On the Mechanism of Sodium Extrusion across the Irrigated Gill of Sea Water-Adapted Rainbow Trout (Salmo gairdneri)

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ABSTRACT Sodium efflux ($J_{Na}^{out}$) across the irrigated trout gill was rapid in sea water (SW), but only about 25% as large in fresh water (FW). The difference correlated with a change in the potential difference across the gill (TEP). The latter was about +10 mV (blood positive) in SW, but -40 mV in FW. Both flux and electrical data indicated that gills in this fish are permeable to a variety of cations including Na+, K+, Mg++, choline, and Tris. They are less permeable to anions; $P_{Na}:P_{K}:P_{Cl}$ was estimated to be 1:10:0.3, and $P_{Cl} > P_{HCO}_3$. The TEP was shown to be a diffusion potential determined by these permeabilities and the extant ionic gradients in SW, FW as well as in other media. $J_{Na}^{out}$ appeared to be diffusive in all of the experiments undertaken. Exchange diffusion need not be posited, and the question of whether there is an active component remains open.

INTRODUCTION

Sodium fluxes across the gills of sea water (SW)-adapted fish have different characteristics than in better known fresh water (FW) epithelia like frog skin and toad bladder. These were described in the previous paper (Greenwald et al., 1974), and recent studies have been directed at attempting to understand the underlying mechanisms. In one series of studies it was shown that unidirectional fluxes are exceptionally rapid in SW and that both influx ($J_{Na}^{in}$) and efflux ($J_{Na}^{out}$) appear to show saturation kinetics when [Na+] is varied (Motais et al., 1966; Motais, 1967). This was ascribed to operation of an exchange diffusion system. In addition, efflux exceeded influx by about 5% in order to extrude the Na+ absorbed from the gut. It was proposed that this fraction of $J_{Na}^{out}$ required active transport, and when it was shown that
these gills contained high levels of the well-known \( \text{Na}^+ + \text{K}^+ \)-ATPase (Epstein et al., 1967) a coupled \( \text{Na}^+ - \text{K}^+ \) transport system appeared to play the key role. The detailed model, and the experimental evidence supporting it were described fully by Maetz (1969, 1971).

Until recently, little attention was paid to the flux-force relationships existing between blood and SW; in particular, electrical phenomena were almost entirely neglected. A few measurements showed that the transepithelial potential (TEP) across the gill was in the range 18-25 mV, blood positive to SW (House, 1963; Maetz and Campanini, 1966; Evans, 1969), and in the eel the TEP reversed (the gill “depolarized”) to about \(-40 \text{ mV}\) when the external medium was changed from SW to FW (Maetz and Campanini, 1966).

The first systematic examination of the relationship between \( \text{Na}^+ \) fluxes and the electrochemical gradient was undertaken, not in fish, but in the brine shrimp \textit{Artemia salina} (Smith, 1969a, b). Like marine teleosts, this animal is hypotonic to SW, drinks the medium to replace water lost osmotically, and must extrude NaCl absorbed from the gut across the gills back into SW (Croghan, 1958a, b, c). The TEP in \textit{Artemia} was \(+23 \text{ mV}\), only slightly below the equilibrium potential for \( \text{Na}^+ \) (\( E_{\text{Na}} = 25-26 \text{ mV} \)). When the animal was transferred to Na+-free SW the gill depolarized, and the TEP reversed as in the eel. Transference numbers were estimated for single ions, and although the quantitative conclusions are suspect for reasons discussed later, they showed that the gill was more permeable to cations than to anions, hence \( J_{\text{Na}^+} \) must be voltage dependent, and rapid fluxes could be explained by diffusive leak; exchange diffusion appeared to play no role. In addition, since \( \text{Na}^+ \) was close to equilibrium there was no need to postulate active transport. A recent study of the electrical behavior of the flounder (\textit{Platichthys flesus}) gill led to some of the same conclusions (Potts and Eddy, 1973). The fish is much more cation than anion permeable \( (P_{\text{Na}^+}:P_{\text{K}^+}:P_{\text{Cl}^-} = 1:2.3:0.03) \), and when ion concentrations are varied in the external medium the TEP varies in a fashion that can be described, to a first approximation, by the constant field equation (Goldman, 1943). However, Potts and Eddy concluded that, while most of the unidirectional efflux was diffusive a small fraction, corresponding to the amount absorbed in the gut, must be due to active transport.

