THE EFFECT OF ANAEMIA ON ION EXCHANGE IN THE SOUTHERN FLOUNDER (*PARALICHTHYS LETHOSTIGMA*)

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Abstract—1. Measurements of branchial sodium efflux rates, radiosodium spaces, and plasma ionic levels were made in southern flounder through use of a catheterization technique which permitted serial blood sampling.

2. Disturbances in hematocrit and plasma sodium concentration were associated with the catheterization procedure.

3. Sodium efflux rate was reduced 23 per cent in fish rendered anaemic through bleeding.

4. Radiosodium spaces and plasma sodium and potassium levels were not significantly changed by anaemia. However, plasma sodium and potassium levels were positively correlated regardless of hematocrit.

5. The results are discussed in terms of present theories of electrolyte balance and blood shunting mechanisms in the gills of teleosts.

INTRODUCTION

THE GILL filament of a teleost fish represents the points of closest proximity of the internal and external environments. As such, it serves as the principal site for both respiration and osmoregulation, the two vital functions most dependent upon the rapid movement of substances between the two media. The design of this exchange area must comprise, therefore, in both structure and physiology, a compromise between the demands of gas diffusion and those of ion transport. In particular, it seems likely that the system of blood perfusion through the filament is an optimum for the co-existence of the two functions under normal conditions, yet retains the flexibility to respond to sudden demands upon either of these processes.

Steen & Kruysse (1964) have demonstrated that at any one time blood can pass from the afferent to efferent filamental arteries through the lacunae of the respiratory lamellae, through a central "lymphatic" sinus in the body of the filament, through direct vascular connection at the tip of the filament, or by a simultaneous combination of all three routes. A large number of studies on excised gill preparations (Krawkow, 1913; Keys & Bateman, 1932; Ostlund & Fange, 1962; Steen & Kruysse, 1964; Richards & Fromm, 1969) have shown that catecholamines decrease vascular resistance of the gills and stimulate blood flow through the respiratory lamellae, while acetyl choline increases resistance and encourages flow through the central sinus and around the tip of the filament.

Data available on the *in vivo* effects of these drugs agree qualitatively with the results from isolated gills (Mott, 1951; Randall & Stevens, 1967; Kirschner, 1969).

The presence of alternate circulatory pathways in the teleost gill has been explained as a compromise between respiratory demand and ionic regulation (Steen & Kruysee, 1964; Randall *et al.*, 1967; Taylor *et al.*, 1968; Kirschmer, 1969). As generally stated, this model postulates that an increased rate of gas exchange during stress or activity may be attained by increasing blood flow through the adrenalin mediated pathway, the thin-walled high surface area respiratory lamellae, at the cost of an increased diffusional exchange of ions and water. However, it seems equally reasonable that branchial blood shunting could affect carrier mediated ionic transport processes as well as exerting simple permeability effects. Histological and cytochemical evidence now assigns electrolyte transport function only to the "interlamellae filamental epithelium" which overlies the central sinus blood pathway (see Conte, 1969), and not to the respiratory epithelium of the lamellae. Increased blood flow through the central filament could unmask or activate more transport sites, while greater perfusion of the respiratory lamellae could decrease the number of carrier sites in operation.

This investigation was designed primarily as an attempt to demonstrate *in vivo* that changes in blood flow through the gill filaments can affect the rate of carrier facilitated ion efflux in a sea water adapted teleost. The euryhaline southern flounder, *Paralichthys lethostigma*, was chosen as an experimental animal because of its availability and its similarity to another euryhaline flounder, *Platichthys flesus*, the teleost species in which ionic exchange mechanisms are perhaps most completely understood (e.g. Motais *et al.*, 1966; Maetz, 1969). The length of time entailed in flux rate determinations necessitated a tool for effecting chronic changes in branchial blood flow, a condition which could not be satisfactorily produced by catecholamine administration or exercise. However recent studies of experimentally induced anaemia in fish (Cameron & Wohlschlag, 1969; Cameron & Davis, 1970) indicate that this condition may produce the desired effect.

Thus this study constituted an examination of the effect of induced anaemia on sodium efflux rates and related parameters of ionic regulation in the southern flounder.

MATERIALS AND METHODS

Specimens of *Paralichthys lethostigma* were collected from the sea in the vicinity of Port Aransas, Texas, by otter trawl and hook and line during April and May 1970. Prior to experimentation the fish were held for a period of 3–14 days in a 100-gal outside aquarium constantly flushed with sea water at ambient temperatures (19–25°C) and salinities (27–32‰). During this time the animals were not fed. All flounder weighed between 150 and 300 g.

