J. exp. Biol. (1976), 64, 461–475
With 5 figures
Printed in Great Britain

THE EFFECTS OF TRANSECTING THE IXTH AND XTH CRANIAL NERVES ON HYDROMINERAL BALANCE IN THE EEL *ANGUILLA ANGUILLA*

BY N. MAYER-GOSTAN AND T. HIRANO*

Groupe de Biologie Marine du Département de Biologie du Commissariat à l'Energie Atomique, Station Zoologique, 06230 Villefranche-sur-Mer, France

(*Received* 1 *September* 1975)

SUMMARY

The IXth and the Xth cranial nerves in *Anguilla anguilla* were transected, and the effects upon ion and water balance were studied in fresh water and sea water, and during transfer from fresh water to sea water and *vice versa*. In fresh water there is a slow demineralization due to an excess loss of Na and Cl ions. During freshwater to seawater transfer the eel survives only for 4–5 days. The fish do not drink and Na efflux does not increase enough to extrude excess ions. In sea water the glossopharyngeal and vagus nerves are necessary for the maintenance of the hydromineral balance. Denervation is followed by an increase in plasma ion concentrations. Na fluxes are not modified and increased water loss is not compensated by drinking.

The rapid reduction of Na efflux during transfer from sea water to fresh water is not modified by denervation.

INTRODUCTION

Although much is known about the electrolyte exchange mechanisms in teleost fish (Maetz, 1970, 1971, 1973), and their hormonal control (Mayer, 1970; Henderson & Chester Jones, 1972; Johnson, 1973), much less work has been devoted to the role of the nervous system in osmoregulation. In fish the participation of the nervous system in hydromineral balance has rarely been considered. Pequignot, Labat & Serfaty (1968) have shown that transection of the cardiac branch of the vagus modifies the heart rate and the ion concentrations in the liver tissue of the freshwater fish *Tinca tinca*. However, Priede (1974) has recently shown that transection of the same branch of the vagus has no effect on the heart rate in the trout *Salmo gairdneri*. In the eel *Anguilla anguilla*, Pequignot, Serfaty & Gas (1969) found that section of the visceral branch of the vagus does not interfere with hydromineral metabolism, while Hirano, Satou & Utida (1972) have shown that the eel *Anguilla japonica* failed to adapt in sea water after complete transection.

The gills and the gut are two of the main osmoregulatory organs in fish. They receive an important innervation through the vagus nerve. The gill is also innervated through the glossopharyngeal nerve. The present investigation deals with the

* Present address: Laboratory of Physiology, Ocean Research Institute, University of Tokyo Nakano, Tokyo 164 Japan.

Table 1. *Experimental design and survival time of the denervated eel*

FW, fresh water, SW, sea water.

Group	Adaptation and operation	After operation	Experiment	Survival time
I	FW	FW (7 days)	FW	Denervated > 15 days Sham-operated > 15 days
II	FW	FW (7 days)	SW	Denervated 4–5 days after transfer Sham-operated > 15 days
III	SW	SW (24 h)	SW	Denervated 3–4 days after operation Sham-operated > 15 days
IV	SW	SW (24 h)	FW	Denervated 10 days after transfer Sham-operated > 15 days

participation of the glossopharyngeal (IXth) and the vagus (Xth) nerves in osmoregulation in the eel *Anguilla anguilla*, when adapted to sea water or fresh water, and also on readjustment of osmoregulatory mechanisms during transfer from fresh water to sea water or *vice versa*.

MATERIAL AND METHODS

Eels *Anguilla anguilla*, weighing about 130 g, were collected in the Rhône valley. They were kept in running fresh water or sea water for more than 3 weeks before use. During that period, temperature was not controlled. Transection of the glossopharyngeal (IXth) and the vagus (Xth) nerves was carried out as described by Hirano *et al.* (1972). The experimental design is shown in Table 1, together with the survival time of the operated fish under different conditions. During the experiments the temperature of the external medium was maintained at 16 ± 1 °C. Sodium and chloride concentrations and osmolality of the plasma and external medium were measured using an Eppendorf flame photometer, Aminco Cotlov chloridometer, and Knauer semi-micro-osmeter. Unidirectional fluxes of sodium ions were measured using ^{24}Na. The electronics described by Tanguy (1970) was used for the influx measurements in fresh water, and for efflux measurements in sea water or during transfer experiments. Flux calculations were done as reported previously by Maetz (1956) and Motais (1967). Sodium influx in sea water was measured using a total body counter as described by Payan & Maetz (1973). The measurements of drinking on the operated eel were made with an apparatus described by Hirano (1974).

RESULTS

Freshwater-adapted eels

Although the denervated eels survived well in fresh water (Table 1), the denervation had some effects on ion balance. As shown in Table 2, transection of the IXth and the Xth cranial nerves caused a significant decrease in plasma Na and Cl concentrations after 8 days in fresh water while the sham-operation had no effect on these parameters. Water balance seems to be unaffected by the operation, since the change in body weight between day 3 and 7 after the operation was found to be very small

Table 2. *Effect of transection of IXth and Xth cranial nerves on plasma ion concentrations in freshwater eels kept in fresh water*

n = number of eels; means are given ±S.E.

	Operation	n	Before operation	8 days after operation	Difference
Na^+ (m-equiv. l^{-1})	Denervation	10	168±1·9	148±3·2	$P<0·001$
	Sham-operation	8	159±2·2	154±2·9	n.s.
Cl^-(m-equiv. l^{-1})	Denervation	10	100±3·5	81±4·4	$P<0·02$
	Sham-operation	8	95±6·7	87±5·0	n.s.

Table 3. *Effect of transection of the IXth and Xth cranial nerves on net fluxes of Na and Cl ions in freshwater eels operated and kept in fresh water*

Negative values indicate a net efflux. * significantly different from zero.
n = number of eels; means are given ±S.E.