Thus, three models have been proposed to describe \( \text{Na}^+ \) transfer across the gill in SW-adapted animals: exchange diffusion plus active extrusion, leak diffusion plus active extrusion, and leak diffusion alone (no active transport). The irrigated trout gill shows all of the phenomena that have led to these models. It has been used here to further examine the relationship between \( J_{\text{Na}^+} \) and the electrochemical gradient.
METHODS

The procedures described in the previous paper were used, but two points should be emphasized. Fluxes in these fish are relatively slow; each measurement took about an hour. As a result, experiments requiring three to four fluxes per animal (e.g., Figs. 1 and 3) took 4–5 h in addition to a 2-h period before the measurements began (see Methods in Greenwald et al., 1974). In contrast, electrical measurements were brief. TEP changes were rapid and stabilized quickly. As a result, although the data shown in Fig. 1 involve the same changes in $[\text{Na}^+]_{\text{out}}$ as those in Fig. 3, the experimental situations differed in that a series of flux measurements on each fish required 6–7 h whereas a more extensive series of TEP's were obtained in less than an hour. For this reason no attempt is made to correlate the data in Figs. 1 and 3 or 4 and 6. Instead simultaneous measurements of $J_{\text{Na}}^{\text{out}}$ and TEP (Fig. 6) were undertaken. For these the cooling system employed was a cold plate on which the irrigation reservoir rested. Thermal control during the long periods needed for flux estimations was probably no better than ±2°C, and this might have introduced some variability into the results. However, the more precise thermal control system employed in the other flux studies could not be used because it introduced large and variable DC signals into the electrical recordings.

RESULTS

Ion Concentrations in Plasma and SW

Plasma ion concentrations are shown in Table I for animals that had been in SW for periods varying from 2 wk to several months. They agree well with the corresponding values for a number of SW-adapted salmonids (Holmes and Donaldson, 1969). Sea water concentrations are those for "Instant Ocean," and were obtained from Aquarium Systems, Inc., Eastlake, Ohio. In the calculations that follow the values used were (in mM), for plasma, Na = 180, Cl = 150, K = 4, and for SW, Na = 450, Cl = 520, and K = 10.

Alkali Metal-Deficient SW

The previous paper showed that changing the gill-irrigation medium from SW to FW resulted in a sharp decrease in $J_{\text{Na}}^{\text{out}}$ and depolarization of the gill.

Table I

| Concentration | Na⁺ | K⁺ | Cl⁻ |
|---------------|-----|----|-----|
| meq/liter*    | 172.4±1.3 | 3.12±0.28 | 142.4±4.9 |
| (N = 202)     | (N = 6)   | (N = 15)    |

*Mean ±SEM.; the number of fish is shown in parentheses.
by 40–50 mV. Table II shows that when only K\(^+\) was deleted neither efflux nor the TEP was affected. On the other hand, when Na\(^+\) (only) was deleted efflux was reduced by about 25\%, and the gill depolarized by about 10 mV. Deletion of both ions resulted in a more substantial inhibition of efflux and greater depolarization. When the external medium contained only 1 mM NaCl (FW) \(J_{\text{out}}^{\text{Na}}\) was lower than when the divalent ions are present, and depolarization was considerably larger. Preliminary experiments indicate that the effects of Ca\(^{2+}\) and Mg\(^{2+}\) are complex, and they will, for the most part, be neglected here. However, one aspect of the action of Mg\(^{2+}\) will be considered below.

### Table II

| Ion(s) delete | \(J_{\text{out}}^{\text{Na}}\) (\(100 \text{ gm}^{-1} \text{ h}^{-1}\)) | TEP \(mV\) |
|---------------|-----------------|-----------|
| K\(^+\)       | Control (SW)    | Test solution | Control (SW) | Test solution |
|               | 131±16 (11)     | 135±14 (11) | +10.1±1.3    | +11.4±1.3    |
| Na\(^+\)      | 104±12 (12)     | 78±10 (12)  | +10.1±1.3    | + 0.6±1.9   |
| Na\(^+\) and K\(^+\) | 144±19 (6)     | 45±7 (6)    | +10.1±1.3    | -14.3±3.0   |
| FW†           | 155±16 (16)     | 40±5.8 (16) | +10.1±1.3    | -41.4±3.6   |

* The TEP was measured for each fish in SW, then in each test solution, and finally in SW. \(N = 15\) for these data. Because flux experiments took much longer sequential measurements on each animal were not made. Each SW control and the corresponding test value (columns 2 and 3) refers to a single group of animals; no fish were exposed to more than one ion-deficient medium. No TEP's were obtained from any of the animals in the flux experiments.

† The FW flux values are from Table II of the previous paper. TEP's were obtained on another group of fish, and the FW value differs from the one in that table.