Two groups of six animals were used in the flux rate and sodium space determinations. Each fish was subjected to the following procedure. After preliminary anaesthesia with MS 222 (1/20,000), the flounder was placed on an operating table and mouth perfused with an anaesthetic solution. An incision was made on the left (upper) surface of the body, originating at the caudal peduncle, parallel and slightly ventral to the lateral line, resulting in exposure of the vertebral column. Cannulation of either the caudal artery or caudal vein

was effected using a regular point No. 22 needle bent at a 120° angle and attached to 50 cm of Clay Adams PE 50 tubing. The catheter was filled with heparinized (100 i.u./ml) Cortland saline (Wolf, 1963) adjusted to 180 m-equiv./l. sodium concentration. Insertion of the needle under a haemal spine prior to entry into the haemal canal ensured durability of the preparation. The wound was then closed with silk stitches and the protruding catheter firmly sutured to the body wall. Successful completion of the procedure entailed virtually no loss of blood. At the end of the operation, the fish was weighed and then placed in a small aquarium with an open circulation of ambient seawater.

The six individuals forming the high hematocrit group were simply allowed to recover while the other six in the low hematocrit group were bled from the cannula either during the operation or sometime thereafter. All blood losses were replaced with an equal volume of saline. A minimum 8-hr recovery period followed all operative or bleeding procedures.

At least 1 hr prior to an experiment, a flounder was placed in a darkened plastic aquarium containing a known volume (approximately 1 l/100 g body weight) of sea water (sodium = 450 m-equiv/l.) thoroughly mixed by aeration. Sea water drawn from a single stock tank was used in all experiments. Temperature was controlled at $20 \pm 1°C$. Starting at zero time, a known amount of $^{22}NaCl$ (approximately 5 μc/100 g body wt.) in an injection volume of 0·18 ml saline/100 g body wt. was slowly infused into the vascular system through the catheter; this was followed by an equal volume of regular saline. Total infusion time was 5 min. Ten-ml aliquots of the external medium were taken immediately following injection and at $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 4 and 6 hr. Blood samples of 100, 150 or 200 μl, dependent upon the size of the fish, were simultaneously withdrawn from the cannula into ammonium heparinized syringes at all times except immediately after injection. The blood samples were then spun in a Clay–Adams microhematocrit centrifuge for 10 min and hematocrits read directly from the centrifuge tubes. Aliquots of plasma were diluted for later ionic and radioactivity analyses.

Duplicate 3-ml samples of the sea water were counted in standard Pyrex test tubes using a Nuclear-Chicago shielded well counter. Plasma samples were made up to 3 ml with sea water and similarly counted. Determinations of total plasma sodium and potassium concentrations were performed on a Beckman model 105 flame photometer using lithium as an internal standard.

Calculations to determine sodium effluxes and sodium spaces were performed as described in Chan *et al.* (1967) and Mayer & Nibelle (1969) respectively after appropriate corrections for loss of radioactivity from the system through water and blood sampling. In brief, sodium effluxes were measured using the formula:

$$f_{out} = \frac{\Delta Q_{et}}{^{22}Na/Na \; pt}$$

where ΔQ_{et} is the rate of change of radioactivity in the water at time t and $^{22}Na/Na \; pt$, the specific activity of the plasma at time t. External activity and plasma specific activity data for each fish were transcribed to graphic form, and the sodium effluxes calculated at 45, 75 and 105 min. It should be noted that the ratio of specific activities between the internal and external media at these times was such that the backflux of ^{22}Na into the animal was insignificant. Radiosodium spaces at each sample time were estimated through use of the relationship:

$$V_{int} = \frac{Q_0 - Q_{et}}{^{22}Na/ml \; pt}$$

where Q_0 is the amount of activity injected at time zero, Q_{et}, the amount of activity in the water at time t, and $^{22}Na/ml \; pt$, the activity per ml of plasma at time t.

In order to determine normal hematocrit and plasma sodium levels in uncannulated animals, an additional five flounder were sampled. After removal of the fish from its holding

aquarium, the caudal peduncle was quickly washed with fresh water and then dried. A No. 22 needle was inserted through the skin in this region and into the hemal canal, and a 200 μl blood volume drawn into an ammonium heparinized syringe. The samples were thereafter treated in the same manner as those of the experimental animals.

