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ACTIVE CHLORIDE TRANSPORT IN THE *IN VITRO* OPERCULAR SKIN OF A TELEOST (*FUNDULUS HETEROCLITUS*), A GILL-LIKE EPITHELIUM RICH IN CHLORIDE CELLS

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SUMMARY

1. The opercular epithelium lining the inside of the gill chamber of the killifish, *Fundulus heteroclitus*, contains Cl^- cells, identical in fine structure to gill Cl^- cells, at the high density of 4×10^5 cells/cm^2. This epithelium can be isolated, mounted in a Lucite chamber, and its ion transport properties studied with the short-circuit current technique.

2. The isolated opercular epithelia of seawater-adapted fish, when bathed on both sides with Ringer and gassed with 100 % O_2, displayed a mean short-circuit current of $136{\cdot}5 \pm 11{\cdot}1$ μA/cm^2, a mean transepithelial potential difference of $18{\cdot}7 \pm 1{\cdot}2$ mV (blood side positive), and a mean transepithelial d.c. resistance of $173{\cdot}7 \pm 12{\cdot}1$ Ω.cm^2 (mean $\pm$ S.E. of mean; $n = 64$).

3. The transepithelial potential difference across the opercular epithelia of seawater-adapted fish was dependent on both Na^+ and Cl^- in the bathing solutions and increased linearly with increasing Cl^- concentrations with a slope of $28{\cdot}3 \pm 2{\cdot}1$ mV/tenfold concentration change. The short-circuit current was Na^+ dependent and increased linearly with increasing Cl^- concentrations with no evidence of saturation kinetics below 142·5 m-equiv/l.

4. When the short-circuited epithelia of seawater-adapted fish, bathed on both sides with Ringer, was gassed with 100 % O_2 the mean Cl^- blood side to seawater side flux was $211{\cdot}7 \pm 27{\cdot}1$ μA/cm^2 and the mean Cl^- seawater side to blood side flux was $48{\cdot}9 \pm 10{\cdot}0$ μA/cm^2. This resulted in a

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net Cl^- blood side to seawater side flux of 162·8 $\mu A/cm^2$ which was not statistically different ($P > 0{\cdot}70$) from the mean short-circuit current of 158·6 ± 16·3 $\mu A/cm^2$ for these flux studies. The mean Na^+ blood side to seawater side flux was 32·2 ± 3·3 $\mu A/cm^2$ and the mean Na^+ seawater side to blood side flux was 34·8 ± 4·1 $\mu A/cm^2$, resulting in no significant ($P > 0{\cdot}20$) net flux of this cation. Similar results were obtained with short-circuited epithelia of seawater-adapted fish when bathed on both sides with Ringer and gassed with 95 % O_2/5 % CO_2.

5. Ouabain (10^{-5} M), furosemide (10^{-3} M), thiocyanate (10^{-2} M), adrenaline (10^{-6} M), and anoxia (100 % N_2) decreased the short-circuit current 92·7, 85·0, 45·3, 62·6, and 83·3 % respectively. Theophylline (10^{-4} M) stimulated the short-circuit current 54·9 %. Increasing the HCO_3^- concentration in the bathing solutions had a stimulatory effect on the short-circuit current and the potential difference across epithelia from seawater-adapted fish.

6. The opercular epithelia of freshwater-adapted *F. heteroclitus*, when bathed on both sides with Ringer, displayed a mean short-circuit current of 94·1 ± 10·4 $\mu A/cm^2$, a mean transepithelial potential difference of 14·8 ± 1·9 mV (blood side positive), and a mean d.c. resistance of 169·0 ± 14·0 $\Omega \cdot cm^2$ (mean ± S.E. of mean; $n = 20$). Isotope flux studies across these short-circuited epithelia revealed a net Cl^- blood side to freshwater side flux of 95·2 ± 16·1 $\mu A/cm^2$ and no significant net flux of Na^+.

7. The opercular epithelia of 200 % seawater-adapted *F. heteroclitus*, when bathed on both sides with Ringer, displayed a mean short-circuit current of 33·5 ± 8·5 $\mu A/cm^2$, a mean transepithelial potential difference of 10·5 ± 2·5 mV (blood side positive), and a mean transepithelial d.c. resistance of 440·7 ± 62·6 $\Omega \cdot cm^2$ (mean ± S.E. of mean $n = 18$). Isotope flux studies across these short-circuited epithelia revealed a net Cl^- blood side to seawater side flux of 96·2 ± 51·5 $\mu A/cm^2$ and a net Na^+ blood side to seawater side flux of 65·3 ± 28·6 $\mu A/cm^2$.