			Hours after operation			
	Operation	n	4	24	48	72
Na^+ (μ-equiv. 100 g^{-1}. h^{-1})	Denervation	5	−25·3±1·25*	−6·5±1·12*	−14·8±3·65*	−8·3±1·36*
	Sham-operation	3	−19·0±2·87*	−2·6±1·05	− 8·0±3·16	−1·7±0·44 (6)
Cl^- (μequiv. 100 g^{-1}. h^{-1})	Denervation	5	−20·9±1·23*	−6·4±1·24*	−14·2±2·16*	−9·8±2·34*
	Sham-operation	3	−16·1±2·30*	−2·6±1·39	− 6·8±3·35	−4·8±1·03 *(6)

and identical in the two groups (denervated fish: +1·39±0·26% per 24 h; sham-operated-fish: +1·12±0·41% per 24 h). On the other hand, both the denervated and the sham-operated eels showed a negative ion balance shortly after operation (Table 3). While the denervated eel kept losing ions for more than 72 h, the sham-operated eels maintained their ion balance after about 24 h; the net flux was not significantly different from zero. In order to know whether the net ion loss observed in the denervated eel was due to a decrease in influx or an increase in efflux, or both, the unidirectional fluxes of Na were measured 72 h after operation. The results obtained are illustrated on Fig. 1. It appears that the influx, or ion pump, was not modified after such an operation, but efflux was significantly higher in the denervated fish. Therefore the decrease in plasma ion concentrations in the operated eels seems to be due to an excess ion loss.

Transfer from fresh water to sea water

Freshwater-adapted eels were operated on and kept in fresh water for 7 days before they were transferred to sea water. The denervated eels died 4–5 days after transfer to sea water (Table 1). Change in plasma ion concentrations are shown in Fig. 2. At the time of transfer the denervated fish showed slightly lower ion concentrations than the sham-operated controls. Transfer to sea water caused a marked increase in plasma ions during 24 h following transfer, and there was no significant difference between the two groups. After 48 h, plasma ions decreased significantly in the sham-operated eel, as compared with the level at 24 h, while those in the

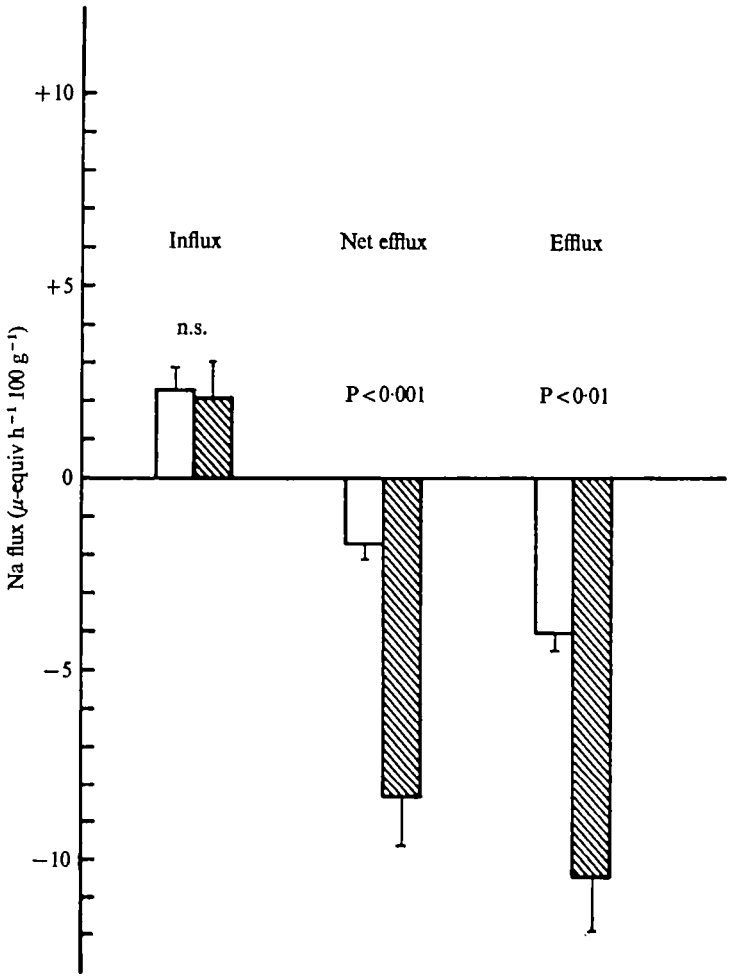

Fig. 1. Unidirectional Na fluxes in freshwater-adapted eels. Open columns indicate fluxes of sham-operated eels ($n = 6$) and shaded columns those of denervated eels ($n = 5$). Vertical bars indicate standard errors of the means.

denervated eel continued to rise significantly. The denervated eels eventually died, 4–5 days after transfer, with very high plasma ion concentrations. Changes in body weights paralleled those of Na and Cl in the plasma. Both groups lost weight during the first 24 h after transfer. The sham-operated eels regained weight, to a slightly lower level than their initial weight, while the denervated eels continue to lose weight (Fig. 2).

Changes in Na efflux, as a function of time, are shown in Fig. 3. In the control fish, Na efflux, which was very low in fresh water, increased considerably after 24 h in sea water and attained the level of fully adapted fish after 48 h. In the denervated eels, an increase of the efflux was also observed during the first 24 h. However, the increment was much less than that with the sham-operated fish, and there was no subsequent increase.

