Sodium Efflux and Potential
Differences across the Irrigated
Gill of Sea Water-Adapted
Rainbow Trout (Salmo gairdneri)

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ABSTRACT Sodium extrusion ($J_{\text{out}}^{\text{Na}}$) was measured across the gills of rainbow trout, Salmo gairdneri, adapted to sea water (SW) using a gill-irrigation system of small volume. The potential difference (TEP) was also measured under similar conditions. $J_{\text{out}}^{\text{Na}}$ was usually between 100-250 µeq (100 g)$^{-1}$ h$^{-1}$, about an order of magnitude faster than in fresh water (FW)-adapted trout, but slower than has been reported for any other marine teleost. The TEP was between 10-11 mV, body fluids positive to SW. When the external medium was changed from SW to FW $J_{\text{out}}^{\text{Na}}$ was reduced to about 25% of the initial value, and the TEP was reduced by 40-50 mV (i.e. body fluids negative by 30-40 mV). Addition of either Na$^+$ or K$^+$ in SW concentrations reversed the changes; $J_{\text{out}}^{\text{Na}}$ increased and the gill repolarized. The electrical behavior and sodium efflux in irrigated trout gill is qualitatively the same as has been reported for unanaesthetized, free-swimming fish of other species. Thus, the irrigated gill provides an adequate model for studying the mechanism of sodium extrusion in marine teleosts.

INTRODUCTION

Although the sea water-adapted fish gill provided the first clear demonstration of active ion transport across an epithelium (Keys, 1932) the preparation was little studied for many years. Instead, attention was directed toward organs such as the frog skin, urinary bladder, and renal tubule. In most of these, NaCl transport is quantitatively, at least, the most important functional event. Further, there is a remarkable similarity in the transport systems of quite different organs, e.g. among surface epithelia of fresh water (FW) animals from different phyla, or between a surface epithelium and the distal
tubule in the kidney. Some of these similarities have been mentioned recently (Kirschner et al., 1973). Of importance here is that in all these cases salt moves into the body fluids, either from a tubular lumen or the external environment. We will use the term "FW epithelia" as a generic designation for this group. Recent work, pioneered by Motais (1967) and extended primarily by Maetz and his collaborators (summarized by Maetz, 1971) on the gill of sea water (SW) fish has delimited a number of fundamental differences between salt transfer across this organ and the FW epithelia. Some of these are: (a) FW epithelia are specialized to transport sodium from an external medium to the blood. The sea water fish gill extrudes sodium from blood to medium. In this regard it resembles the avian salt gland and elasmobranch rectal gland.

(b) Unidirectional fluxes are about two orders of magnitude faster across the SW gill than across FW gills and amphibian skins. Both influx ($J_{in}$) and efflux ($J_{out}$) in SW epithelia appear to show saturation kinetics when external sodium ([Na$^+$]$_{out}$) is varied.

(c) SW epithelia contain large numbers of specialized cells characterized by an extensive system of intracellular channels (smooth ER). The latter are open at the base of the cell, but not at its apex (Philpott and Copeland, 1963). These cells were called "chloride cells" by Keys and Wilmer (1932) who proposed that they were responsible for salt extrusion. Homologous FW epithelia contain fewer such cells.

(d) SW epithelia have a much higher concentration of (Na$^+$ + K$^+$)-activated ATPase than comparable FW systems (Epstein et al., 1967; Jampol and Epstein, 1970; Kamiya and Utida, 1969; Zaugg and McLain, 1970).

(e) The transepithelial potential difference (TEP) generated by most FW epithelia is positive on the side of the ion sink (i.e. blood). In SW epithelia the blood is also positive (House, 1963; Maetz and Campanini, 1966) although salt transport is in the opposite direction, from blood to medium.