INTRODUCTION

Marine teleosts are continually confronted with the problem of maintaining an internal salt concentration far below that of their external seawater environment. This problem is even more pronounced in those teleosts inhabiting waters with salinities greater than seawater (Maetz, 1971). Their ability to maintain this concentration difference depends primarily on their extrarenal osmoregulatory mechanisms, particularly those of the gills. Freshwater teleosts, on the other hand, have the problem of maintaining an internal salt concentration far above that of their external environment. Although the kidneys of freshwater teleosts play a major

osmoregulatory role, considerable attention had been given to their gills because they are supplementary sites of salt and water regulation. In the past, methods employed to study these extrarenal salt-transporting mechanisms in teleosts consisted of two experimental approaches: the intact fish and the isolated, perfused gill. These two preparations have revealed many insights into teleost osmoregulation but 'the study of gill transport phenomena is dominated by frustration because of the difficulty of obtaining clear thermodynamic data' (Maetz, 1971; Maetz & Bornancin, 1975). The short-circuit current technique of Ussing & Zerahn (1951), commonly employed in the study of ion transport across epithelia, can provide some of this thermodynamic data but has never been applied to the teleost gill because of the complex histology of this tissue.

Since the initial description by Keys & Willmer (1932), gill chloride cells have been assumed to play a primary osmoregulatory role in seawater teleosts. The definitive proof of this assumption is lacking but an abundance of histological data (reviewed by Conte, 1969) and biochemical data (reviewed by Maetz & Bornancin, 1975) strongly suggest that these cells are actively engaged in ion transport. The opercular epithelium lining the inside of the gill chamber of the teleost *F. heteroclitus* contains an abundance of chloride cells (Burns & Copeland, 1950; Karnaky & Kinter, 1977) identical in fine structure to the chloride cells of the gill epithelium (Karnaky & Kinter, 1977). In a preliminary report, Karnaky, Degnan & Zadunaisky (1977) mounted the opercular epithelium of 100% seawater-adapted *F. heteroclitus* as a flat sheet between two halves of a Lucite chamber and demonstrated that the measured short-circuit current was equivalent to the net active secretion of Cl^- ions from the blood side to the seawater side of this epithelium. This third alternative approach to the study of teleost osmoregulation, the isolated, short-circuited opercular epithelium of *F. heteroclitus*, avoids many of the technical problems involved in intact fish and isolated, perfused gill preparations and can provide some of the needed biophysical data required for a better understanding of teleost osmoregulation. The so-called 'laboratory diuresis' and other complicating systemic influences are eliminated with the isolated opercular epithelium preparation. The question of adequate perfusion of the gill vasculature and the presence of unstirred layers surrounding the intricate gill structure are also eliminated. Finally, the passive electrical and chemical forces can be nullified and the isotope fluxes in both directions accurately measured with the isolated opercular epithelium preparation thus providing a better thermodynamic approach to teleost osmoregulation, particularly Cl^- cell function.

Since the killifish, *F. heteroclitus*, is euryhaline and will adapt to a wide range of salinities from freshwater to 200% sea water (Karnaky, Kinter,

Kinter & Stirling, 1976), this epithelium can serve as a model to study comparative aspects of extrarenal ion transport mechanisms in euryhaline teleosts. This paper describes our findings on the opercular epithelium of 100 % seawater-adapted *F. heteroclitus* and some comparisons to epithelia of freshwater-adapted and 200 % seawater-adapted *F. heteroclitus*.