Na influx was measured during the first 30 min in sea water and during another

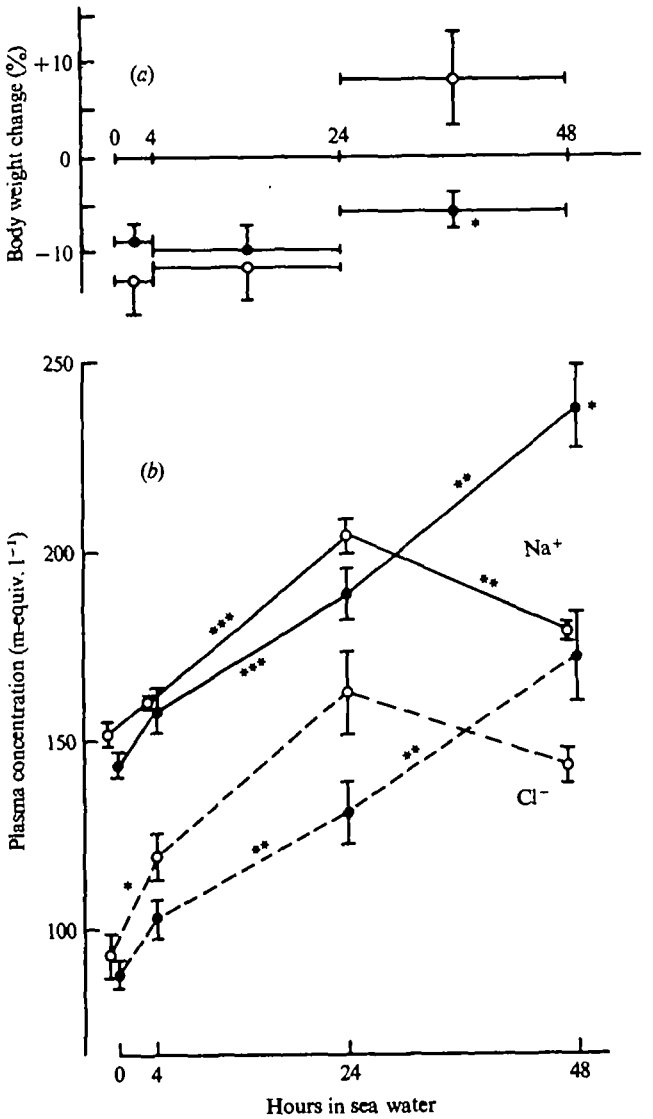

Fig. 2. Effect of the transection of the IXth and Xth cranial nerves on body weight change (*a*) and on plasma ion concentrations (*b*) during transfer from fresh water to sea water. Vertical bars = ±S.E. Horizontal bars indicate duration of the change in body weight. ●, denervated eels ($n = 6$); ○, sham-operated eels ($n = 5$). Asterisks indicate either a significant difference from the sham-operated eel or significant increase or decrease measurements. * $P < 0.02$ ** $P < 0.01$ *** $P < 0.001$.

30 min period, 24 h after transfer. Since the eel is known to drink a considerable amount of water during the course of adaptation to sea water, especially at the time of transfer, Na influx was measured in eels with the oesophagus ligated in order to avoid any influx of sodium due to drinking. As shown in Table 4, there was no significant difference in the gill Na influx between sham-operated and denervated eels either during the first 30 min in sea water or 24 h later ($P > 0.5$). The Na influx was also measured in the intact fish. The difference between the intact fish and the

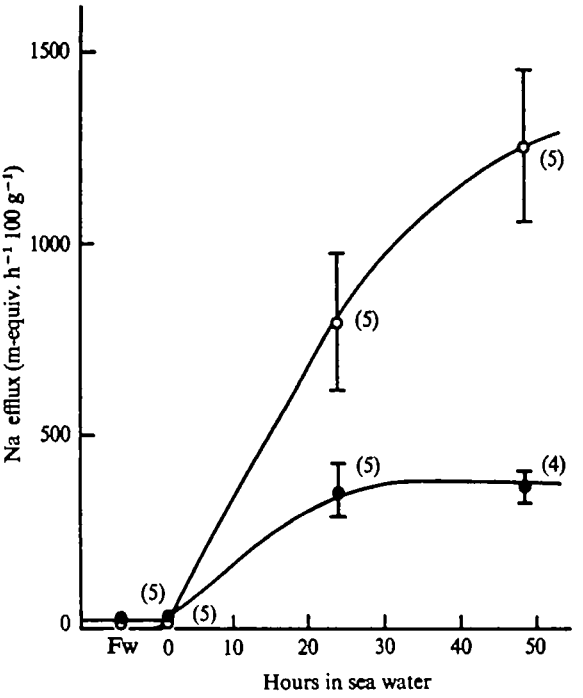

Fig. 3. Changes with time of the sodium efflux during adaptation to sea water. ○, flux of the sham-operated eels: ●, flux of the denervated eels. Vertical bars = ±S.E. Figures in brackets = number of eels.

Table 4. *Effect of transection of the IXth and Xth cranial nerves on Na influx during transfer from fresh water to sea water*

Figures in brackets = numbers of eels; means given ±S.E.

	Operation	Hours in sea water: 0–1/2 h		Hours in sea water: 24 h	
Gill influx μ-equiv. h^{-1}. 100 g^{-1}	Denervation + oesophagus ligation	232 ± 60·8	(5)	461 ± 106·2	(4)
	Sham-operation + oesophagus ligation	378 ± 191·2	(3)	647 ± 95·6	(3)
Total influx μ-equiv. h^{-1}. 100 g^{-1}	Intact	714 ± 362	(4)	653 ± 196	(2)

oesophagus-ligated and sham-denervated fish seems to be due to entry via the gut. During the first 30 min, the difference was about 300 μ-equiv./h^{-1}. 100 g^{-1}, corresponding to drinking 0·6 ml of sea water. The ability of the fish to drink sea water at the time of transfer was tested using oesophagus – cannulated eels. No drinking response was observed in the denervated eels. Therefore it appears that sham-operated fish suffered a Na loading for the first 24 h (dehydration, Na influx greater than efflux, important drinking of sea water). At 24 h and afterwards, the gill seems to have been able to pump sodium out efficiently and water balance was re-established; resulting in a decrease in plasma ion concentration. On the other hand, after the transection of the