Potts and Eddy (1973) showed that the flounder gill behaved like a cation-permeable membrane with a very low anion permeability, and that at least some of its electrical behavior could be described by the Goldman equation (Goldman, 1943),

\[
E = \frac{RT}{zF} \ln \frac{[\text{Na}^+]_{\text{sw}} + \alpha[\text{K}^+]_{\text{sw}} + \beta[\text{Cl}^-]_{\text{sw}}}{[\text{Na}^+]_{\text{pt}} + \alpha[\text{K}^+]_{\text{pt}} + \beta[\text{Cl}^-]_{\text{pt}}},
\]

where \(\alpha\) equals the ratio of K\(^+\) to Na\(^+\) permeability coefficients \(P_{\text{K}}/P_{\text{Na}}\), and \(\beta\) is the corresponding ratio of coefficients for Cl and N \(P_{\text{Cl}}/P_{\text{Na}}\). For the flounder \(\alpha\) was estimated to be 2.25 from Maetz’s (1969) measurements of \(J_{\text{in}}^{\text{Na}}\) and \(J_{\text{in}}^{\text{K}}\). The parameter \(\beta\) was estimated as follows. When the gill potential is less than the equilibrium potential for sodium there must be a net diffusional influx of sodium across the gill given by \(J_{\text{in}}^{\text{Na}} = P_{\text{Na}}(E_{\text{Na}} - E)\). To preserve electrostatic neutrality there is an equivalent net influx of chloride,
the major anion in sea water, given by $J_{\text{in}}^{\text{Cl}} = P_{\text{Cl}}(E - E_{\text{Cl}})$. Since the fluxes are equal it follows that

$$\frac{P_{\text{Cl}}}{P_{\text{Na}}} = \frac{E_{\text{Na}} - E}{E - E_{\text{Cl}}},$$

from which they calculated that $\beta = 0.03$. For our fish $E_{\text{Na}} = 22.5 \text{ mV}$, $E_{\text{Cl}} = -30.4 \text{ mV}$ and the TEP was about 10.5 mV. These values give $\beta = 0.29$ for the irrigated trout gill in anaesthetized animals. Although this suggests that our preparation is relatively much more anion permeable than the unanaesthetized flounder an independent estimate gives approximately the same value (cf. gluconate hyperpolarization in *Effect of Other Ions* below). $J_{\text{in}}^{K}$ has not been measured in our system, hence there is no direct method for estimating $\alpha$. However, having estimated $\beta$ a value for $\alpha$ is chosen which approximates that found in SW. For $\alpha = 10$ Eq. 1 predicts a TEP of $+11.2$ mV in SW which is close to the value shown in Table II and in the previous paper. We will take $P_{\text{Na}}:P_{K}:P_{\text{Cl}} = 1:10:0.3$.

Using these parameters Eq. 1 predicts that in FW the TEP should fall to $-38.5 \text{ mV}$ in good agreement with the value in Table II. The other calculated values are: for K-free SW, $+7.3 \text{ mV}$, for Na-free SW, $-10.6 \text{ mV}$, and for alkali metal-free SW, $-38.5 \text{ mV}$. All three values are lower than those observed, but the most striking difference between the values predicted and observed was for alkali metal-free SW. The constant field equation was derived for a membrane permeable only to monovalent ions, hence it predicts that the TEP should be the same in alkali metal-free SW and in FW. This is clearly not the case. Since $\text{Na}^+$ and $\text{K}^+$-free SW contains divalent ions at SW concentrations one possible explanation of the disagreement might be that the gill is permeable to divalent ions. In particular, the concentration of $\text{Mg}^{2+}$ is very high in SW ($100 \text{ meq/liter}$; plasma contains only about 2 meq/liter), and if $P_{\text{Mg}}$ is appreciable the TEP would be more electropositive than predicted by Eq. 1. The discrepancy would be larger when $\text{Mg}^{2+}$ was a major proportion of the total cation concentration in the external medium; i.e. in Na-free and alkali metal-free SW. This is exactly what is observed in Table II. We will present independent evidence for $\text{Mg}^{2+}$ permeability below (section on *Effect of Other Ions*). It suffices here to note that quantitative differences are explicable if the gill is permeable to $\text{Mg}^{2+}$, and that in the absence of divalent ions agreement between experimental and calculated values is quite good.

*Single Alkali Metal Salts in the Medium*

The observations in the previous paper show that $J_{\text{out}}^{\text{Na}}$ was suppressed when the irrigating medium was switched from SW to FW. When 500 mM Na$^+$ is added to a FW medium efflux is stimulated. $J_{\text{out}}^{\text{Na}}$ was measured on four fish,
first in SW, then in FW, and finally in 500 mM NaCl. The control value in SW was 144 ± 24 μeq (100 g)^{-1} h^{-1}. It dropped to 34 ± 8 in FW and increased to 125 ± 25 when the NaCl was added. No other constituent of sea water was required. In flounders and eels the magnitude of $J_{out}^{Na}$ varied with [Na+]_{out} (Motais, 1967; Motais et al., 1966), and the data were fitted to a rectangular hyperbola by assuming a very high $K_m$ (400 mM). We studied the concentration dependence in trout, but were limited to four flux measurements per fish because of the relatively slow extrusion (5 min is adequate for determining $J_{out}^{Na}$ in most SW fish, but an hour is required in the trout under our conditions). To define the relationship between $J_{out}^{Na}$ and [Na+]_{out}, six concentrations, between 0-500 mM, were used. Efflux was determined in every animal in the absence of external Na+ and was denoted $J_{o}$. It was then determined in each fish at three concentrations from the set 50, 100, 250, 400, and 500 mM. In order to reduce the variability that is inherent in comparing absolute flux values among animals the results are expressed as the ratio $J_{(Na)}/J_{(o)}$, the efflux at a particular sodium concentration to that in the absence of Na+. The results are shown in Fig. 1. Efflux increased with [Na+]_{out}, and stimulation was nearly two-fold at the lowest concentration used, 50 mM. The relationship appears to be nonlinear, although there is no sign of an upper, limiting rate.