METHODS

Adaptation of the fish and the preparation of the isolated opercular epithelium

Specimens of the euryhaline killifish, *F. heteroclitus*, weighing 4–14 g, were collected from the estuaries near Mt. Desert Island Biological Laboratory during the months of April to October and adapted for a minimum of 4 weeks to either 100 % artificial seawater (SW), freshwater (FW), or 200 % artificial seawater (2SW) by the technique described by Karnaky *et al.* (1976). The FW fish were adapted and maintained in local pond water which contained Na^+ 5 m-equiv/l., K^+ 0·5 m-equiv/l., Cl^- < 0·5 m-equiv/l. The experiments were performed primarily during the months of May and July with a few additional experiments performed during November. The fish were sacrificed by pithing and decapitation. The head was then bisected and each half placed in a petri dish in which a layer of hardened Sylgard (Dow Corning Corp., Midland, Mich.) had been deposited and the dish filled with Ringer. The head was then pinned to the Sylgard and the opercular epithelium exposed by cutting away the gills and the branchiostegal rays of the operculum. With the aid of a dissecting microscope the epithelium was freed from the underlying bony operculum by gently teasing it away with microforceps (Dumont no. 5). The epithelium was then floated onto a piece of X-ray film and positioned across a small centrally located aperture. This film with the tissue was then secured in a special Lucite holder and the holder placed between two halves of a Lucite chamber. This chamber was designed specifically for small area tissues of the eye and has been described in detail elsewhere (Zadunaisky & Degnan, 1976). Briefly, the area of exposed tissue was 0·07 cm^2 and the volume of each chamber half was 2·5 ml. Fluid circulation and gassing were accomplished with a bubble lift. Each fish yielded two isolated opercular epithelia which were mounted in matching chambers within 20 min from the initial pithing.

Composition and gassing of the Ringer used to bath the isolated opercular epithelium

The solution used to bathe both sides of the opercular epithelium when mounted in the chamber was a modified version of that described by Forster (1948). It consisted of the following (in m-equiv/l.): Na^+, 151·0; K^+, 2·5; Mg^{2+}, 1·0; Ca^{2+}, 1·5; Cl^-, 142·5; HCO_3^-, 16·0. Glucose and Trizma base were added to give final concentrations of 5 and 20 mM respectively and the pH adjusted to 8·25 with dilute HCl. The final Na^+ and Cl^- concentrations of all solutions were determined with a flame photometer (G. K. Turner Assoc., Rochester, N.Y.) and chloridometer (Buchler Instruments, Inc., Fort Lee, N.Y.) respectively. The experiments were performed at room temperature (22–24° C) and the solutions gassed with either 100 % O_2 (pH 8·25) or with 95 % O_2/5 % CO_2 (pH 7·25). In the anoxia experiments the 100 % O_2 was replaced with 100 % N_2 on both sides of the chamber. In the Cl^- substitution experiments, Cl^- was replaced with equimolar amounts of the methylsulphate anion and the pH adjusted with H_2SO_4. In the Na^+ substitution experiments, Na^+ was replaced with equimolar amounts of the choline cation and the pH adjusted with HCl.

Electrical measurements

In all instances any electrical potential asymmetries between the recording electrodes were nullified with a bucking current and the fluid resistances compensated for before mounting the epithelia in the chambers. At the termination of each experiment these parameters were checked to ascertain any significant changes. Drifts greater than $\pm$ 0·5 mV were considered significant and the data from these experiments were not included in the results presented in this paper.

The potential difference measurements were made through polyethylene-agar Ringer bridges connecting each side of the chamber to a calomel electrode (Beckman Instruments Inc., Fullerton, Calif.) and displayed on the digital voltmeter of the short-circuiting unit. The short-circuiting was accomplished with automatic voltage clamp units connected to each side of the chamber via polyethylene-agar Ringer bridges and the current output continuously recorded on a chart recorder. Preliminary studies with these isolated opercular epithelia showed that the voltage deflexion across these preparations varied linearly with the applied current, indicating that these tissues behaved like ohmic resistors. The transepithelial d.c. resistance (R) was therefore taken as the ratio of the open-circuited potential difference (p.d.) and the applied short-circuit (I_{sc}) and expressed as $\Omega.cm^2$. The preparations were kept short-circuited throughout the time course of most of the experiments except for a few seconds every 30 min to record the potential difference and to calculate the d.c. resistance.