Although a number of fish species have been investigated, most of this information has been drawn from studies on two euryhaline groups, the eel (Anguilla anguilla) and the flounder (Platichthys flesus). Comparable information for salmonids is sparse; one paper has been published on sodium fluxes (Potts et al., 1970) and one on chloride fluxes (Gordon, 1963). We have examined the sodium flux-force relationships in the rainbow trout (Salmo gairdneri), and we propose to use the data to examine the Na$^+$ flux model. However, instead of working with unanaesthetized animals in small aquaria, we have used the gill-irrigation system described by Kerstetter et al. (1970) for FW-adapted animals. The fish are anaesthetized and external medium is recirculated past the gills from a small-volume reservoir. Since this procedure has never been used with marine fish our initial work was directed toward characterizing the preparation and comparing its behavior with that reported
for unanaesthetized, free-swimming animals. It is these data that are reported below. The paper is thus primarily methodological stressing comparison with data obtained in other fish studied under different conditions. Some of these observations appeared earlier in a preliminary report (Greenwald and Kirschner, 1971).

METHODS

Salmo gairdneri, 150–350 g, were obtained from a commercial hatchery near Soap Lake, Washington. They were held in FW at 12°C for at least a week, after which they were adapted to artificial sea water (ASW) (“Instant Ocean,” Aquarium Systems, Inc., Eastlake, Ohio) in two steps. The animals were first transferred to 50% SW for at least a week, then to full strength SW where they were held for at least 10 days before being used. Although mortality in the closed SW systems was higher than in FW tanks, the majority of the trout could be maintained unfed for extended periods (up to several months) at 12°C.

The gill-irrigation system used in flux measurements was described in a previous study on FW animals (Kerstetter et al., 1970). Briefly, the fish was anesthetized in 0.03% neutralized tricaine methane sulfonate (TMS) in SW. The animal was suspended dorsal side down in a “hammock” of plastic webbing, a 15-gauge hypodermic needle inserted through the lower jaw into the mouth, and the external medium was pumped from a reservoir through a stainless steel cooling coil into the mouth via the hypodermic needle. Although the fish was anesthetized, respiratory movements were normal and ensured that the medium entering its mouth passed over the gills after which it was returned to the reservoir. The pumping rate was 100–150 ml/min, and the total reservoir volume was 500 ml.

The basic irrigating solution was Instant Ocean which contained Na⁺ 443.7 mM; K⁺ 9.5 mM; Ca²⁺ 9.2 mM; Mg²⁺ 49.4 mM; Cl⁻ 519.0 mM; SO₄⁻ 26.0 mM, and HCO₃⁻ 2.3 mM. The term “SW concentration” of an ion will refer to its concentration in this medium. FW is a designation for 1 mM NaCl. The irrigating solution was maintained at 12–13°C and constantly aerated.

In one series of experiments fluxes were measured on unanaesthetized trout in small (2.5-liter) aquaria. In this system the animals swam against a vigorous current created by recirculating the SW rapidly through the aquaria. Temperature was maintained at 12°C.

In the experiments reported below sodium extrusion (J⁺ Na) was measured after injection of about 5 μC 22Na into the peritoneal cavity. Samples of the external medium were taken for estimation of the appearance of isotope, and periodic blood samples (about 20 μl) were taken from the caudal vein for measuring plasma-specific activity. Isotope measurements were made on a Nuclear-Chicago Autogamma scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) and plasma sodium concentrations were determined by atomic absorption spectrophotometry on suitably diluted samples. Fig. 1 shows typical data obtained from a single fish in an extended experiment. Several features are notable. The isotope was injected 1 h before any samples were taken, and the specific activity of 22Na in the plasma did not decrease much through the entire experiment. The lower graph shows the appearance of iso-
FIGURE 1. Sodium extrusion from SW-adapted trout. About 5 μCi of $^{22}$NaCl (carrier free) were injected into the peritoneal cavity about 15 min before measurements were begun. At 0 h the gill-irrigation reservoir was refilled with 500 ml of SW. Samples were taken every hour from the reservoir (2.0 ml) and caudal vein (50 μl) for determination of $^{22}$Na$^+$ and [$Na^+]_{plasma}$. Plasma-specific activity (sp act) is shown by the bars in the upper part of the figure. Cumulative appearance of $^{22}$Na$^+$ in the external medium is shown in the lower portion. The sp act of the medium after 5 h was < 1 cpm/μmol, hence backflux of isotope was neglected in calculating $J_N^{Na}$. The efflux for each hour is shown in parentheses (μmol (100 g)$^{-1}$ h$^{-1}$).

