing to cellular euryhanility eels are able to survive the critical phase of adaptation when the mechanisms of peripheral ion exchange are unable to assure the homeostasis of the internal medium.

Sea-water adapted eels show a strict homeostasis, and the plasma concentrations of Cl^- and Na^+ fluctuate very little. The very high plasma concentrations of sodium and chloride noted by Keys (1933) and by Sharratt *et al.* (1964) for sea-water eels probably characterize eels in a hypermineralization phase and, therefore, incompletely adapted.

II. Chloride space

The FW–SW adaptation of eels causes a modification in the internal distribution of chlorides and a reduction in chloride space.

The kinetics of loss of radioactivity from the plasma made it possible to distinguish two chloride compartments, A and B, but we were unable to ascertain their anatomical significance. Chan *et al.* (1967) observed an increase in the ratio of extracellular to total chloride when eels go from fresh water to sea water, and we observed the reverse for the ratio $V_A/(V_A+V_B)$. Since compartment A includes the blood and is, therefore, a part of the extracellular space, compartments A and B cannot correspond respectively to the extracellular and intracellular chlorides. The increase in V_A in sea water could be explained by an incorrect estimation of compartment B, due to the presence of an important quantity of intra-intestinal chlorides (sea water drunk by eels). However, according the observations of Skadhauge (1969) on the maximal amount

of intestinal chloride transport, the digestive component represents a mere fraction of about 5% of the exchange flux between *A* and *B*. The intra-intestinal chloride therefore has very little effect on the estimation of V_B.

Compartments *A* and *B* probably do not correspond to a definite anatomical distribution. Chan *et al.* (1967) observed important differences in the distribution of water and electrolytes between the tongue and the parietal muscle. This indicates that the organism probably includes a mosaic of numerous elementary ion compartments which our kinetic study re-grouped differently in *A* and *B* according to their exchange rate with the interstitial medium.

The major aim of our kinetic study is to show that sea-water adaptation does not only cause the well known stimulation of ion exchanges in the peripheral membranes (gills, intestine), but a much more important activation of the internal ion exchanges.

The reduction in chloride space after the FW–SW transfer goes against all previously reported observations on the changes in sodium space in teleosts (Motais, 1967 in *Platichthys*; Lahlou, Henderson & Sawyer, 1969, in *Carassius*), and especially in eels (Mayer & Nibelle, 1969). Sodium space increases by around 30% of its initial value and we have noted 10% reduction in chloride space. The increase in sodium space was interpreted as a favourable mechanism enabling the fish to limit the increase in plasma concentration and thus to reduce the injurious effects of an ion overload of the internal medium. Moreover, the increase in sodium space is parallel to a water transfer from the intracellular space poor in sodium to the extracellular space rich in sodium (Thorson, 1961; Evans, 1967). The chloride concentration in the extracellular medium is also much greater than in the intra-cellular medium (Chan *et al.* 1967) and changes in chloride space should parallel changes in sodium space. We have, however, previously noted a very large cellular euryhalinity in eels. In addition, Schlieper (1933) showed, *in vitro*, that the increase in chloride concentration in the internal medium causes a reduction in branchial permeability to water. Mayer & Nibelle (1970) established, *in vivo*, that the increase in plasma concentration of sodium activates the branchial outflux. The fast increase in plasma concentrations of electrolytes therefore promotes water economy and electrolyte excretion through the gills. These two reactions are helpful to sea-water adaptation. The reduction in chloride space can thus be considered as positive to sea-water adaptation, since it brings about a fast increase in plasma concentrations while limiting quantitatively the mineral overload which has to be eliminated.

The difference between our observations on chloride space and those of Mayer & Nibelle on sodium space can be accounted for in several ways:

(i) A systematic difference between the distribution of chlorides and sodium. By measuring chloride and sodium spaces simultaneously in two eels in sea-water, we found sodium space to be slightly larger than chloride space (ratios of 1·04 and 1·08).

(ii) A difference in the techniques of measurement. The intra-intestinal electrolytes are included in their totality in the measurements of Mayer & Nibelle and very little in ours.

(iii) Fundamentally a physiological difference between the eels studied. Those of Mayer & Nibelle were smaller than ours (75–250 g as opposed to 589·8 ± 30·9 g).

As regards the reduction, by about 10%, of the chloride space in sea-water eels, it can be calculated that their exchangeable chloride corresponds to that of fresh-

water eels having 126 m-equiv l^{-1} plasma chloride. This concentration can frequently be observed in well-fed eels captured in late spring. The exchangeable chloride of eels is, therefore, not essentially different in fresh water and in sea water.

SUMMARY

1. New intra-vascular cannulation techniques are described, and also an extra-corporal blood circuit containing an artificial heart and a counting cell. This makes possible a continuous study of the radioactivity of the blood.

2. Plasma chloride concentration varies greatly in fresh-water eels despite good sodium regulation.

3. The fresh-water to sea-water adaptation of eels is frequently accompanied by a temporary hypermineralization of the internal medium. This necessitates a high degree of cellular euryhalinity.

4. The sea-water-adapted eel maintains strict homeostasis of its plasma chloride and sodium.

5. The chloride distribution space decreases by 10% when eels are transferred from fresh water to sea water. The internal distribution of chloride is also modified and its fluxes between the ion compartments of the body are considerably increased.

REFERENCES

BOUCHER-FIRLY, S. (1935). Recherches biochimiques sur les Téléostéens apodes. *Annls Inst. océanogr., Monaco* **15**, 127–240.