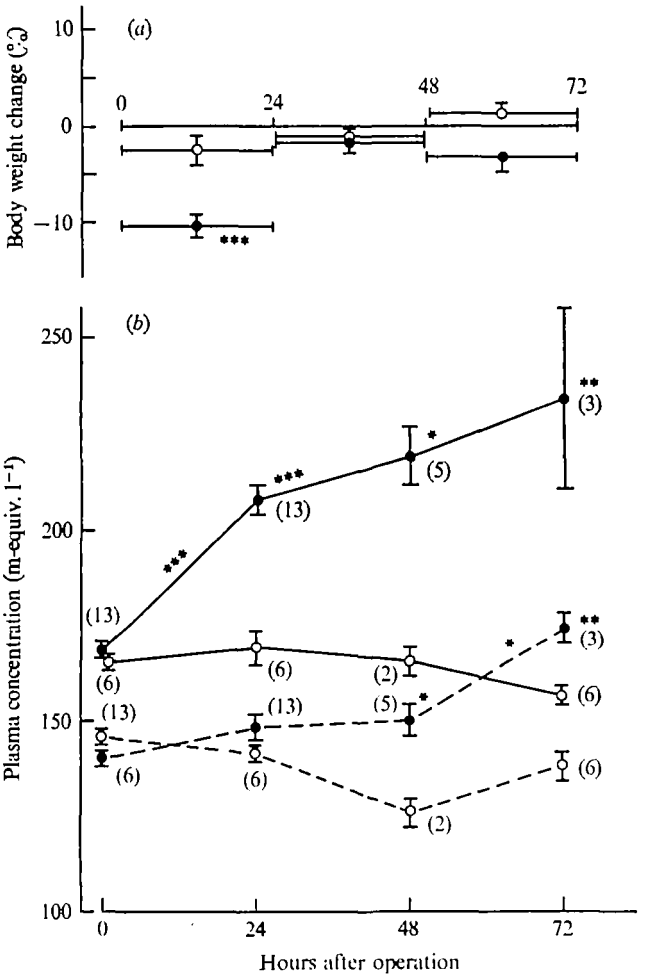

Fig. 4. Effect of the transection of the IXth and Xth cranial nerves on body weight change (*a*) and on plasma ion concentrations (*b*) in sea-water-adapted eels. Display as in Fig. 2. Figures in brackets = number of eels.

IXth and Xth cranial nerves for the first 24 h, there was a salt load comparable to that of the sham-operated eels (even if no drinking of sea water occurred). But at 24 h and afterwards, dehydration was still very important and the fish was unable to adjust its excretion of ions. This resulted in a high plasma ion concentration and the fish started to die.

Seawater-adapted eels

The effect of transection of the IXth and the Xth cranial nerves was examined in seawater-adapted eels in order to assess the role of these nerves on the maintenance of the water and ion balances in a steady state. As shown in Table 1, the operated fish survived only 3–4 days after the transection of the nerves when they were kept in sea water. The effect of denervation on plasma ion concentrations is illustrated in Fig. 4. Sham-operation did not cause any significant change in plasma ion concentration, whereas denervation caused a marked increase. Plasma sodium in the denervated

Table 5. *Effect of transection of the IXth and Xth cranial nerves on Na fluxes in seawater eels kept in sea water*

Figures in brackets = numbers of eels; means given ±S.E.

		Hours after operation		
	Operation	24	48	72
Efflux (μ-equiv./100 g^{-1}.h^{-1})	Denervation	1469±255 (8)	1561±172 (5)	1529±327 (3)
	Sham-operation	1053±213 (10)	—	—
Influx (μ-equiv./100 g^{-1}.h^{-1})	Denervation	737±84 (4)	—	—
	Sham-operation	527±37 (4)	—	—

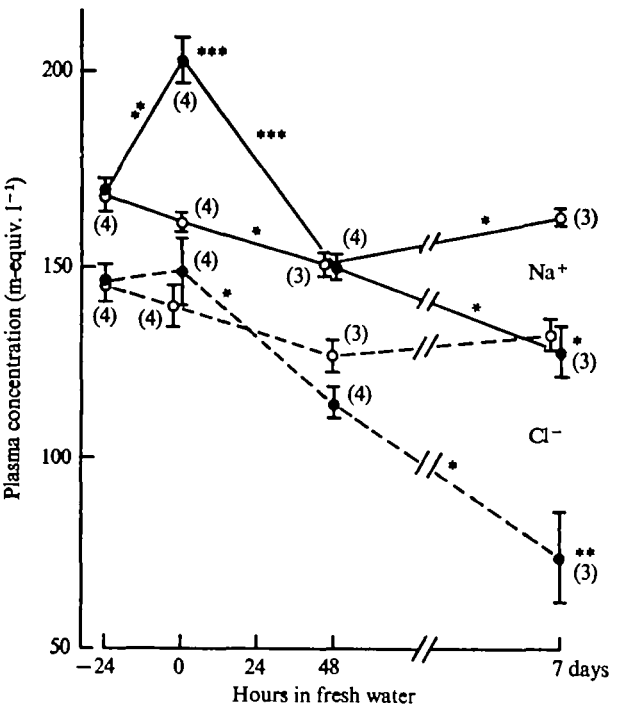

Fig. 5. Effect of transection of the IXth and Xth cranial nerves on plasma ion concentrations during transfer from sea water to fresh water. Display as in Fig. 2. Figures in brackets = number of eels.

eel increased to more than 200 m-equiv. l^{-1}, and kept increasing up to 72 h, when the fish started to die. The effect of denervation on plasma Cl concentration was less marked than on Na; a significant increase was seen only 48 h after denervation. Plasma osmolality, which was the same in the two groups of fish before operation (312, S.E. 3·4; 316, S.E., 3·2 mOsmol. l^{-1}), increased considerably 24 h after denervation and reached 422 S.E., 9·1 mOsmol. l^{-1} while there was no change after the sham-operation (321, S.E., 11·3). The difference is highly significant ($P < 0{\cdot}001$). Furthermore, in the denervated eel, there was an increase in plasma osmolality which was greater than that expected from the increase in Na and Cl concentrations.

Sham-operation did not cause any significant change in body weight while denervation caused a loss of about 10% during the first 24 h (Fig. 4). Na efflux in the operated

Table 6. *Effect of transection of the IXth and Xth cranial nerves on relative Na effluxes of eels during rapid transfer from sea water to fresh water and to fresh water with* 10 mM *KCl*

n = numbers of eels; means given ±S.E.