Addition of NaCl also resulted in rapid repolarization of a gill irrigated by FW. When [Na+]_{out} was increased in steps, as in Fig. 2, it was apparent that the TEP was a continuous function of external concentration. Changes in the range 1-10 mM had little effect, but above 10 mM each increment caused

![Graph](https://example.com/graph.png)

**Figure 1.** $J_{out}^{Na}$ as a function of [Na+]_{out}. A total of 17 fish were used. $J_{out}^{Na}$ was measured on every animal in the absence of Na+ (i.e. in DW). In addition, it was measured at three different [Na+]_{out} for each animal. Fluxes were corrected by 10% for each experimental period as described in Greenwald et al. (1974). The ordinate is the efflux at a particular concentration ($J_{(Na)}$) relative to the efflux for the same animal in DW ($J_{(o)}$), and the values shown are means ±1 SEM. The number of fish used at each concentration is shown in brackets.
FIGURE 2. The effect of external NaCl on the TEP. The gill was initially depolarized in FW. Addition of 3 and 10 mM caused additional depolarization, but this was rarely noted. These concentrations usually had no perceptible effect on the TEP. Stepwise addition of NaCl to the reservoir caused increasing repolarization over the range 30-400 mM. The TEP for this fish was +12 mV with SW irrigating the gills.

the gill to repolarize to a new stable value. At 500 mM the TEP was close to that in SW. The concentration dependence was clearly nonlinear in this animal, and in Fig. 3 mean values for a group of fish are plotted against [Na⁺]out on a logarithmic scale. Over the range 30-400 mM the relationship appears to be logarithmic with a slope of about 27 mV/10-fold concentration change. At lower concentrations the dependence is less steep, and below 10 mM becomes negligible. This is the behavior expected from a membrane more permeable to Na⁺ than to Cl⁻, and the dotted line shows the relationship predicted by the constant field equation with the parameters α = 10 and β = 0.36. The inset shows the experimental points plotted on linear coordinates, and the resemblance to a rectangular hyperbola is obvious.

Maetz (1969) also showed that after $J_{Na}^N$ was suppressed in FW it could be stimulated by adding KCl alone. In six experiments on SW-adapted trout 10 mM K⁺-stimulated $J_{Na}^N$ from 33 ± 7 to 163 ± 35 μeq (100 g)$^{-1}$ h$^{-1}$. The control value for this group (in SW) was 176 ± 32 μeq (100 g)$^{-1}$ h$^{-1}$. It is worth noting that KCl added to a fish adapted to FW had no effect on efflux. In the absence of Na⁺ $J_{Na}^N$ in SW fish varied with [K⁺]out. The concentration dependence shown in Fig. 4 is clearly nonlinear and appears to be near maximal at 10 mM, although there may be some additional increase in the range 10-50 mM. The half-maximal value ($K_w$) appears to be 2-3 mM which is about the same as in the flounder (Maetz, 1969) and in Dormitator maculatus (Evans et al., 1973).

Fig. 5 shows that external K⁺, like Na⁺, repolarized the gills but was effec-
Figure 3. Dependence of the TEP on [NaCl]_out. Mean values (±1 SEM) of the TEP at different NaCl concentrations are shown. The measurements were made as in Fig. 2, and the plateau TEP used at each concentration. N = 5 (fish) except at 500 mM where only two measurements were made. An initial measurement in SW was made on each animal, and the mean value for the group is also shown (X). The dashed line shows the solution of Eq. 1 for P_{Na} : P_{K} : P_{Cl} = 1 : 10 : 0.36. The same points are shown on linear coordinates in the inset.