RESULTS

Mean hematocrits significantly declined as a result of the bleeding procedure in the treated group of flounder (Table 1). Table 1 also illustrates that caudal catheterization was associated with a significant depression of packed red cell volume and elevation of plasma sodium levels. Values for cannulated fish are based upon blood samples taken 25 min after the [22]Na saline injection. However

TABLE 1—SUMMARY OF BASIC PARAMETERS FOR THE THREE GROUPS OF FLOUNDER

		Cannulated	
	Uncannulated (1)	Not bled (2)	Bled (3)
N	5	6	6
Body weight (g)	$223 \cdot 2 \pm 25 \cdot 0$	$260 \cdot 8 \pm 7 \cdot 1$ $P_1 = $ n.s.	$205 \cdot 6 \pm 21 \cdot 0$ $P_2 = 0 \cdot 05$
Initial hematocrit (%)	$19 \cdot 50 \pm 0 \cdot 84$	$15 \cdot 92 \pm 0 \cdot 52$ $P_1 = 0 \cdot 01$	$8 \cdot 12 \pm 0 \cdot 97$ $P_2 = 0 \cdot 001$
Average hematocrit (%)	—	$14 \cdot 85 \pm 0 \cdot 64$	$6 \cdot 66 \pm 0 \cdot 97$ $P_2 = 0 \cdot 001$
Plasma Na(m-equiv./l.)	$151 \cdot 94 \pm 7 \cdot 35$	$178 \cdot 41 \pm 7 \cdot 21$ $P_1 = 0 \cdot 02$	$186 \cdot 67 \pm 9 \cdot 58$ $P_2 = $ n.s.

Values = means ± standard error. P_1 = significance level of difference between means of columns 1 and 2: P_2 = significance level of difference between means of columns 2 and 3.

the differences observed between catheterized and control animals are too great to result simply from additive or dilutant effects of the saline infusion, and are supported by non-systematic observations on uninjected but cannulated flounder. Neither the original exsanguination to produce the low hematocrit condition, nor the serial blood sampling of the experimental procedure, caused any marked change in plasma sodium or potassium concentrations (Table 2). These values ranged widely among different members of the same treatment group but remained remarkably constant in any one animal throughout the sampling period. A close positive correlation ($r = 0 \cdot 83$, $P = 0 \cdot 001$) existed between the steady-state levels of sodium and potassium regardless of hematocrit (Fig. 1). However the relationship does not appear to be fully linear and the line has been fitted by eye.

Sodium efflux rates were originally calculated on a per fish basis. As expected, a definite positive relationship ($r = 0 \cdot 70$, $P = 0 \cdot 02$) existed between body weight and average efflux rate regardless of hematocrit (Fig. 2). As the bled flounder had a slightly inferior mean weight, it was especially important to reduce all flux rate

TABLE 2—PLASMA SODIUM AND POTASSIUM CONCENTRATION AT EACH SAMPLE TIME FOR THE TWO EXPERIMENTAL GROUPS

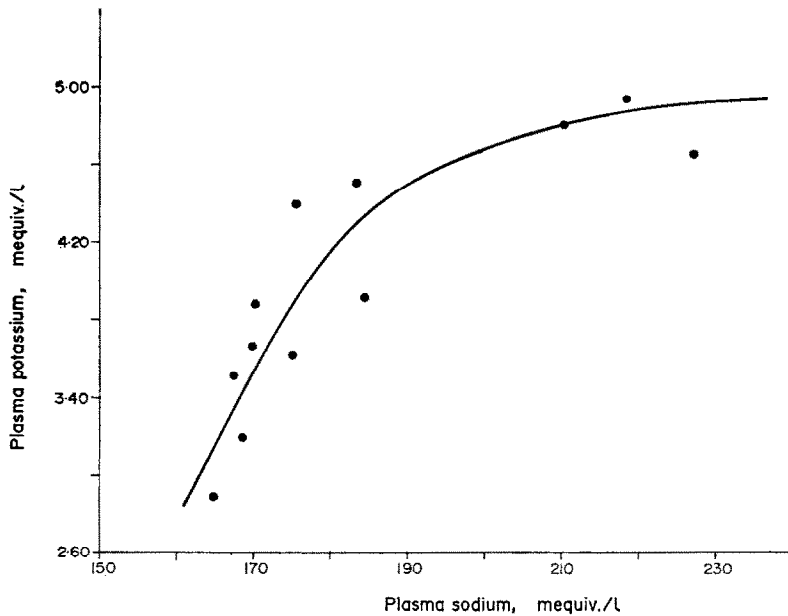| Sample time (hr)	Not bled		Bled	
	Plasma Na (m-equiv./l.) ($N = 6$)	Plasma K (m-equiv./l.) ($N = 6$)	Plasma Na (m-equiv./l.) ($N = 6$)	Plasma K (m-equiv./l.) ($N = 6$)
$\frac{1}{2}$	178·41 ± 7·21	3·88 ± 0·25	186·67 ± 9·58	3·92 ± 0·29
1	183·00 ± 10·61	3·99 ± 0·24	186·78 ± 10·52	3·89 ± 0·41
$1\frac{1}{2}$	184·80 ± 10·86	4·04 ± 0·29	185·44 ± 10·38	3·93 ± 0·32
2	183·33 ± 10·97	4·04 ± 0·36	189·32 ± 8·54	4·24 ± 0·36
4	179·85 ± 9·59	4·02 ± 0·24	187·13 ± 8·89	4·11 ± 0·30
6	182·71 ± 8·28	3·93 ± 0·26	187·40 ± 8·60	3·94 ± 0·27