to pe in the external medium. A rough estimate of the efflux for each 1-h period was obtained by dividing the total radioactivity appearing in the medium by the plasma-specific activity, and the values (μeq (100 g)$^{-1}$ h$^{-1}$) are shown for each period. $J_N^{Na}$ was substantially higher (25–30%) during the first hour of sampling than in the second period. It continued to decrease with time, but slowly enough to permit us to run experiments lasting 4–5 h. It will be shown below that the behavior in Fig. 1 is representative, and hence it has conditioned experimental design and data handling.

To eliminate the initial period of high efflux, often noted in aquatic animals, most flux measurements were not commenced until 2 h after the isotope injection. The basis for the subsequent slow decline in $J_N^{Na}$ is not understood. It was not noted in our previous work with FW trout. Although the gill-irrigation system delivers water more slowly than is characteristic of unanaesthetized fish (Wood and Randall, 1973), O$_2$ delivery did not appear to be limiting, since there was no difference when the irrigating solution was equilibrated with 100% O$_2$. Accumulation of H$^+$ or NH$_4^+$ excreted by the fish was ruled out, because changing the medium to fresh SW at the beginning of each flux period did not prevent it. Whatever the cause, the decrease was strictly time dependent (about 10% per hour after the initial period of high efflux), and was not conditioned by intervening manipulations (e.g. changes in medium composition). Hence, the data were corrected in one of two ways depending on experimental design. Where an experimental treatment was to be compared with a control value the experimental period was preceded and followed by control meas-
urements. The average of the two control measurements was compared with the value obtained under the experimental condition. When the experiment involved a series of measurements, as in studies on $J_{\text{Na}}^{\text{out}}$ at different $[\text{Na}^+]_{\text{out}}$, which are described in the following paper, the measured effluxes were simply corrected (increased) by 10% for each hour from the beginning of the series. In most of the experiments on single animals uncorrected data are shown. These will be noted where they occur.

The technique for measuring TEP in intact aquatic animals has been described before (Dietz et al., 1967; Kirschner, 1970). It consists of introducing an agar-Ringer or agar-saturated KCl bridge through a small hole (made with a probe) in the body wall. This bridge was connected through a saturated-KCl reservoir and calomel electrode to a recording potentiometer. The external bridge was agar-saturated KCl and was also connected to the potentiometer through a calomel electrode. The internal bridge was made by filling flexible plastic tubing, tip diameter about 2 mm, with the agar-salt solution. The position of the internal bridge had no effect on the TEP; intravenous placement gave the same values as intraperitoneal, and moving it to different locations in the peritoneal cavity gave the same readings. Fig. 2 shows the recording arrangement.

Metal-SW junctions introduced large DC signals into the recording circuit. The needle used to introduce the medium into the fish's mouth was replaced by plastic tubing, and the refrigeration system and cooling coils were replaced by a thermoelectric cold plate on which the reservoir rested. Temperature control was much less precise, but the electrical measurements were made rapidly and thermal changes were probably not an important factor.

One possible source of artifact in such electrical measurements is shunting through the wound made to introduce the internal bridge. This appears to cause no problems in FW; the TEP in intact frogs and its dependence on $[\text{Na}^+]_{\text{out}}$ is about the same as has been reported for isolated skins (Kirschner, 1970). However, the high conductance of SW makes the possibility of shunting through the incision more likely than

![Diagram of recording arrangement for measuring the TEP across the trout gill. The external bridge is a Pasteur pipette filled about half way to the top with agar-KCl (2.5 M) above which was a solution of 2.5 M KCl in contact with a calomel electrode. The internal bridge was agar-KCl-filled Tygon tubing inserted through an incision in the ventral midline or ventrolateral surface. Fluid exiting from the operculum fell vertically and did not contact the incision.](image-url)
in FW. This was minimized in our experiments since the animals were not immersed, and fluid irrigating the gill did not contact this region. As a further precaution the incised area was flushed with distilled water several times during an experiment.