Figure 4. J_{Na}^\text{ext} as a function of [K+]_out. The procedure was as in Fig. 1 except that [K+]_out was varied in the absence of Na+.

tive at much lower concentrations. The relationship is approximately logarithmic for the range 1–20 mM with a slope of about 20 mV/10-fold concentration change. At low concentrations the dependence is less steep. The relationship predicted by the constant field equation (α = 10, β = 0.36) is shown by the dashed line, and the agreement is good. The inset shows the experimental values on linear coordinates. Here too, the TEP appears to approach an upper limiting value at high [K+]_out, and it is interesting that
Figure 5. Dependence of the TEP on [KCl]out. The procedure was as in Fig. 3 except that KCl was added to the reservoir. N = 7 for 0.1–10 mM; N = 4 at 20 mM. The mean SW TEP for this group is shown (X). The dashed line is predicted by Eq. 1 for $P_{Na}:P_{K}:P_{Cl} = 1:10:0.36$.

The concentration at which repolarization was half maximal is about 2–4 mM, close to that found for half-maximum stimulation of $J_{Na}$. The data in Figs. 1–5 show a close correspondence between the behavior of sodium efflux and the TEP, and they point to the possibility that in these experiments at least, sodium efflux is strictly diffusive. This can be approached experimentally as follows. Using the constant field constraint the unidirectional efflux of sodium in this system can be written

$$J_{Na} = P_{Na}[Na^{+}]_{plasma} \frac{FE}{RT} \exp \left( \frac{FE}{RT} \right) \exp \left( \frac{FE}{RT} \right) - 1 \quad (3)$$

The equation is not useful in this form because the parameter $P_{Na}$ is unknown. However, if we manipulate the voltage across the gill, fluxes can be determined at two values of $E$, and the ratio of the two effluxes is given by (House, 1963),

$$\frac{J_{(x)}}{J_{(0)}} = \frac{\frac{FE_{(x)}}{RT} \exp \left( \frac{FE_{(x)}}{RT} \right)}{\frac{FE_{(0)}}{RT} \exp \left( \frac{FE_{(0)}}{RT} \right)} \exp \left( \frac{FE_{(x)}}{RT} \right) - 1$$

From the experiments described above it is obviously possible to change the TEP by varying external $Na^{+}$ or $K^{+}$ and hence to test the proposition that
efflux is purely diffusive. Simultaneous measurements of flux and TEP over extended periods of time is difficult but feasible, and the experiments have been performed as follows. $J_{\text{out}}^{\text{Na}}$ and the TEP were measured simultaneously while the gill was being irrigated with FW. The flux is denoted $J_{(o)}$ and the voltage $E_{(0)}$. The external medium was then changed to one containing a concentration of $K^+$ in the range 1–10 mM, and the measurements repeated. The variables were measured again and are denoted $J_{(x)}$ and $E_{(x)}$. From the two TEP measurements we can calculate an expected ratio $J_{(x)}/J_{(o)}$ based on the assumption that $J_{\text{out}}^{\text{Na}}$ is purely diffusive. The calculated ratio can then be compared with the ratio determined from the flux measurements. This was done for six fish, and the results are presented in Fig. 6. Although there is considerable variability the points appear to cluster about the line of identity with no tendency to fall above it (which might indicate active transport outward).

**Effect of Other Ions**

A change in the external medium from 500 mM NaCl to FW depolarized the gill by 40–50 mV, but changing from NaCl to 500 mM choline chloride depolarized it by only 15–20 mV. The observation suggests that choline can contribute to the diffusion potential. Fig. 7 shows that this is, in fact, the case. In this experiment the gill was irrigated by FW, and the TEP was about

![Image](https://jgp.rupress.org/)

**Figure 6.** The dependence of $J_{\text{out}}^{\text{Na}}$ on the TEP. Sodium efflux was measured in FW ($J_{(o)}$) and in the presence of external $K^+$ ($J_{(x)}$), and the TEP was monitored throughout. The ordinate shows the flux ratio $J_{(x)}/J_{(o)}$, and the abscissa the same value calculated from Eq. 3. For three fish (△) a single ratio was obtained from measurements at $[K^+] = 0$ and 10 mM. For three other fish fluxes and TEP were measured successively at $[K^+] = 0$, 1, 3, and 10 mM. A common symbol (□, ○, or ●) is used for the three flux ratios on a single animal in this group.
-25 mV. Addition of choline (500 mM) caused it to repolarize by about 15 mV, an effect that was readily reversible by washing. Choline also stimulates sodium efflux. Fig. 8 shows an experiment in which $J_{\text{Na}}^{\text{out}}$ was measured in SW, then in FW with the usual result. When choline-Cl (500 mM) was added to the external medium $J_{\text{Na}}^{\text{out}}$ was increased substantially though it was clearly lower than in SW. The same phenomena were demonstrated on two animals using Tris hydroxymethyl aminomethane at pH 7 (Tris-HCl which is more than 90% dissociated at this pH).