Isotope flux studies

$^{22}Na^+$ and $^{36}Cl^-$ were obtained from New England Nuclear, Boston, Mass., as $^{22}NaCl$ and $H^{36}Cl$ solutions respectively. They were diluted into 1 or 2 ml. Ringer, titrated to pH 8·25 with NaOH, and made isotonic again to Ringer by adding the appropriate amounts of NaCl. Aliquots (25 or 50 μl.) of these isotope stock solutions, containing 5 μc, were added to the Ringer bathing one side of the epithelium, after an equivalent volume was removed and used to determine the background, and the system allowed to equilibrate for 1 h to insure a linear flux of isotope during the cold side sampling. After this initial hour, 100 μl. samples of Ringer were taken from the cold side every 30 min and 25 μl. samples taken from the hot side every 60 min with constriction pipettes (H. E. Pedersen, Denmark). These samples were dissolved in a scintillation cocktail (Yorktown Research, Hackensack, N.J.) and counted in a liquid scintillation counter (Searle, Mark II) for 10 min each. Samples taken from the cold side were replaced with equal volumes of non-isotopic Ringer to keep the fluid volume constant on this side of the chamber. Isotope efflux (blood side to seawater side) and influx (seawater side to blood side) studies were performed on paired epithelia from the same fish mounted in matching chambers. The average unidirectional fluxes were determined from a minimum of four and a maximum of six half-hour flux periods. The net flux was taken as the difference between the two average unidirectional fluxes of the paired preparations.

Ion substitution and pharmacological experiments

In the Na^+ and Cl^- substitution experiments the epithelia were mounted in the chambers as described and bathed on both sides with the normal Ringer until the I_{sc} or potential difference reached some steady-state level. The chambers were then rinsed several times with the ion-free Ringer until the electrical parameter under study ceased to decline with successive washings. The Na^+ or Cl^- was then titrated to both sides of the chamber by removing aliquots of this ion-free Ringer and replacing

them with equal volumes of the normal Ringer. The final step in each titration experiment was the removal of all the fluid in each chamber half and the introduction of the normal Ringer. In some experiments, after establishing a steady-state in the ion-free Ringer, the ion was replaced by evacuating the chambers and reintroducing the normal Ringer.

The pharmacological experiments were carried out by making up stock solutions of the drugs to be tested in Ringer and readjusting the pH to that of the Ringer when necessary. Small aliquots (10–100 μl.) of the Ringer bathing both sides of the epithelium were removed and replaced with equal volumes of the drug stock which, when diluted into the 2·5 ml. bathing the epithelium, gave the concentration desired.

Calculations

The electrical parameters and isotope fluxes reported are those for steady-state conditions only. The average I_{sc} for any control or experimental period was calculated from 5 min readings and the average potential difference from 30 min readings. The transepithelial d.c. resistance in virtually all experiments increased steadily throughout the course of the experiments at a rate of $< 10\%$ per hour and the average d.c. resistance for any period was taken as the ratio of the average potential difference and the average I_{sc}. The isotope flux calculations contained corrections for background and the successive removal of isotope from the system with each cold side sample. Volume corrections were eliminated by replacing the 100 μl. cold side sample volumes with equal volumes of non-isotopic Ringer. Data were handled by a digital computer (Digital Equipment Corp., Maynard, Mass.) and the results expressed as the mean $\pm$ the standard error of the mean. The number of experiments is given in parentheses and statistical significance was taken at the level $P < 0{\cdot}01$.

Preparation of the isolated opercular epithelium for morphological investigation

Opercular epithelia from SW-adapted fish were mounted in chambers as described and the I_{sc} and potential difference allowed to reach steady-state levels (45–60 min). The tissues were fixed in the chambers by removing aliquots of the Ringer bathing both sides of the epithelia and replacing them with equal volumes of concentrated (50 or 70 %) glutaraldehyde which, when diluted into the total fluid volume, gave a final concentration of 6 % glutaraldehyde. With this introduction of glutaraldehyde the I_{sc} and potential difference across the epithelia were reduced to zero values within 5 min and the tissues allowed to fix under these conditions for 60 min. The tissues were then rinsed 6 times with the normal Ringer over the next 30 min. They were then removed from the chambers, rinsed in 0·2 M cacodylate buffer (Gomori, 1955) at 0–5° C, and post-fixed for 90 min in 1 % OsO_4 in 0·2 M cacodylate buffer (pH 7·4) at 0–5° C. After three quick rinses in tap water at 0–5° C, the tissues were rapidly dehydrated through increasing concentrations of ethanol and embedded in Spurr low-viscosity epoxy (Spurr, 1969).

Sections for light microscopy (0·8–1·2 μm) were cut with glass knives and stained with methylene blue and Azure II (Richardson, Jarrett & Finke, 1960). Thin sections were cut with a diamond knife, stained for 10 min with 2 % aqueous uranyl acetate adjusted to pH 5 with 1 N-NaOH, washed in distilled H_2O, and counter stained for 10 min with lead citrate (Reynolds, 1963). These sections were examined with a Philips 300 electron microscope.