Electrode and bridge pairs were measured for electrical asymmetry with both bridges in the same solution. This was rarely more than 5 mV and changed little during an experiment. The results reported are corrected for such asymmetry. The question of whether junction potentials existed when the bridges were in place could not be answered. The internal bridge was usually agar-Ringer's and should generate no signal when immersed in body fluids of about the same composition. But the external agar-KCl bridge is not symmetrical with SW. The high medium NaCl concentration is not really negligible compared with saturated KCl, and a junction potential may develop. We have assumed that this is small enough to be disregarded.

RESULTS

Sodium Efflux Into Sea Water

Since no information has been published on sodium fluxes in SW-adapted rainbow trout, and because the recirculating gill-irrigation system has never been used with SW fish, we examined sodium efflux under a number of different conditions. The data in Fig. 1 show that plasma-specific activity drops relatively slowly after an injection of Na. This suggests that turnover across the gills cannot be very rapid. Mean \( J_{out}^{Na} \) values for a group of animals are presented in the first line in Table I; they show that the data in Fig. 1 are not unique; fluxes are generally higher during the first experimental hour, then decrease about 10% per hour for several hours. The values are much higher than those noted earlier for FW trout measured in FW which are in the range 10-15 \( \mu \text{eq} \ (100 \text{g})^{-1} \text{h}^{-1} \). However, they are the lowest ever reported for marine teleosts. It was necessary to eliminate the possibility that our small-volume gill-irrigation system was suppressing a normally rapid efflux, and hence some measurements were made on free-swimming trout, unanaesthetized and confined to small aquaria through which water was rapidly recir-

| Adaptation medium | Condition                  | N | 1  | 2  | 3  | 4  | 5  |
|-------------------|----------------------------|---|----|----|----|----|----|
| SW water          | Anaesthetized, gills irrigated | 8 | 254±31 | 186±21 | 180±20 | 152±20 | 112±11 |
| SW water          | Free swimming              | 6 | 373±73 | 294±69 | —      | —      | —      |
| FW water          | Anaesthetized, gills irrigated | 4 | 22.9±4.5 | 25.0±5.9 | —      | —      | —      |
culated. The second row of data in Table I show $J^\text{Na}_{\text{out}}$ in these animals through two experimental periods. The mean values were about 50% higher than for the previous group, but the differences were barely significant statistically. The data show that $J^\text{Na}_{\text{out}}$ in the unanaesthetized rainbow trout is lower than in any SW-adapted fish previously studied (cf. Motais, 1967; Lahlou and Sawyer, 1969; Potts et al., 1970), and also that efflux is only a little depressed when the fish are anaesthetized and the gills irrigated.

To emphasize the last point a few measurements were made on FW-adapted trout under similar conditions, i.e. anaesthetized, and gills perfused with SW recirculated from a small reservoir. $J^\text{Na}_{\text{out}}$ in these animals was an order of magnitude smaller than in the SW-adapted fish. The same phenomenon (very low $J^\text{Na}_{\text{out}}$ values) was reported by Motais et al. (1966) for unanaesthetized FW-adapted flounder measured in SW. These data show that the gill-irrigation system yields values in SW trout somewhat lower than, but approximating those in free-swimming fish for periods of at least 4-5 h.

The TEP in anaesthetized trout was in the range 5-20 mV when the gills were irrigated with SW. For 15 fish the average value was $10.4 \pm 0.9$ mV (mean $\pm$ SEM), body fluids always positive to SW. This is lower than has been reported for the few other fish on which measurements have been made (+18 to +25 mV), but the order of magnitude and polarity is the same.

**Efflux and TEP in SW and FW**

When the external medium was changed from SW to FW (or to distilled water, DW) $J^\text{Na}_{\text{out}}$ was markedly reduced. Fig. 3 shows that the reduction was rapid; about 10 min elapsed between the last SW measurement and the beginning of measurements in DW. It also shows that addition of sucrose to...
make the medium isosmotic with SW had little effect on $J_{\text{out}}^{Na}$ which remained low. There was a large osmotic gradient favoring inward water flow in DW. After adding sucrose the gradient was even larger, but reversed, favoring osmotic water loss. The fact that $J_{\text{out}}^{Na}$ was unchanged indicates that flow-entrained sodium fluxes (solvent-drug effects) can be neglected.