We noted earlier that the change in TEP and inhibition of $J_{\text{Na}}^{\text{out}}$ are greater when all ions are removed from SW than when only NaCl and KCl are deleted (Table II). Further, the diffusion equation accounted quantitatively for the TEP change only when all ions were deleted. Since the previous experiments show that the gill is permeable to organic cations as well as Na$^+$ and K$^+$ it appeared possible that divalent cations might also be permeant. Fig. 9 provides evidence that this is indeed the case. With the gill irrigated by FW the TEP was about -47 mV. Addition of MgSO$_4$ to the medium (25 mM) caused it to become nearly 15 mV more positive, and increasing the concentration to that in sea water in FW (50 mM) repolarized it a few millivolts more. The values in Table II show that when K$^+$ was deleted the measured TEP is a few millivolts higher than predicted by the constant field equation; when Na$^+$ was deleted it was about 10 mV higher; and in the absence of both
Na⁺ and K⁺ the difference was even greater. It is clear that much, if not all, of the discrepancy can be accounted for by the fact that Mg²⁺ (and Ca²⁺?) is permeant. It is not possible to calculate the alkaline earth metal contribution to the TEP, because there is no simple solution of the diffusion equation corresponding to Eq. 1 for electrolytes with valences other than 1:1. But it is clear that the discrepancies between theory and results in Table II may be accounted for on this basis.

Figure 9

Figure 9. The effect of MgSO₄ on the TEP. The TEP was approaching -47 mV in FW when 25 mM MgSO₄ was added (first arrow). The concentration was increased to 50 mM at the second arrow and reduced to zero at the third arrow.

Figure 10

Figure 10. A gill model in which NaCl might be extruded into SW without participation of an active Na⁺ transport system. It is worth noting that the shunt pathway need not be in or even adjacent to the chloride cell. All that is necessary is that the electrical space constant be large compared with the distance between pump site and leak pathway.

The fact that the constant field equation accounts for the electrical behavior of this gill when β = 0.3 indicates that Cl⁻ is appreciably permeant. Independent evidence for this was obtained by comparing the TEP's in solutions of Cl salts and when part or all of the Cl⁻ was replaced by larger, presumably less mobile anions. In one experiment the TEP increased (gill hyperpolarized) by several millivolts when ASW containing only sulfate salts was substituted for Instant Ocean. In three other experiments the gill hyperpolarized by 3 mV when the medium was changed from 500 mM NaCl to an equimolar solution of NaCl and Na-gluconate. When 500 mM NaCl was completely replaced by Na-gluconate the hyperpolarization amounted to 11 mV (five measurements on three fish). If gluconate ion is completely nonpenetrating (which is not known), the constant field equation predicts a hyperpolariza-
tion of 13 mV. Thus, these experiments provide independent evidence that the irrigated trout gill is appreciably chloride permeable and that $\beta$ is about 0.3.

**DISCUSSION**

**Ionic Basis of the TEP**

The data reported above lead to several conclusions about the irrigated trout gill. First, it is more permeable to alkali metal cations than to chloride. The ratio $P_{Cl}/P_{Na}$ was estimated by two independent methods and found to be about 0.3. Thus, the relative chloride permeability is somewhat greater than in *Artemia* ($P_{Cl}/P_{Na} = 0.1$; Smith, 1969 a) and an order of magnitude greater than in the flounder ($P_{Cl}/P_{Na} = 0.03$; Potts and Eddy, 1973). The ratio $P_{K}/P_{Na}$ was not estimated directly. Qualitatively KCl had the same effect, when added to the external medium, as Na$^+$; it repolarized a gill irrigated by FW and stimulated $J_{Na}^{\text{out}}$. But K$^+$ was effective at concentrations at least an order of magnitude lower than those needed for Na$^+$, suggesting that $P_{K}$ is considerably larger than $P_{Na}$. Quantitatively, with $P_{Cl}/P_{Na}$ fixed by experiment at 0.35, data such as those in Fig. 5 are described reasonably well if $P_{K}/P_{Na}$ is about 10, and this also gives the correct value in SW. The flounder gill was also found to be more permeable to K$^+$ than to Na$^+$, although the ratio was somewhat lower (about 2.5).

In addition, the electrogenic effects of choline (or Tris) and Mg$^{2+}$ show that the gill is permeable to a variety of cations. Both choline and Mg$^{2+}$ have been used in work of the type described here on the premise that they are impermeant. Choline is often used as a nonpenetrating replacement for Na$^+$. Its use in electrical measurements on the *Artemia* gill (Smith, 1969 a) might explain why the sum of the transference numbers for Na$^+$, K$^+$, and Cl$^-$ (assumed to be the sole permeant ions in SW and blood) was substantially less than 1. If this is correct, then $P_{Cl}/P_{Na}$ may be even lower than 0.11 in this animal, and might approach zero. In the work on flounder gill MgSO$_4$ was used to maintain the external medium isosmotic with SW in experiments where the total salt concentration would otherwise be lowered. The rationale was that the procedure would minimize contributions to the TEP of osmotic flow through charged pores. However, the electrogenic effect of Mg$^{2+}$ may introduce more error, if the ion is assumed impermeant, than changes in TEP caused by modifying the osmotic gradient. The discrepancies in Table II between the TEP and values predicted on the assumption that only Na$^+$, K$^+$, and Cl$^-$ are permeant may well be due to the electrogenic effect of 50 mM Mg$^{2+}$ in the alkali metal-deficient media.