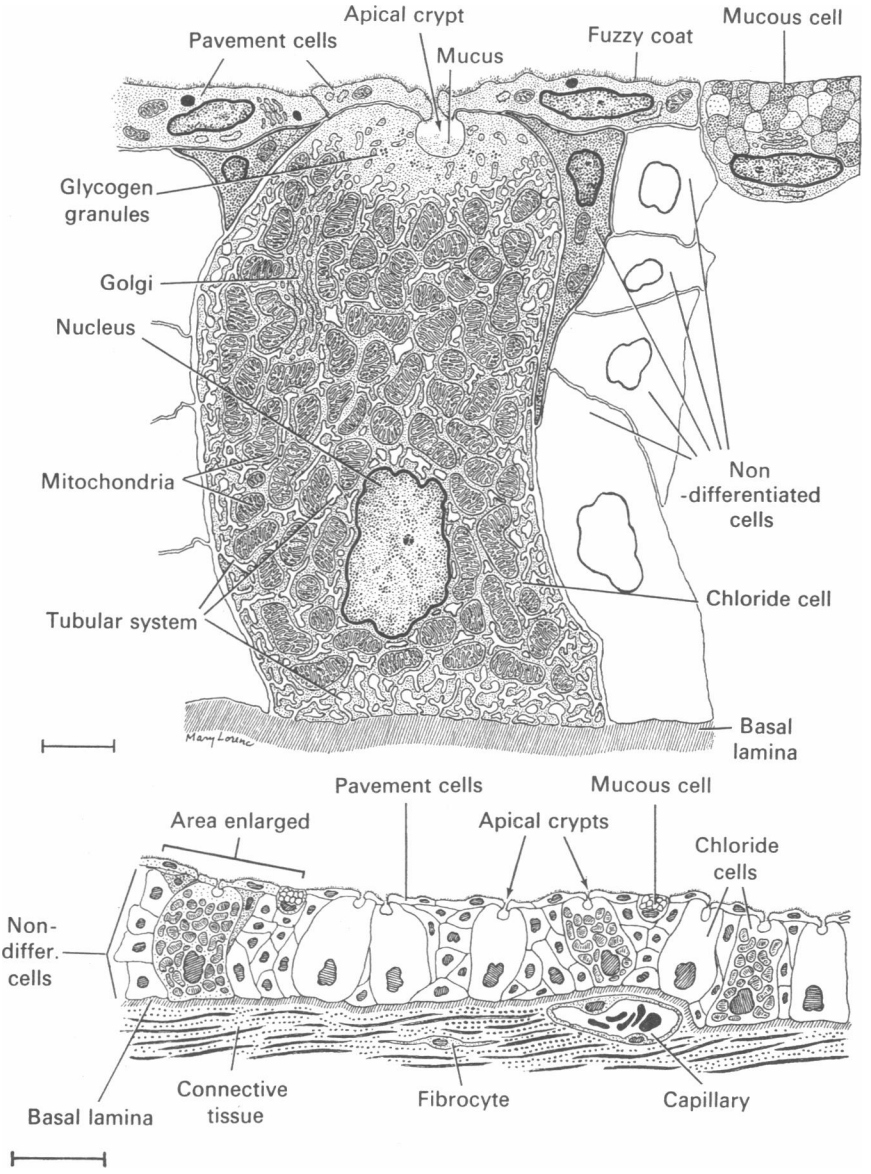
RESULTS

Morphology of the isolated, short-circuited opercular epithelium of 100 % seawater-adapted F. heteroclitus

The morphology of the *F. heteroclitus* opercular skin has been described previously (Burns & Copeland, 1950; Karnaky & Kinter, 1977). Briefly, the skin consists of a stratified epithelium with an underlying layer of connective tissue as illustrated schematically in Text-fig. 1. The stratified epithelium consists of four major cell types (Karnaky & Kinter, 1977): mucous cells, pavement cells, non-differentiated cells, and Cl^- cells. The pavement cells form a continuous external layer except where the mucous and Cl^- cells contact the external environment. The non-differentiated cells comprise the stratification which is interrupted by the Cl^- cells extending from the basal lamina to the external environment.