Values = means ± S.E.

FIG. 1. Plasma potassium concentrations (m-equiv/l.) plotted against plasma sodium concentration (m-equiv./l.). Data from both unbled and bled fish. Each point represents the mean of six determinations of each parameter for an individual flounder. The line has been fitted by eye.
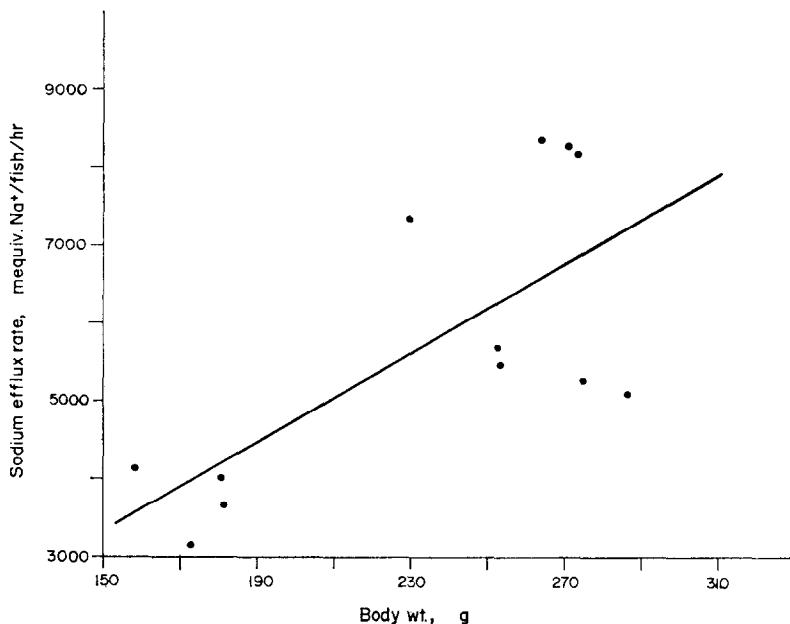
FIG. 2. Sodium efflux rate (μ-equiv. Na/fish per hr) plotted against body weight (g). Data from both unbled and bled fish. Each point represents the mean of three determinations of flux rate for an individual flounder. Equation of regression line: Sodium efflux rate $= -920\cdot4 + 28\cdot4$ (body wt.).

values to a common weight basis. The individual sodium efflux rates per 100 g body weight are presented for each animal in Table 3. Depression of hematocrit was associated with a 23 per cent reduction of sodium efflux rate ($P = 0\cdot01$).

The evolution of the ^{22}Na distribution volume within the flounder as a function of time is presented in Fig. 3. At 6 hr the radiosodium space reached the remarkably high value of approximately 63 ml/100 g; the variability of the estimate at this time was larger because only a small percentage of the original injected dose of ^{22}Na remained within the fish. The expansion of the distribution volume with time was somewhat retarded in the low hematocrit group. The differences were not large enough for statistical confirmation, but in light of the small sample size used, did seem great enough to warrant further examination. As the cause of these differences could not be detected by simple inspection of the data, the distribution and movement of ^{22}Na were analyzed in the following manner.