The picture that emerges is that the TEP is a diffusion potential generated by extant ionic gradients across an epithelium that is more permeable to
cations than to anions. The main determinants in SW are the concentrations of NaCl in blood and SW and the value of $\beta$. If $\beta$ approaches zero, as in the flounder, the TEP will approximate $E_{Na}$, while in gills that are appreciably Cl permeable it will be lower. Contributions of other ions are small because their concentrations are relatively low (more correctly their activities; in solutions as concentrated as SW and plasma the distinction may be important as will be noted later). In FW the same factors, [NaCl] and $\beta$, determine the TEP. In Na+-free solutions other ions (e.g. K+, Mg++) may play a major role in electrogensis.

The behavior of $J_{Net}$ in the experiments described above is almost entirely predictable on the basis of the diffusion model. A relatively large efflux is expected in SW because the voltage gradient favors it, and depression in FW is simply a consequence of reversing the polarity of the TEP. When single salts with permeant cations (i.e. NaCl, KCl, choline chloride) are added to FW efflux is stimulated. The dependence of $J_{Net}^{Na}$ on [NaCl] and [KCl] is clearly nonlinear, and appears, especially with KCl, to approach an upper limiting value. This “trans effect”, which appears to saturate at high external concentrations, has been ascribed to an exchange diffusion system in the presence of NaCl (Motais et al., 1966; Motais, 1967), and in the presence of external KCl to the operation of a conventional Na+ + K+-ATPase-mediated active sodium extrusion across the apical membrane. But Smith (1969 b) showed that apparent saturation is predicted by the constant field flux equation for a cation diffusing across a preferentially cation-permeable membrane. The reason is that unidirectional tracer efflux is a function of plasma concentration and the voltage across the gill (Eq. 3), and the latter approaches an upper limit as the external salt concentration increases. That this, in fact, occurs is shown in the insets to Figs. 3 and 5.

Since diffusion theory adequately describes the behavior of $J_{Net}^{Na}$ there appears to be little or no exchange diffusion occurring in the irrigated trout gill. A straightforward diffusion model also explains the stimulation of $J_{Net}^{Na}$ by choline which would otherwise require a remarkably unselective exchange carrier. The same conclusion, that sodium fluxes are predominantly diffusive, was reached earlier by Smith (1969 b) for Artemia and by Potts and Eddy (1973) for the flounder.

Active or Passive Sodium Extrusion?

NaCl, equivalent to that absorbed in the gut, must be extruded across the gill into SW. Chloride is clearly actively transported; both chemical and electrical gradients favor net inward movement. But the situation for Na+ is equivocal, although the mechanism of operation of a transport system has received a lot of attention recently. Most SW-adapted teleosts have a higher level of Na+ + K+-activated ATPase in the gill than their FW counterparts.
It has been proposed (Maetz, 1971) that the enzyme operates on the apical membrane, extruding Na\(^+\) from the cell in exchange for K\(^+\) from SW. We will not examine the model in detail here, but one aspect relates to data presented above. This is worth discussing, because it illustrates how weak is the support for this model. The dependence of \(J_{0N}^N\) on [K\(^+\)]\(_{out}\) shown in Fig. 4 was first described in experiments on the flounder (Maetz, 1969). Stimulation of efflux and saturation kinetics suggested that the Na\(^+\) + K\(^+\)-ATPase was located on the apical membrane with the K-site exposed to the medium. The interpretation is supported by the fact that the [K\(^+\)]\(_{out}\) at which \(J_{0N}^N\) is half maximal (the \(K_m\)) is about the same as that giving half-maximal ATPase activity in microsomal preparations. In the trout \(K_m\) for flux stimulation is 2–3 mM (from Fig. 4), while for the enzyme at 13°C it was 0.8 mM (Pfeiler and Kirschner, 1972), and the same correlation has been noted in another fish, Dormitator maculatus (Evans et al., 1973). However, when one examines the inset in Fig. 5 it is clear that the half-maximum change in TEP occurs at about the same [K\(^+\)]\(_{out}\). It was argued above that the TEP is a diffusion potential which will increase and appear to approach an upper limit as [KCl]\(_{out}\) increases in the absence of other ions. The half-maximum concentration is a function of \(P_K\) and need have nothing to do with the operation of an enzyme. The ATPase is clearly present in these gills, and it may or may not play a role in active Na\(^+\) extrusion. However, this experiment casts no light on the question since the results can be explained on a nonenzymatic basis.