Karnaky & Kinter (1977) estimated that the Cl^- cells represent 50–70 % of the total cell population of the opercular epithelium of *F. heteroclitus*, and reported an approximate Cl^- cell density of 4×10^5 cells/cm^2. This provided a field of Cl^- cells, oriented in the same direction, across the aperture of the chamber as shown in Pl. 1. These opercular epithelium Cl^- cells (Text-fig. 1) are identical to those of the gill epithelium (Karnaky & Kinter, 1977) and morphological studies on epithelia fixed *in vitro* in the chambers showed no difference from those of SW-adapted fish fixed immediately after removal from the opercular bone. Seawater teleost Cl^- cells exhibit a characteristic apical crypt which is open to the external environment (Philpott & Copeland, 1963). They also contain a rich population of mitochondria and an extensive branching tubular system which has been shown to be continuous with the basal and lateral plasma membranes (Philpott, 1966; Ritch & Philpott, 1969). Surveys of epithelia in the chambers revealed that the apical crypt persisted *in vitro* when these epithelia were bathed on both sides with Ringer. Electronmicrographic surveys of the isolated, short-circuited opercular epithelium (Pl. 2) readily revealed the abundance of mitochondria and the extensive tubular system of the Cl^- cells of this tissue.

Frequently the connective tissue underlying this epithelium was torn away during dissection leaving an intact transparent opercular epithelium. When mounted in the chamber, the epithelial layer exhibited all the electrical, flux and pharmacological properties of the entire skin. This indicated that the epithelial layer with the chloride cells was responsible for the generation of the electrical properties and the active transport of ions.



Text-fig. 1. Above, schematic representation of ultrastructural details of a Cl^- cell from the opercular epithelium of a SW-adapted *F. heteroclitus*. The Cl^- cell is characterized by an apical crypt, a rich population of mitochondria, and a branching tubular system continuous with the basal and lateral plasma membrane. Scale, 2·5 μm. Below, schematic representation of opercular epithelium, the gill-like epithelium used in the present *in vitro* studies. Four major cell types found in the teleost gill, pavement, mucous, non-differentiated, and chloride cells are present in this epithelium but respiratory lamellae are totally absent. Chloride cells extend from the basal lamina to the external environment of the fish. Scale, 20 μm.

Observations on seasonal variations in the electrical parameters across the opercular epithelium of 100 % seawater-adapted F. heteroclitus

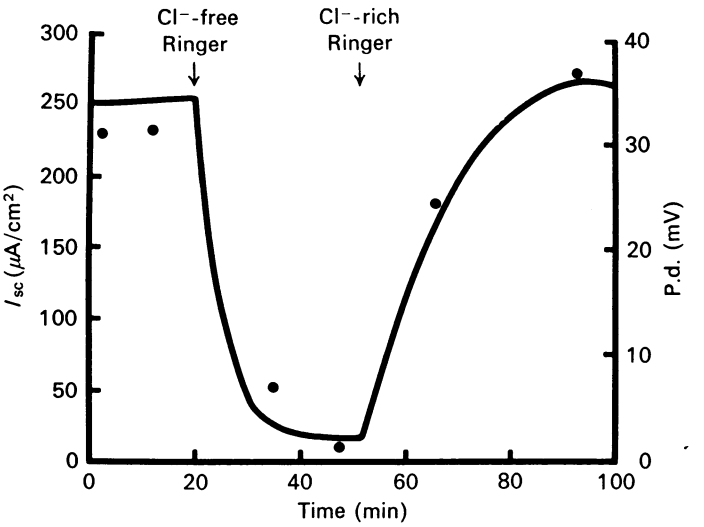
Definite seasonal variations in the electrical parameters across the isolated epithelia of SW-adapted *F. heteroclitus* were observed. In May the I_{sc}s and potenial differences increased to relatively high steady-state levels and remained constant for many hours after mounting in the chambers. In July the I_{sc}s and potential differences displayed initial increases but then decayed at a rate of about 10 %/hr and frequently stabilized at some lower levels. In May the mean I_{sc}, potential difference, and d.c. resistance were $190{\cdot}1 \pm 13{\cdot}1$ μA/cm^2, $24{\cdot}0 \pm 1{\cdot}6$ mV (blood side positive), and $138{\cdot}5 \pm 9{\cdot}2$ $\Omega.\text{cm}^2$ respectively ($n = 32$). In July these means were $75{\cdot}2 \pm 9{\cdot}2$ μA/cm^2, $12{\cdot}2 \pm 1{\cdot}1$ mV (blood side positive), and $213{\cdot}9 \pm 21{\cdot}6$ $\Omega.\text{cm}^2$ respectively ($n = 32$). This represented a decrease of 60·4 and 49·2 % in the mean I_{sc} and potential difference respectively and a 54·4 % increase in the d.c. resistance from May to July. These changes were significant ($P < 0{\cdot}005$) but for neither month was there a significant difference between the mean I_{sc} and the mean net Cl^- secretion ($P > 0{\cdot}60$). The difference in the mean I_{sc} from May to July appeared to reflect a change in the rate of active Cl^- secretion since no significant changes were noted in the Cl^- influx.