Operation	n	Relative Na efflux (% of the SW efflux)			Na effluxes, in μ-equiv. h^{-1} 100 g^{-1}		
		SW	FW 6′	FW+K 6′	0′–12′	12′–24′	24′–36′
Denervation	3	100	26·6±7·42	61·2±3·60	117±29·6	100±31·2	86±34·2
Sham-operation	3	100	11·9±1·97	63·9±7·07	131±50·7	86±21·5	76±36·6

fish did not increase significantly, although the efflux 24 h after the operation was slightly greater than the efflux in the sham-operated fish. The influx, the total of Na entry through the gill and the gut, was slightly greater in the denervated eel than in the control but the difference was not significant. In both groups of fish, the influx was less than the efflux, the fish being apparently in a negative balance (Table 5).

Transfer from sea water to fresh water

Seawater-adapted eels were maintained in sea water for 24 h after the transection of the IXth and Xth cranial nerves and were then transferred to fresh water. The denervated fish survived in fresh water for more than 10 days (Table 1). Changes in the plasma ion concentrations are shown in Fig. 5. As observed in the previous experiment, plasma Na concentration in the denervated fish increased significantly during the first 24 h in sea water while there was no change in the Cl concentration. When these eels were transferred to fresh water, plasma Na and Cl concentrations declined significantly after 48 h, in both the operated and the sham-operated animals, but the rate of decrease was more marked in the denervated fish. After 7 days in fresh water, plasma ion concentrations continued to decrease in the denervated fish, while those in the sham-operated fish increased slightly, as compared with the level at 48 h in fresh water. The differences between the two groups of fish are statistically significant. As shown in Table 2, when freshwater-adapted eels were denervated and kept in fresh water for 7 days, plasma Na and Cl decreased by about 20 m-equiv. l^{-1}. There was a decrease of 75 m-equiv. l^{-1}, for both sodium and chloride, after transfer from sea water to fresh water.

When transferred from sea water to fresh water, the sham-operated eels did not lose or gain any weight during 48 h after transfer (−0·33±1·2% per 48 h), while the denervated fish gained about 10% (10·6±1·76% per 48 h). However, no further change was observed in both groups of fish, as in the previous experiment with the freshwater-adapted eel kept in fresh water.

Sodium efflux was studied during rapid transfer from sea water to fresh water (Table 6). The rate of instantaneous reduction of the efflux was slightly less in the denervated eels than in the sham-operated controls but the difference was not significant. When potassium is present at the concentration in sea water (10 mM) the instantaneous reduction in Na efflux in fresh water is much less or not apparent (Maetz, 1969; Motais & Isaia, 1972). No difference in this K-dependent Na efflux was

observed between the two groups of fish; the glossopharyngeal and the vagus nerves do not seem to be involved in the instantaneous modification of the sodium efflux.

The Na efflux was also followed for 3 consecutive periods of 12 min after transfer into fresh water (Table 6). In both groups of fish, the efflux 30 min after transfer was still greater (75–85 μ-equiv. h^{-1} 100 g^{-1}) than that in fully adapted freshwater fish ($\simeq$ 4 μ-equiv. h^{-1} 100 g^{-1}) (Fig. 1). Net sodium flux was measured 48 h after transfer to fresh water and was significantly ($P < 0{\cdot}02$) greater in the denervated eels (17·6, S.E., 2·16 μ-equiv. h^{-1} 100 g^{-1}; $n = 4$) than in the sham-operated eels (5·5, S.E., 0·3, μ-equiv. h^{-1} 100 g^{-1}; $n = 3$). The observed decrease in the plasma Na concentration of the denervated fish (Table 2) correspond to a mean net loss of 13 μ-equiv. h^{-1} 100 g^{-1} during the first 7 days in fresh water, which is in accordance with the measured net flux.

DISCUSSION

The glossopharyngeal nerve innervates the first gill arch and the buccal cavity in the eel although nothing is known about its electrical activity in relation to osmotic and ionic changes of the external medium. The eel's vagus nerve has fibre endings in the oesophagus, the stomach, the heart and all four gill arches (Young, 1936; Burnstock, 1958). In fish, it was shown that vagal nerve stimulation evokes contraction and relaxation of the stomach (Ito & Kuriyama, 1971), and that the movement of the stomach and the alimentary sphincters is dependant on cholinergic or adrenergic nerves (Nilsson & Fänge; 1969; Ng, Tey & Yan 1973). Thus, transection of the vagus nerve as in the present study, would probably affect oesophagus and stomach movements. The heart function was found to be different in stenohaline and euryhaline fish (Labat, 1966) as a consequence of a different vagal control. In the eel (Pequignot, 1972), no change in the heart beat was observed after transection of the cardiac branch of the vagus. Thus, in the present study, the observed effects on the gill should be considered as direct parasympathetic innervation of the gill and not a secondary haemodynamic effect. The terminal site of branchial nerves in the eel gill was described by Gilloteaux (1969). Nerve endings were observed around the afferent and efferent arteries, at the neuromuscular junctions and at the base of the lamaellae around the pilar cells. Nerve endings were never found in connexion with the chloride cells which are accepted as specialized sites for ion exchange with the external medium. However, a decrease in chloride cell number, after transection of the visceral branch of the vagus, was observed in *Tinca tinca* (Pequignot & Gas, 1971) but not in the eel (Peguignot, 1972). No histological study of the gill epithelium was undertaken after complete transection of the glossopharyngeal and vagus nerves.

The participation of the nervous system is discussed below in relation to what is known of the mechanisms for adaptation to changes in external salinity and to the maintenance of the ionic and water balance in fresh water or sea water.