From an arbitrary estimate of blood volume (5 ml/100 g) and knowledge of the hematocrit, a fish was divided into a simple two compartment system, plasma and tissues. At any one sample time, the amounts of ^{22}Na in each compartment were calculated from the plasma activity and the total activity of the fish. These parameters were related between different animals by expressing all values as a percentage of the original injected dose (Fig. 4). As calculated by this analysis, the ratio between the amount of radioactivity in the plasma and that in the tissues

TABLE 3—SUMMARY OF INDIVIDUAL SODIUM EFFLUX RATE DETERMINATIONS AND HEMATOCRITS
FOR THE TWO EXPERIMENTAL GROUPS

	Not bled			Bled	
Fish	Average hematocrit (%)	Na efflux (μ-equiv./100 g per hr)	Fish	Average hematocrit (%)	Na efflux (μ-equiv./100 g per hr)
8	16·25	2245·5	11	10·37	2916·5
		1968·2			2193·5
		1517·8			1571·5
4	16·00	2688·6	10	8·55	1438·9
		2523·5			1961·6
		3777·8			1939·8
5	16·00	3259·9	14	6·13	1689·6
		2639·6			2317·3
		3254·7			1996·4
12	14·75	2239·8	13	6·13	1782·7
		2056·1			1952·9
		2188·0			1719·7
6	13·75	3495·0	9	4·47	2784·7
		2964·6			2426·2
		3163·2			2673·8
7	12·33	2730·8	15	4·30	2092·4
		3236·9			2339·0
		3508·2			2132·9
Mean	14·85	2747·7		6·66	2107·1
S.E.	0·64	147·6		0·97	97·2
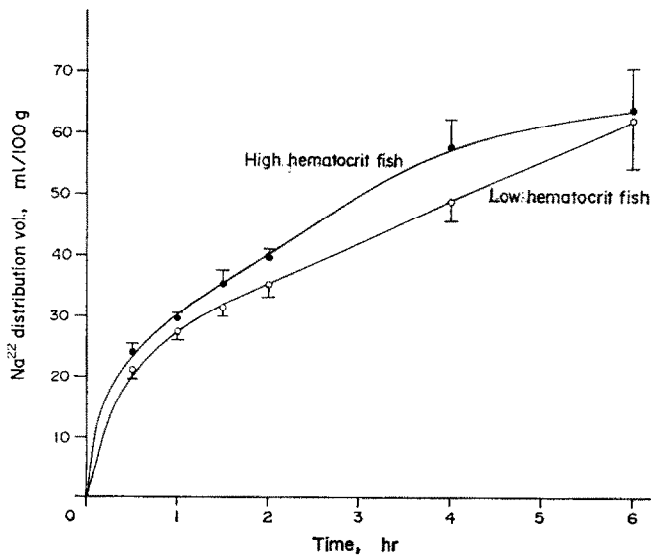
FIG. 3. Volume of distribution of ^{22}Na (ml/100 g) plotted against time after
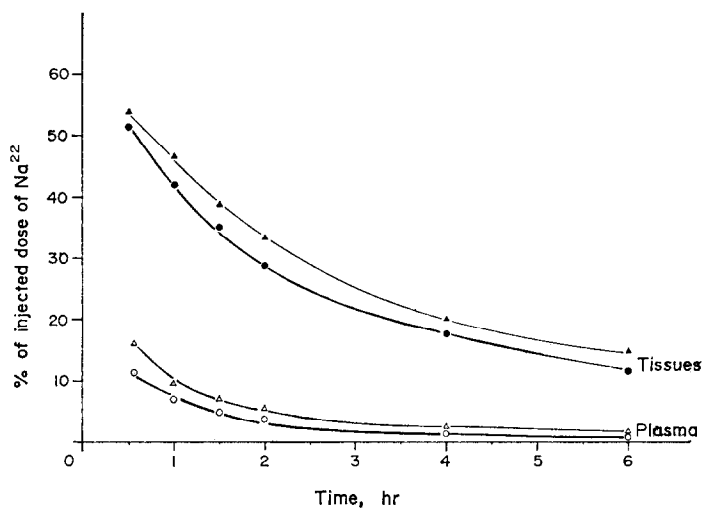injection (hr) for each treatment group. Bar = 1 standard error.