July was coincidental with these fishes' breeding period. During this time they displayed a yellow-pigmented abdomen whereas during May their abdomens were white. Most likely there were hormonal changes in these fish from May to July which may have influenced the rate of Cl^- secretion and the d.c. resistance across their opercular epithelia. Since the rate of Cl^- efflux was the only difference in the ion transporting properties of these epithelia, the data were pooled and presented without reference to the month. For all studies ($n = 64$), the mean I_{sc} was $136{\cdot}5 \pm 11{\cdot}1$ μA/cm^2, the mean potential difference was $18{\cdot}7 \pm 1{\cdot}2$ mV (blood side positive), and the mean transepithelial d.c. resistance was $173{\cdot}7 \pm 12{\cdot}1$ $\Omega.\text{cm}^2$. The opercular epithelia studied in November ($n = 8$) resembled more closely those of May than those of July.

The relationships between the [Cl^-] and the [Na^+] and the electrical properties of the isolated opercular epithelium of 100 % seawater-adapted F. heteroclitus

When the Cl^- in the Ringer bathing both sides of the opercular epithelium of SW-adapted *F. heteroclitus*, gassed with 100 % O_2, was replaced with equimolar amounts of methylsulphate anions, the I_{sc} and potential difference declined to lower steady-state levels near zero within 30 min as illustrated in Text-fig. 2. The d.c. resistance also declined to about 50 % of its control level but these measurements were complicated by the fact that

the I_{sc} or potential difference or both were sometimes reduced to zero. The reintroduction of Cl^- to the bathing solutions quickly reversed these inhibitory effects and stimulated the electrical parameters to near their control levels. The combined data from six Cl^- substitution experiments is presented in Table 2.

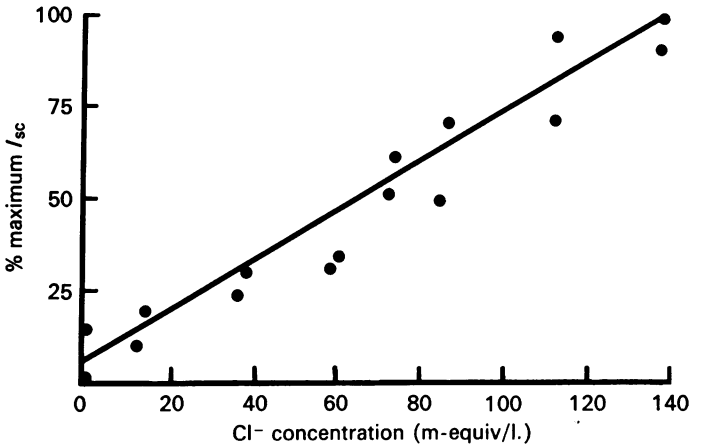


Text-fig. 2. The relationship between the I_{sc} and potential difference across an epithelium from SW-adapted *F. heteroclitus* and the Cl^- ions in the bathing solution. The continuous line represents the continuous I_{sc} recording and the dots represent the spontaneous open-circuited potential difference measurements. This preparation was bathed with Ringer on both sides and gassed with 100 % O_2. The Cl^- was replaced with equimolar amounts of methylsulphate anions on both sides of the epithelium.

The relationship between the Cl^- concentration and the I_{sc} across the opercular epithelium of SW-adapted *F. heteroclitus*, when bathed on both sides with Ringer and gassed with 100 % O_2, is presented in Text-fig. 3. The results of two experiments, the I_{sc} normalized by expressing them as percentages of the maximum I_{sc} obtained for each preparation, were combined and fitted by the method of least squares. The I_{sc} increased linearly with increasing Cl^- concentrations over the entire range (0–142·5 m-equiv/l.) with a slope of 0·65 (correlation coefficient = 0·97). No saturation kinetics for the I_{sc}, frequently seen with transporting epithelia, were observed.