Freshwater-adapted fish

In the present study, the denervated eel survived well in fresh water, as observed by Hirano *et al.* (1972) in *Anguilla japonica*. Water balance seems to be unaffected by the operation, if a change in body weight is considered as a criterion for net water movements. This may indicate that denervation does not cause any change in

the water permeability through the body surface or that increased or decreased entry of water is compensated by modification of kidney function. The latter could be occurring since Kirsch (1972*b*) has shown that an experimental perfusion of fresh water into the stomach of *Anguilla anguilla* stimulated a parallel increase in urinary flow. On the other hand, plasma Na and Cl concentrations decreased significantly 8 days after the operation. Similar changes are also observed in the 'decerebrate' eel (Hirano *et al.* 1972) or after hypophysectomy (Olivereau & Chartier Baraduc, 1966). The negative ion balance which is observed in both operated and sham-operated eels during the first 24 h after the operation seems to be due to a loss through the wound. The sham-operated eels subsequently restored their ion balance but in the denervated fish this ion loss persisted due to increased efflux. Since the urinary bladder was not catheterized, it is not known whether the increased loss in the denervated eels takes place through the gills, kidneys or both. It appears that the ion pump in the gills was not modified by transection of the nerves. Similar increases in the Na efflux, without change in the Na influx, were also found in the hypophysectomized eel (Maetz, Mayer & Chartier-Baraduc 1967). The observed electrolyte losses may be a result of the transection of the glossopharyngeal nerve since Hirano *et al.* (1972) did not find any change in plasma Na and Cl concentrations when the Xth cranial nerve alone was cut in eels in fresh water. Thus, the denervated fish suffered a slow electrolyte loss although this did not cause the death of the fish. The eel can withstand a relatively large decrease in the concentration of its plasma ions: chloride as low as 40–50 m-equiv. l^{-1} (Kirsch, 1971), sodium down to 120 m-equiv l^{-1} (Mayer & Nibelle, 1970).

Seawater-adapted fish

Fish adapted to sea water experience a continual loss of water across the body surface. Urinary and faecal water losses are reduced to a minimum and the osmotic net efflux is compensated for by drinking the external medium. As shown in the present experiment, the glossopharyngeal and the vagus nerves are necessary to maintain water balance of eels in sea water. Two mechanisms may be deficient; denervation may result in an inability to drink water and/or an increase in water permeability. The normal drinking rate, compensating for net losses is about 3–5% of the body weight per day (Kirsch & Mayer-Gostan, 1973; Maetz, 1970). During the 24 h following denervation, the fish lost 10% of its body weight so that an absence of drinking alone cannot explain the loss. Water permeability thus is also increased. A loss of water and hyperosmolality of plasma have been shown to stimulate drinking in the eel (Hirano, 1974) and this process seems to be under the control of the nervous system. It is difficult to assess from the present experiments whether information from the 'milieu intérieur' is missing or whether mechanical swallowing is not possible in the absence of the intact nerves. It has been shown in the mullet that adrenaline will stop drinking (Pic, Mayer-Gostan & Maetz 1974). It is possible that there are opposing cholinergic and adrenergic effects and that cutting the IXth or Xth cranial nerves leaves the effector under adrenergic control. In sea water, the plasma Na concentration increased rapidly for 24 h after denervation while the chloride concentration was not altered. The discrepancy between the changes in Na and Cl levels excludes the possibility that the increase in plasma concentration is

due to a net loss of water alone. It is likely that Na extrusion decreases after denervation while the Cl extrusion or the chloride space increase. However the effect of denervation on chloride exchanges was not examined in the present study. In intact fish and in the sham-operated fish, Na and Cl ions were the principal contributors to the plasma osmolality. After denervation, however, the expected osmolality, calculated from Na and Cl concentrations, was significantly less than the measured osmolality. Presumably another unknown osmotic solute increases in concentration after denervation.

There was no change in Na efflux after denervation. Mayer & Nibelle (1970) and Mayer-Gostan & Kirsch (in preparation) have shown that Na efflux in seawater-adapted eels is not maximal and that perfusion of hypertonic NaCl solution instantly induces long-lasting increases in the ion effluxes. It is likely that the controlling feedback mechanism is lost after transection of the glossopharyngeal and vagus nerves. That there is no decrease of the ion efflux after denervation suggests that the normal flux is not under a constant cholinergic control but that the branchial nerves are necessary to stimulate ion extrusion.

In seawater-adapted eels, the glossopharyngeal and the vagus nerves are necessary to maintain hydromineral balance; they are unable to drink and plasma ion concentrations increase as a result of an increased net loss of water and probably a net influx of ion through the gill, without any change in the sodium extrusion.

Transfer experiments

When the denervated eels were transferred from sea water to fresh water, they failed to readjust water permeability during the first 48 h. However, subsequently, there was no further change of weight suggesting that the kidney was then able to remove the excess water that is gained across the body surface. It is also probable that the normal water permeability seen in fresh water is then restored. On rapid transfer there was no difference between the sham-operated and the denervated eels in instantaneous reduction of Na efflux and its restoration by K ions; the glossopharyngeal and the vagus nerves are apparently not involved in the immediate adaptation of the Na flux or the exchange diffusion mechanisms.

After transfer from fresh water to sea water, the denervated fish survived for only a few days, confirming the results of Hirano *et al.* (1972). The drinking reflex, which is normally observed when the fish come into contact with sea water (Kirsch, 1972 *a*; Kirsch & Mayer-Gostan, 1973; Hirano, 1974), was abolished. Hirano *et al.* (1972) showed that denervated fish suffer from dehydration due to reduced drinking and a low absorption of fluid from the gut.