FIG. 4. Amount of ^{22}Na in each compartment (tissues and plasma) expressed as percentage of injected dose plotted against time after injection (hr) for each experimental group. Method of calculation is described in the text. Triangles, Low hematocrit fish. Circles, high hematocrit fish.

approached constancy at 6 hr, the condition which would apply when an equilibrium distribution volume is attained. In addition, the amounts of ^{22}Na in both compartments were greater in the low hematocrit fish than in the unbled individuals; this effect was much more pronounced in the plasma. For example, at $\frac{1}{2}$ hr, the ^{22}Na concentration of the plasma in the bled animals was 1·40 times greater than in the high hematocrit fish, while that of the tissues was only 1·05 times greater. These differences can account for the lower sodium spaces in the anaemic group. Similar calculations of the movements of radioactivity out of the fish (i.e. plasma to water) and into the blood (i.e. tissues to plasma) indicated that the ^{22}Na distribution differences between the two treatment groups were caused by a much lower initial efflux of the isotope into the water from the low hematocrit animals during the first 30 min, and especially during the first 5 min, the period when isotope concentrations were maximal in the blood stream. This reduced loss of ^{22}Na may have been caused by the lower sodium efflux rates measured in the bled individuals. After 30 min, the ^{22}Na distribution differences decreased very slowly because the movement of the tracer from the tissue to plasma, and from plasma to water, was similar in two groups of fish.

Finally it should be noted that whether the catheter entered the caudal artery or caudal vein was largely a matter of chance owing to the small size and close proximity of these vessels at the level of the peduncle. However, no effect of cannulation site on sodium space measurements, flux rate determinations, or plasma ion levels could be detected in the results. The vessel cannulated could easily be distinguished by either blood pressure on blood oxygen tensions. Mean

pressures recorded from the artery ranged from 24 to 32 mm Hg but were negligible in the vein; arterial pO_2's were 90–105 mm Hg while venous values ranged from 11 to 16 mm Hg.

DISCUSSION

Vascular catheterization techniques, although a popular tool for the elucidation of cardiorespiratory function in teleosts (e.g. Randall et al., 1967; Cameron & Davis, 1970), have found only sparse application to problems of electrolyte balance (e.g. Mayer & Nibelle, 1969). In fact, nearly all recent advances in the understanding of branchial ionic exchange mechanisms have utilized radiotracer methods which necessitate no more than a single terminal sample of the internal environment (e.g. Motais et al., 1966; Maetz et al., 1967). Such practices involve only a minimal disturbance of the experimental animal but often yield essentially "indirect" estimates of flux rates, or but a single "direct" determination for each fish (Maetz et al., 1967). Chronic vascular intervention, as in this study, provides a means for repeated direct flux rate measurements on one animal, and allows a simultaneous following of changes in plasma constituents. However, the depression of hematocrit and elevation of plasma sodium levels in cannulated flounder (Table 1) indicates the possible long term trauma caused by this procedure; similar effects have been reported in the fresh water trout, Salvelinus fontinalis (Houston et al., 1969). The rise in internal sodium concentrations may be explained by an increased branchial permeability following stress; the hemodilution is more puzzling for an increase in blood volume seems unlikely under these circumstances, and no hemolytic effects were observed. Houston et al. (1969) suggested that localized pooling of erythrocytes could be involved. Thus parameters measured in cannulated animals may not be quantitatively representative of the normal condition. It seems likely however, that differences demonstrated between two groups of similarly catheterized fish should still represent effects independent of those caused by catheterization.

The distribution volume for ^{22}Na evolves more slowly in the anaemic flounder, but the data provides no evidence that the final equilibrium values differ between the two groups. The radiosodium spaces measured after 6 hr in the present study are greater than those reported for any other sea water teleost. As noted earlier, the estimates of this space taken at the end of the experimental period are less reliable than those taken during the first few hours. Nevertheless, distribution volumes of over 60 ml/100 g may well be real under the conditions of this study. Recent determinations in P. flesus yielded a value of 45·9 ml/100 g (MacFarlane, personal communication) 1·5 hr after isotope injection, considerably greater than the original estimate of 34 ml/100 g (Motais, 1967) taken 1–2 hr after injection. It seems probable that relatively low sodium spaces may result from blood samples taken too soon after infusion of the isotope, which would yield values inferior to that of the equilibrium space. The serial blood sampling of the present study indicates that at least 6 hr must be allowed for dispersal of the isotope within the internal compartment of the flounder. It is also possible that high distribution

volumes may be associated with the increased internal sodium levels seen in catheterized animals; Mayer & Nibelle (1969) have suggested that augmentation of the sodium space may act to limit large increases in the plasma sodium concentration.