The TEP also responded to the change from SW to FW as is shown for a different fish in Fig. 4. The SW value was +12 mV; on changing to FW there was an abrupt decrease and polarity change which will be called de-

![Figure 4](image1.png)

**Figure 4.** The effect of SW → FW change on the TEP. An unaltered tracing of a recording shows that the TEP was +12 mV with SW bathing the gill. At the first arrow the reservoir was changed to one containing 1 mM NaCl (FW). The first 100 ml returning from the gills were discarded before beginning recirculation. At the second arrow the reservoir was exchanged for one containing the same FW solution. A third reservoir change, not shown, brought the TEP to −43 mV.

![Figure 5](image2.png)

**Figure 5.** The effect of external NaCl on $J_{\text{out}}^{Na}$. The procedure was the same as in Fig. 3 except that 14 g NaCl was added to the reservoir at the end of the second hour (final concentration 500 mM). $J_{\text{out}}^{Na}$ (uncorrected) was 82% of the value in SW.

polarization. The TEP approached a plateau in a few minutes, but changing to a new reservoir (also FW) lowered it further. After a third medium change (not shown) the TEP was −43 mV. It could be maintained at this level for at least an hour, although a slow drift downward was noted in some long experiments. Addition of sucrose or mannitol to make the FW medium isosmotic with SW shifted the TEP toward the SW value, but by only about 5 mV. Therefore, flow-entrained potentials appear to be small compared to those associated with the presence or absence of alkali metal ions in the external medium.

The data in Table II summarize flux and TEP values for groups of animals exposed to the two media. On changing from SW to FW or DW the mean depolarization was 44 mV and $J_{\text{out}}^{Na}$ was reduced by about 75%.
Single Salt Replacement

Figs. 3 and 4 show that efflux was reduced and the gill depolarized when the preparation was bathed by FW. When NaCl was added to the medium to a final concentration of 500 mM there was a prompt increase in \( J_{\text{out}}^{Na} \) as shown in Fig. 5. The first panel shows \(^{22}\)Na extrusion into SW. The external medium was then changed to DW, and efflux was reduced as expected. NaCl was added to the medium at the arrow, and \( J_{\text{out}}^{Na} \) increased from 25–82% of the original value in SW. If the 10% per hour correction is applied, efflux in 500 mM NaCl was essentially the same as in SW. No other constituent of sea water was required, and it is apparent in Fig. 5 that any delay was within the limits of our sampling procedure, since no inflection appears in the efflux line after adding NaCl.

Fig. 6 shows that the gill also repolarized when NaCl was added to a FW medium. The final value, about +8 mV, is about that noted in full SW even

| TABLE II | \( J_{\text{out}}^{Na} \) AND TEP IN SW AND FW |
|----------|------------------------------------------|
| Medium   | \( J_{\text{out}}^{Na} \) \( (\mu g \text{g}^{-1} h^{-1}) \) | TEP \( (mV) \) |
| SW       | 155±16 (16)                             | +10.4±0.9 (15) |
| FW       | 39.6±5.8 (16)                           | −34.9±1.4 (15) |

Figure 6. The effect of NaCl on the TEP. The gill, previously irrigated by SW, was exposed to FW, and the TEP approached −40 mV. At the first arrow NaCl (500 mM) was added to the reservoir and caused repolarization to +8 mV (the SW TEP was +5 mV). At the second arrow the NaCl was replaced by FW.
though none of the other constituents were present. It is also apparent that the electrical response was very rapid.

Fig. 7 shows that external KCl also stimulates sodium efflux. $J_{out}^{Na}$ was measured first in SW, then in DW which reduced it to 27% of the control. At the arrow, K$^+$ was added to the medium to give a final concentration of 10 mM, about that in SW. Sodium efflux increased, with no noticeable delay, to 69% of the original value, and the corrected value in this fish would be about 80% of that in SW. It is worth noting that K$^+$ added to a fish adapted to FW had no effect on efflux. External K$^+$ also caused partial repolarization of the gill as shown (for a different animal) in Fig. 8, although the final TEP was rarely as large as in SW.