The absence of drinking at the time of transfer to sea water may result either from the absence of neural informations, resulting from local dehydration, or sensory stimuli from the gill or mouth region. Such stimuli may trigger the drinking reflex *via* afferent branchial nerves. Alternatively, swallowing may be impaired since the muscles involved may be normally under the control of the visceral branch of the vagus. Sodium influx through the gills during the first hour after transfer from fresh water to sea water was measured and it appears that in the sham-operated fish, as well as in the denervated fish, there is a net influx of sodium during this period. Kirsch (1971) also observed that there is a net influx of chloride at the time of transfer

sea water but this rapidly decreases during the next 5 h. This net influx of sodium and chloride was also observed in the *in vitro* perfused gill of the eel during the first 3 h in sea water (Shuttleworth & Freeman, 1973, 1974). From the present study in sham-operated fish, there are about 200–300 μ-equiv. h^{-1} 100 g^{-1} of Na entering through the gill during the first hour in sea water. During the same period Kirsch & Mayer-Gostan (1973) have shown that a similar amount of salt is taken in as a result of drinking. Since both the denervated and the sham-operated fish had similar sodium influx, it seems that Na permeability at the time of transfer is not controlled by the nervous system. On the other hand, Na efflux in the denervated fish only increased to 350 μ-equiv. h^{-1} 100 g^{-1} after 24 h in sea water, which is much less than in the sham-operated fish where it reaches a sea water level. This suggests that the gill needs an intact nerve supply, either efferent or afferent, to initiate salt excretion. Fänge, Schmidt-Nielsen & Robinson (1958) and Hanwell, Linzell & Peaker (1972) showed in birds that nasal gland secretion in response to salt loading is prevented by cutting the vagus nerves and that afferent fibres from receptors to the central nervous system lie in the vagus nerve. In fish the vagus nerve also contents sensory fibres so that the same type of reflex may exist. It has been shown in eels that during adaptation from fresh water to sea water, both the number of chloride cells and Na-K-ATPase activity in the gills are increased (Utida, Kamiya & Shirai 1971; Bentley, 1971) and that there is an induction of the synthesis of ribosomal proteins in the gill (Conte & Morita, 1968). Maetz *et al.* (1969) found that actinomycin D, which inhibits mRNA synthesis in the nucleus, also inhibits the adjustment of the Na efflux when the eel is transferred from fresh to sea water. In the present experiments, the time course in Na efflux found following the transfer to sea water of the denervated eel is comparable to the one observed in eels treated with the antibiotic. In the salt gland of the goose, during adaptation to drinking salt water, Hanwell & Peaker, (1973, 1975) have shown that nervous activity is necessary to initiate the rise in RNA levels and their results also indicate that even if hormones have a role in this process they cannot alone be responsible for the hypertrophy of the gland. As the glossopharyngeal and vagus nerves are the main nerves innervating the gill they may also be necessary for the protein synthesis which takes place in the gill during adaptation of the fish to sea water.

In conclusion nerves seem to be involved in the maintenance of water permeability and drinking in the eel. They are also necessary for the initiation of Na extrusion during adaptation to sea water. The nerves may be afferent fibres which send information from receptors to the brain and regulate the release of hormones. It is also possible that efferent nerves that control drinking or the synthesis of regulatory proteins in the gills are involved.

We are grateful to Dr P. Bentley for reading the manuscript, and we wish to thank Dr Maetz for providing research facilities for Dr T. Hirano. Dr Hirano received a grant from the C.E.A.

REFERENCES

BENTLEY, P. (1971). *Endocrines and Osmoregulation.* New York: Springer-Verlag.

BURNSTOCK, G. (1958). The effect of drugs on spontaneous motility and on response to stimulation of the extrinsic nerves of the gut of a teleostean fish. *Br. J. Pharmac. Chemother.* **13**, 216–26.

CONTE, F. P. & MORITA, N. (1968). Immunochemical study of cell differentiation in gill epithelium of euryhaline *Oncorhynchus* (Walvaum). *Comp. Biochem. Physiol.* **24**, 445–54.

FÄNGE, R., SCHMIDT-NIELSEN, K. & ROBINSON, M. (1958). Control of secretion from the avian salt gland. *Am. J. Physiol.* **195**, 321–6.

GILLOTEAUX, J. (1969). Note sur l'innervation des branchies chez *Anguilla anguilla* L. *Experientia* **25**, 270–1.

HANWELL, A., LINZELL, J. L. & PEAKER, M. (1972). Nature and location of the receptors for salt gland secretion in the goose. *J. Physiol., Lond.* **226**, 453–72.

HANWELL, A. & PEAKER, M. (1973). The effect of post ganglionic denervation on functional hypertrophy in the salt gland of the goose during adaptation to salt water. *J. of Physiol.* **234**, 78–80.

HANWELL, A. & PEAKER, M. (1975). The control of adaptative hypertrophy in the salt gland of geese and ducks. *J. Physiol. Lond.* **248**, 193–205.

HENDERSON, I. CHESTER JONES, J. (1972). Hormones and osmoregulation in fishes. *Annals. Inst Michel Pacha* **5**, 69–235.

HIRANO, T., SATOU, M. & UTIDA, S. (1972). Central nervous system control of osmoregulation in the eel (*Anguilla japonica*). *Comp. Biochem. Physiol.* **43A**, 537–44.

HIRANO, T. (1974). Some factors regulating water intake by the eel, *Anguilla japonica*. *J. exp. Biol.* **61**, 737–47.

ITO, Y. & KURIYAMA, H. (1971). Nervous control of the motility of the alimentary canal of the silver carp. *J. exp. Biol.* **55**, 469–87.

JOHNSON, D. W. (1973). Endocrine control of hydromineral balance in teleosts. *Am. Zool.* **13**, 799–818.

KIRSCH, R. (1971). Echanges d'eau, de chlorures et de sodium au niveau des différents effecteurs de l'osmorégulation chez l'anguille (*Anguilla anguilla* L.) en eau douce et au cours de l'adaptation d'eau douce à eau de mer. Thèse de Doctorat d'Etat, Université de Strasbourg.

KIRSCH, R. (1972*a*). The kinetics of peripheral exchanges of water and electrolytes in the silver eel (*Anguilla anguilla*) in fresh water and in sea water. *J. exp. Biol.* **57**, 489–512.

KIRSCH, R. (1972*b*). Corrélation entre perméabilité branchiale, perméabilité disgestive et diurèse chez l'anguille européenne en eau douce. *J. Physiol., Paris* **65**, 428A.

KIRSCH, R. & MAYER-GOSTAN, N. (1973). Kinetics of water and chloride exchanges during adaptation of the european eel to sea-water. *J. exp. Biol.* **58**, 105–21.