The antecedent question is whether there is convincing evidence that active transport need be invoked at all for sodium. The TEP has been reported for only a few fish in SW, and most of the values have been close to the equilibrium potential for sodium (\(E_{Na}\)) as shown in Table III. In three of the fish and in Artemia the TEP is only 2–3 mV below the estimated \(E_{Na}\). It is difficult to measure stable potentials in biological systems with an absolute accuracy of 2–3 mV for a number of reasons, among them the existence of junction potentials of the same order of magnitude. Additional problems are created by uncertainties about the magnitude of activity coefficients in

### TABLE III

| Animal            | TEP  | \(E_{Na}\) | Reference                  |
|-------------------|------|------------|----------------------------|
| Pholis gunnellus  | 18±1 | 20.5       | Evans, 1969                |
| Platichthys flesus| 19±0.8| 20.5      | Potts and Eddy, 1973       |
| Anguilla anguilla | 18±2.9| ~28       | Maetz and Campanini, 1966  |
| Blennius pholis   | 23±1 | 26         | House, 1963                |
| Salmo gairdneri   | 10.1±1.3| 22.5     | This paper                 |
| Artemia salina    | 23.4±0.6| 26        | Smith, 1969 a              |
solutions like SW and blood plasma, with the result that they are usually neglected. Data from the recent study of Potts and Eddy (1973) illustrate this point. They found an average TEP of $19 \pm 0.8$ mV across the gills of flounders in SW. $E_{\text{Na}}$ was calculated from SW and plasma sodium concentrations and found to be $20.5$ mV. No variance estimate was given for $E_{\text{Na}}$, but it is probably about the same as for the TEP, since the standard error of plasma Na determinations was about 3% of the mean; hence the two values cannot be distinguished. Moreover, if account is taken of activity coefficients, the mean $E_{\text{Na}}$ is lower than $20.5$ mV. The coefficient in $0.5$ M NaCl is 0.68 and may be even lower in SW because of the high $\text{SO}_4^{2-}$ concentration ($>50$ meq/liter). It is about 0.73 in $0.2$ M NaCl, the concentration in plasma. At $10^\circ\text{C}$ $E_{\text{Na}}$ will be lower by about $2$ mV if chemical activities are used instead of concentrations or about $18.0$-$18.5$ mV for the flounder. This TEP would sustain a diffusion steady state with $J_{\text{out}}^{\text{Na}} > J_{\text{in}}^{\text{Na}}$, the flux ratio $J_{\text{out}}/J_{\text{in}}$ would be $1.02$-$1.04$ compared with the value $1.03$ deduced by Maetz (1969). The only fish in which TEP's substantially lower than $E_{\text{Na}}$ have been reported are the eel ($+18$ mV, Maetz and Campanini, 1966) and the trout ($+10.1$ mV in our work). But in both cases the fish were anaesthetized, and there is no assurance that they were in a steady state during the measurements.

In fact, it is possible to develop a model, based on our information about the gill, that can account for passive net extrusion of $\text{Na}^+$ (Fig. 10). A chloride extrusion pump, presumed to be located in the "chloride cells," generates inward current flow by transporting $\text{Cl}^-$ from blood to sea water. To maintain bulk electrical neutrality outward current flows through a parallel leak pathway which is cation permeable. Most of the current through the leak is carried by $\text{Na}^+$, because it is the most abundant cation in the blood. Such a system will generate net extrusion of NaCl. It will also generate a small net extrusion of $\text{K}^+$ if the leak is $\text{K}$ permeable. Net $\text{K}^+$ extrusion across flounder gill was suggested by the data of Maetz (1969).

If $R^i \ll R^p$ in such a system, the TEP will be small when the epithelium is bathed on both sides by solutions of the same composition as has been discussed for the gut (Frizzell and Schultz, 1972) and proximal tubule of the mammalian nephron (Boulpaep, 1967). Fig. 3 shows that when external NaCl is $180$ mM, about equal to the plasma concentration, the gill TEP is also near zero. Moreover, the TEP in such a system should be determined by the ionic gradients present when the solutions bathing the epithelium are asymmetric. The constant field equation has been shown to describe the dependence of TEP on external concentration, hence $E'$ in Fig. 10 is simply Eq. 1 in the text.

The model in Fig. 10 is presented as a viable, not a necessary alternative to one requiring active sodium extrusion. It does not address the role of the membrane ATPase which is certainly present in high concentration in chlo-
ride cells (Shirai, 1972; Kamiya, 1972). But it is consistent with the physico-
chemical data and is presented to emphasize the importance of an unambigu-
ous answer to the question of whether active sodium transport must be
posited. This can only be answered when a flux ratio analysis is undertaken
in unanaesthetized fish, a technique that is conceptually feasible, but tech-
ically formidable (double-labeling with two isotopes of Na+ has never been
reported for intact animals).

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