The sodium balance of *P. lethostigma* (Hickman, 1968) appears to be very similar to that of *P. flesus* (Maetz, 1969), and the sodium efflux rates recorded in the present study (2747·7 μ-equiv/100 g per hr at 20°C) were nearly identical to those reported for *P. flesus* (2623 μ-equiv/100 g per hr at 17°C). It is probable that similar regulatory mechanisms are found in the two species. In *P. flesus*, both the high exchange diffusion and lower net extrusion of sodium at the gills seems to be mediated by a common carrier substance with a much greater affinity for potassium than for sodium (Maetz, 1969). Net branchial sodium excretion is effected by the sodium/potassium exchange at a rate sufficient to just balance the influx of sodium across the intestinal surface; urinary sodium loss is insignificant. Thus measurement of the sodium efflux rate, rather than the sodium influx rate, should give the best estimate of the number of carrier sites in operation, which was the purpose of this study.

The high positive correlation between plasma sodium and potassium concentrations is indicative of a direct relationship between their regulatory mechanisms. In *P. lethostigma*, the slight but significant uptake of potassium through the gut must be balanced by extrarenal excretion (Hickman, 1968). Measurements of branchial potassium exchange rates in *P. flesus* yielded similar values for influx (exchanged against a net sodium excretion) and efflux components (Maetz, 1969); however, the difference between the means was sufficient to account for a net potassium extrusion comparable to the net intestinal uptake. The nature of the branchial potassium efflux mechanism is not yet understood. Thus both plasma potassium and sodium levels are probably set by the dynamic balance between their absorption through the gut and excretion at the gills. Net branchial efflux rates of the two ions may result from independent mechanisms, but the uptake of both should be determined by the drinking rate, a function which has been shown to vary widely between individual animals in *P. lethostigma* (Hickman, 1968). Thus the drinking rate seems the most probable cause for correlation of plasma sodium and potassium concentrations, while variations in this parameter may account for the large plasma ionic level differences among individual flounder.

Augmentation of cardiac output through increases in both heart rate and stroke volume enables teleosts to survive severe anaemia with no reduction in metabolic rate or modification of ventilatory parameters (Cameron & Wohlschlag, 1969; Cameron & Davis, 1970). The increase in cardiac output is probably associated with a redistribution of blood in the gills to maintain the rate of gas transfer. Such changes occur during exercise (Randall *et al.*, 1967), a condition characterized by increased concentrations of plasma catecholamines (Nakano & Tomlinson, 1967), substances which have been shown to stimulate lamellar blood flow (Steen & Kruysse, 1964). It is the assumption in this study that anaemia results in similar changes in the pattern of branchial blood perfusion, i.e. vasodilation and

augmentation of blood flow to the lamellar region and away from the central filamental sinus.

If such a concept is valid, then the 23 per cent reduction in branchial sodium efflux rate observed in anaemic flounder may be explained by the diversion of blood flow away from the interlamellar epithelial areas associated with ion transport activity and served by the central sinus pathway. This decreased efflux rate could be caused simply by an absence of sufficient sodium ions at non-perfused binding sites, or more complexly, by a reduction in the concentration or synthesis of carrier in the affected cells. Of course, it is also possible that unknown blood-borne substances liberated during anaemia may directly inhibit the exchange mechanism. Whatever the cause, the anaemic fish is probably subject to a decreased net sodium excretion as well as reduced exchange diffusion of this ion for both processes seem to utilize a common carrier substance (Maetz, 1969). In addition, greater perfusion of the respiratory blood pathway would entail an increased exchange of salts and water by simple diffusion, thus compounding the osmotic problems of the anaemic fish. The results of the present study in fact show a slightly, although nonsignificantly, higher internal sodium level in the low hematocrit flounder. Large excursions of plasma sodium concentration may be prevented by adjustment of the drinking rate or internal distribution of the ion, or may be simply masked in our data by the post-operative disturbances in plasma levels.

In light of these results, it is interesting to speculate whether blood shunting in the gills could have important consequences for electrolyte balance under more normal physiological conditions. For example, if similar changes occur during activity and hypoxia as during anaemia, then disturbances in ionic homeostasis may become a limiting factor in oxygen or exercise tolerance. Furthermore, the rapid masking or unmasking of carrier sites through changes in central sinus perfusion could help a euryhaline teleost, such as the southern flounder, cope with sudden variations in the external salinity.

REFERENCES

CAMERON J. N. & DAVIS J. C. (1970) Gas exchange in rainbow trout (*Salmo gairdneri*) with varying blood oxygen capacity. *J. Fish. Res. Bd Can.* **27**, 1069–1085.