DISCUSSION

Although intensive research on ion movement through the gills of marine teleosts commenced only during the past decade, a number of studies show that both stenohaline and euryhaline forms have very high sodium fluxes in
SW, with flux ratios close to unity (Mullins, 1950; Motais, 1967; Evans, 1967; Potts et al., 1967; Lahlou and Sawyer, 1969). Most of these fish have unidirectional fluxes in the range 1,000–3,000 \( \mu \text{eq} \) (100 g\(^{-1}\)) h\(^{-1}\), which is an order of magnitude faster than in the irrigated gill of the rainbow trout. Therefore, it is important to summarize the reasons for believing that our preparation produces data that are not largely artifactual. These are as follows:

(a) A number of observations indicate that when salmonid fish are adapted to SW NaCl exchange is much slower than in most other marine teleosts. \( J_{\text{out}}^{\text{Na}} \) in unanaesthetized trout, swimming in small aquaria, is also lower than has been reported for most other fish and is only about 50% higher than for the anaesthetized, gill-irrigated animals (Table I). Even this difference may be due simply to the fact that one group was actively swimming, the other completely anaesthetized; Randall et al. (1972) have shown that increased activity in fish can double \( Na^+ \) efflux. \( J_{\text{out}}^{\text{Cl}} \) is also low in these animals, and the value reported, about 380 \( \mu \text{eq} \) (100 g\(^{-1}\)) h\(^{-1}\), is comparable to our sodium fluxes (Gordon, 1963). Finally, \( J_{\text{out}}^{\text{Na}} \) in *Salmo salar* has been reported to be about 500 \( \mu \text{eq} \) (100 g\(^{-1}\)) h\(^{-1}\) (Potts et al., 1970). This is closer to our values for *Salmo gairdneri* than to those reported for other forms. The authors suggested that the low value may have been due to "incomplete smoltification." Perhaps older animals will prove to have higher fluxes than younger ones, but the difference can hardly be due to incomplete adaptation to SW since our animals, at least, survived for months in that medium. Apparently young salmonids can adapt to SW without developing fluxes as high as have been reported for other marine teleosts, although the general proposition that SW-adapted animals have higher fluxes than FW-adapted holds for this group, too.

(b) Modifying the composition of the external medium elicits the same efflux changes in our preparation as those reported in free-swimming animals. Thus, suppression of efflux attending a rapid change from SW to FW has been demonstrated in the flounder (Motais et al., 1966; Motais, 1967; Potts and Eddy, 1973) as well as the eel (Motais, 1967). Maetz showed that in the eel addition of NaCl to a FW bath immediately restored \( J_{\text{out}}^{\text{Na}} \) to its value in SW, and that 10 mM KCl was about 60% as effective (Maetz, 1969), and this single salt effect has also been demonstrated recently in the flounder (Potts and Eddy, 1973) and in *Dormitator maculatus* (Evans et al., 1973). Even the relative magnitude of these changes is about the same in the irrigated trout gill as in intact eel and flounder.

(c) The electrical behavior of the irrigated gill is also qualitatively the same as that in the eel (Maetz and Campanini, 1966) and flounder (Potts and Eddy, 1973). The TEP in SW-adapted trout is somewhat lower than in the eel (+18 mV) or flounder (+19 mV). Depolarization in FW has been de-
scribed for both, and repolarization by NaCl (complete) and KCl (partial) was noted in the flounder.

The irrigated gill preparation is not completely normal in two regards: fluxes are somewhat lower than in free-swimming animals, and they decrease slowly in long experiments. But the behavior described above is consistent with that reported for animals studied under more normal conditions. It is especially significant that the results supporting exchange diffusion and active Na⁺ extrusion (stimulation of $J_{out}^Na$ by external K⁺) occur. The data indicate that such a preparation can be useful for studying the mechanism underlying sodium extrusion from SW-adapted fish.

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