LABAT, R. (1966). Electrocardiologie chez les poissons téléostéens. Influence de quelques facteurs écologiques. *Ann. Limn.* **2**, 1–175.

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. (1969). Sea water teleosts: evidence for a sodium–potassium exchange in the branchial sodium-excreting pump. *Science, N.Y.* **166**, 613–15.

MAETZ, J. (1970). Mechanisms of salt and water transfer across membranes in teleosts in relation to the aquatic environment. *Memoirs of the Soc. for Endocrin.* **18**, 3–29.

MAETZ, J. (1971). Fish gills: mechanisms of salt transfer in fresh water and sea water. *Phil. Trans. Roy. Soc. Lond.* B, **262**, 209–49.

MAETZ, J. (1973). Transport mechanism in sea water adapted fish gills. Alfred Benson Symposium V. *Transport mechanisms in Epithelia* pp. 427–41. Copenhagen: Munksgaard.

MAETZ, J., MAYER, N. & CHARTIER-BARADUC, M. M. (1967). La balance minérale du sodium chez *Anguilla anguilla* en eau douce, en eau de mer et au cours du transfert d'un milieu à l'autre: effets de l'hypophysectomie et de la prolactine. *Gen. Comp. Endocrin.* **8**, 177–88.

MAETZ, J., NIBELLE, J., BORNANCIN, M. & MOTAIS, R. (1969). Action sur l'osmorégulation de l'anguille de divers antibiotiques inhibiteurs de la synthèse des protéines ou du renouvellement cellulaire. *Comp. Biochem. Physiol.* **30**, 1125–51.

MAYER, N. (1970). Controle endocrinien de l'osmorégulation chez les Téléostéens. Rôle de l'axe hypophyse-interrénale et de la prolactine. *Bull. Inf. Sci. Techn. C.E.A.* **166**, 45–75.

MAYER, N. & NIBELLE, J. (1970). Kinetics of the mineral balance in the eel *Anguilla anguilla* in relation to external salinity changes and intravascular saline infusion. *Comp. Biochem. Physiol.* **35**, 553–66.

MOTAIS, R. (1967). Les mécanismes d'échanges ioniques branchiaux chez les Téléostéens. *Ann. Inst. Oceanogr. Paris XLV* (1), 1–84.

MOTAIS, R. & ISAIA, J. (1972). Evidence for an effect of oaubain on the branchial sodium-excreting pump of marine teleosts: interaction between the inhibitor and external Na and K. *J. exp. Biol.* **57**, 367–73.

NG, S., TEH, Y. & TAN, C. (1973). Cholinergic and adrenergic receptors in the oesophagel, pyloric, and special sphincters of the puffer fish, *Tetraodon immaculatus*. *Comp. gen. Pharmacol.* **4**, 43–6.

NILSSON, S. & FÄNGE, R. (1969). Adrenergic and cholinergic vagal effects on the stomach of a teleost (*Gadus morhua*). *Comp. Biochem. Physiol.* **30**, 691–4.

OLIVEREAU, M. & CHARTIER-BARADUC, M. M. (1966). Action de la prolactine chez l'anguille intacte et hypophysectomisée II. Effet sur les électrolytes plasmatiques (Na K Ca). *Gen. Comp. Endocrin.* **7**, 27–36.

PAYAN, P. & MAETZ, J. (1973). Branchial sodium transport mechanisms in *Scyliorhinus canicula*: evidence for Na^+/NH_4^+ and Na^+/H^+ exchanges and for a role of carbonic anhydrase. *J. exp. Biol.* **58**, 487–502.

PEQUIGNOT J. (1972). Réactions comparées de deux téléostéens, Tanche et Anguille, lors d'un changement de salinité: influence du parasympathique. Thèse de Doctorat d'Etat.

PEQUIGNOT, J. & GAS, N. (1971). Modifications histologiques de l'épithélium branchial sous l'influence de la vagotomie chez la *Tanche*. *C. R. Soc. Biol.* **165**, 1172–6.

PEQUIGNOT, J., LABAT, R. & SERFATY, A. (1968). Vagotonicité et résistance à la salinité chez quelques téléostéens d'eau douce. *Hydrobiologia* **32**, 570–6.

PEQUIGNOT, J., SERFATY, A. & GAS, N. (1969). Modifications ioniques chez l'Anguille argentée: influence de la vagotomie et de la surcharge sodique. *Experientia*, **25**, 936.

PIC, P., MAYER-GOSTAN, N. & MAETZ, J. (1974). Branchial effects of epinephrine in the sea water adapted mullet. I. Water permeability. *Am. J. Physiol.* **226**, 689–702.

PRIEDE, I. G. (1974). The effect of swimming activity and section of the vagus nerves on heart rate in rainbow trout. *J. exp. biol.* **60**, 305–19.

SHUTTLEWORTH, T. J. & FREEMAN, R. F. H. (1973). The role of the gills in sea water adaptation in *Anguilla dieffenbachii*. I. Osmotic and ionic composition of the blood and gill tissue. II. Net ion fluxes in isolated perfused gills. III. The relative significance of the gills. *J. Comp. Physiol.* **86**, 323–30.

SHUTTLEWORTH, T. J. & FREEMAN, R. F. (1974). Net fluxes of water in the isolated gills of *Anguilla dieffenbachii*. *J. exp. Biol.* **60**, 769–81.

TANGUY, R. (1970). Ensembles électroniques destinés à l'étude de l'osmorégulation d'animaux aquatiques. *Bull. Inf. Sc. Techn. C.E.A.* **144**, 11–15.

UTIDA, S., KAMIYA, M. & SHIRAI, N. (1971). Relationship between the activity of Na^+–K^+ activated ATPase and the number of chloride cells in eel gills with special reference to sea water adaptation. *Comp. Biochem. Physiol.* **38**, 443–7.

YOUNG, J. Z. (1936). The innervation and reaction to drugs of the viscera of Teleostean fish. *Proc. Roy. Soc.* B **120**, 303–18.