CAMERON J. N. & WOHLSCHLAG D. E. (1969) Respiratory response to experimentally induced anemia in the pinfish (*Lagodon rhomboides*). *J. exp. Biol.* **50**, 307–317.

CHAN D. K. O., PHILLIPS J. G. & CHESTER JONES I. (1967) Studies on electrolyte changes in the lip-shark, *Hemiscyllium plagiosum* (Bennet), with special reference to hormonal influence on the rectal gland. *Comp. Biochem. Physiol.* **23**, 185–198.

CONTE F. P. (1969) Salt secretion. In *Fish Physiology* (Edited by HOAR W. S. & RANDALL D. J.), Academic Press, New York.

HICKMAN C. P., JR. (1968) Ingestion, intestinal absorption, and elimination of seawater and salts in the southern flounder, *Paralichthys lethostigma*. *Can. J. Zool.* **46**, 457–466.

HOUSTON A. H., DEWILDE M. A. & MADDEN J. A. (1969) Some physiological consequences of aortic catheterization in the brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd Can.* **26**, 1847–1856.

KEYS A. & BATEMAN J. B. (1932) Branchial responses to adrenaline and pitressin in the eel. *J. Physiol. Lond.* **75**, 327–336.

KIRSCHNER L. B. (1969) Ventral aortic pressure and sodium fluxes in perfused eel gills. *Am. J. Physiol.* **217**, 596–604.

KRAWKOW N. P. (1913) Über die Wirkung von Giften auf die gefasse isolierter Fischkiemen. *Pflügers Arch. ges. Physiol.* **151**, 583–603.

MAETZ J. (1969) Seawater teleosts: Evidence for a sodium–potassium exchange in the branchial sodium-excreting pump. *Nature, Lond.* **166**, 613–615.

MAETZ J., SAWYER W. H., PICKFORD G. E. & MAYER N. (1967) Evolution de la balance minérale du sodium chez *Fundulus heteroclitus* au cours du transfert d'eau de mer en eau douce: Effets de l'hypophysectomie et la prolactine. *Gen. comp. Endocr.* **8**, 163–176.

MAYER N. & NIBELLE J. (1969) Sodium space in fresh-water and sea-water eels. *Comp. Biochem. Physiol.* **31**, 589–597.

MOTAIS R. (1967) Les mécanismes d'échanges ioniques branchiaux chez les Téléostéens. *Ann. Inst. Océan. Paris* **45**, 1–83.

MOTAIS R., ROMEU F. GARCIA & MAETZ J. (1966) Exchange diffusion effect and evryhalinity in teleosts. *J. gen. Physiol.* **50**, 391–422.

MOTT J. C. (1951) Some factors affecting the blood circulation in the common eel (*Anguilla anguilla*). *J. Physiol., Lond.* **114**, 387–398.

NAKANO T. & TOMLINSON N. (1967) Catecholamine and carbohydrate metabolism in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. *J. Fish. Res. Bd Can.* **24**, 1701–1715.

ÖSTLUND E. & FANGE R. (1962) Vasodilation by adrenalin and noradrenalin; and the effects of some other substances on perfused fish gills. *Comp. Biochem. Physiol.* **5**, 307–309.

RANDALL D. J., HOLETON G. F. & STEVENS D. E. (1967) The exchange of oxygen and carbon dioxide across the gills of rainbow trout. *J. exp. Biol.* **46**, 339–348.

RANDALL D. J. & STEVENS E. D. (1967) The role of adrenergic receptors in cardiovascular changes associated with exercise in salmon. *Comp. Biochem. Physiol.* **21**, 415–424.

RICHARDS B. D. & FROMM P. O. (1969) Patterns of blood flow through filaments and lamellae of isolated-perfused rainbow trout (*Salmo gairdneri*) gills. *Comp. Biochem. Physiol.* **29**, 1063–1070.

STEEN J. B. and KRUYSSE A. (1964) The respiratory function of teleostean gills. *Comp. Biochem. Physiol.* **12**, 127–142.

TAYLOR W., HOUSTON A. H. & HORGAN J. D. (1968) Development of a computer model simulating some aspects of the cardiovascular-respiratory dynamics of the salmonid fish. *J. exp. Biol.* **49**, 477–494.

WOLF K. (1963) Physiological salines for fresh water teleosts. *Progve. Fish. Cult.* **25**, 135–140.