CALLAMAND, O. (1943). L'anguille européenne (*Anguilla anguilla* L.). Les bases physiologiques de sa migration. *Annls. Inst. océanogr., Monaco* **21**, 361–440.

CHAN, D. K. O., CHESTER JONES, I., HENDERSON, I. W. & RANKIN, J. C. (1967). Studies on the experimental alteration of water and electrolyte composition of the eel (*Anguilla anguilla* L.). *J. Endocr.* **37**, 297–317.

CHESTER JONES, I., HENDERSON, I. W., CHAN, D. K. O., RANKIN, J. C., MOSLEY, W., BROWN, J. J., LEVER, A. F., ROBERTSON, J. I. S., TREE, M. (1966). Pressor activity in extracts of the corpuscles of Stannius from the european eel (*Anguilla anguilla* L.). *J. Endocr.* **34**, 393–408.

DEJOURS, P. (1969). Variations of CO_2 output of a fresh-water Teleost upon change of the ionic composition of water. *J. Physiol., Lond.* **202**, 113*P*–14*P*.

DUVAL, M. (1925). Recherches physico-chimiques et physiologiques sur le milieu intérieur des animaux aquatiques. Modifications sous l'influence du milieu extérieur. *Annls Inst. océanogr., Monaco* **2**, 233–407.

EVANS, D. H. (1967). Sodium, chloride and water balance of the intertidal teleost, *Xiphister atropurpureus*. I. Regulation of plasma concentration and body water content. *J. exp. Biol.* **47**, 513–17.

HIRANO, T. & UTIDA, S. (1968). Effects of ACTH and cortisol on water movement in isolated intestine of the eel, *Anguilla japonica*. *Gen. comp. Endocr.* **11**, 373–80.

ISTIN, M. (1964). In Maetz, J. *et al.* (1964). Recherches sur la perméabilité aux électrolytes de la branchie des Poissons et sur sa régulation endocrinienne. *Bull. inf. scient. tech. Saclay* **86**, 11–70.

KEYS, A. B. (1933). The mechanism of adaptation to varying salinity in the common eel and the general problem of osmotic regulation in fishes. *Proc. R. Soc. Lond.* B **112**, 184–99.

LAHLOU, B., HENDERSON, I. W. & SAWYER, W. H. (1969). Sodium exchanges in goldfish (*Carassius auratus* L.) adapted to a hypertonic saline solution. *Comp. Biochem. Physiol.* **28**, 1427–33.

MAETZ, J. (1956). Les échanges de sodium chez le poisson *Carassius auratus* L. Action d'un inhibiteur de l'anhydrase carbonique. *J. Physiol. ,Paris* **48**, 1085–99.

MAETZ, J. & GARCIA ROMEU, F. (1964). The mechanism of sodium and chloride uptake by the gills of a fresh-water fish: *Carassius auratus*. II. Evidence for NH_4/Na^+ and HCO_3/Cl^- exchanges. *J. gen. Physiol.* **47**, 1209–27.

MAETZ, J., MOTAIS, R. & MAYER, N. (1969). Isotopic kinetic studies on the endocrine control of Teleostean ionoregulation. III. International Congress of Endocrinology. Mexico. *Excerpta Med. Int. Congress Series* no. 184, 225–32.

MAYER, N. & NIBELLE, J. (1969). Sodium space in fresh-water and sea-water eels. *Comp. Biochem. Physiol.* **31**, 589–97.

MAYER, N. & NIBELLE, J. (1970). Kinetics of the mineral balance in the eel, *Anguilla anguilla* L., in relation to external salinity changes and intra-vascular saline infusions. *Comp. Biochem. Physiol.* **35**, 553–56.

MOTAIS, R. (1967). Les mécanismes d'échanges ioniques branchiaux chez les Téléostéens. *Annls Inst. océanogr. Monaco* **45**, 1–84.

OIDE, M. & UTIDA, S. (1967). Changes in water and ion transport in isolated intestines of the eel during salt adaptation and migration. *Mar. Biol.* **1**, 102–6.

SANDERSON, P. H. (1952). Potentiometric determination of chloride in biological fluids. *Biochem. J.* **52**, 502–5.

SCHLIEPER, C. (1933). Über die Permeabilität der Aalkiemen. I. Die Wasserdurchlässigkeit und der angebliche Wassertransport der Aalkiemen bei hypertonischem Aussenmedium. *Z. vergl. Physiol.* **19**, 68–83.

SHARRATT, B. M., CHESTER JONES, I. & BELLAMY, D. (1964). Water and electrolyte composition of the body and renal function of the eel (*Anguilla anguilla* L.). *Comp. Biochem. Physiol.* **11**, 9–18.

SKADHAUGE, E. (1969). The mechanism of salt and water absorption in the intestine of the eel (*Anguilla anguilla*) adapted to waters of various salinities. *J. Physiol., Lond.* **204**, 135–58.

THORSON, T. B. (1961). The partitioning of body water in Osteichthyes: phylogenetic and ecological implications in aquatic vertebrates. *Biol. Bull. mar. biol. Lab., Woods Hole* **120**, 238–54.

UTIDA, S., OIDE, M., SAISHU, S. & KAMIYA, M. (1966). Préétablissement du mécanisme d'adaptation à l'eau de mer dans l'intestin et les branchies isolées de l'anguille argentée au cours de sa migration catadrome. *C. r. Séanc. Soc. Biol.* **61**, 1201–4.