The results of four experiments studying the relationship between the Cl^- concentration and the transepithelial potential difference were combined, fitted by the method of least squares, and presented in Text-fig. 4*A*. With

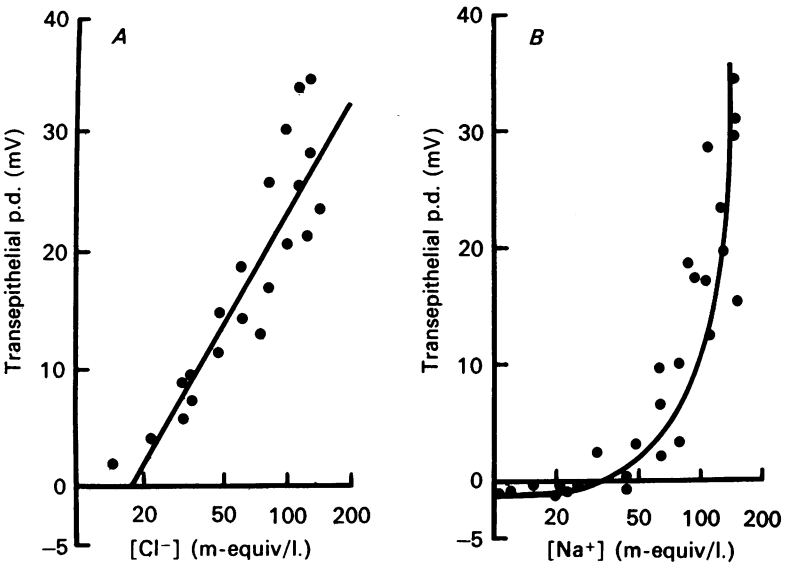
each successive Cl^- titration the potential difference increased to some new steady-state level which was taken as the representative potential difference for that particular Cl^- concentration. In no instance was there a potential difference reversal across the epithelia when bathed on both sides with Cl^- free Ringer. The transepithelial potential difference increased linearly with the log of the Cl^- concentration with a slope of $28{\cdot}3 \pm 2{\cdot}1$ mV/tenfold $[Cl^-]$ change (correlation coefficient = 0·85). This slope was about half of



Text-fig. 3. The relationship between the I_{sc} and the Cl^- concentration across epithelia from SW-adapted *F. heteroclitus*. The epithelia were bathed on both sides with Ringer and gassed with 100 % O_2. Cl^- was replaced with equimolar amounts of methylsulphate anions. The I_{sc}s were normalized by expressing them as percentages of the maximum I_{sc} observed for each preparation.

the theoretical Nernst slope of 58·0 for a pure Cl^- electrode, indicating that this tissue had a substantial permeability to other ion(s). The results of five experiments studying the relationship between the Na^+ concentration and the transepithelial potential difference were combined and presented in Text-fig. 4*B*. The replacement of Na^+ with choline cations in the Ringer bathing both sides of the opercular epithelium caused the potential difference to reverse its orientation in every instance. This reversal ranged from 0·8 to 3·7 mV with a mean of $1{\cdot}8 \pm 0{\cdot}5$ mV. Increasing the ambient Na^+ concentration initially had no effect on the potential difference until a concentration of 8–10 m-equiv/l. was reached. At this point, increasing the Na^+ concentration caused a gradual depolarization which reached zero around 25 m-equiv/l. Further increases in the Na^+ concentration caused larger, non-linear increases in the transepithelial potential difference.

When epithelia of SW-adapted fish were bathed externally with seawater and internally with Ringer, the potential difference increased keeping the same orientation of blood side positive. When bathed externally with 0·1 Ringer and internally with Ringer the potential difference reversed its orientation to blood side negative. These observations suggested that these



Text-fig. 4. *A*, the relationship between the transepithelial potential difference and the Cl^- concentration of the bathing solutions. *B*, the relationship between the transepithelial potential difference and the Na^+ concentration of the bathing solutions. The epithelia were from SW-adapted *F. heteroclitus* bathed on both sides with Ringer and gassed with 100 % O_2. Cl^- was replaced with equimolar amounts of methylsulphate anions and Na^+ was replaced with equimolar amounts of choline cations in the Ringer on both sides of the epithelia.

epithelia were considerably permeable to cations, most likely Na^+. In addition to these observations, it was noted that Na^+ were necessary to maintain the I_{sc} across these epithelia. In the absence of Na^+ the I_{sc} was completely abolished and gradually returned as Na^+ was reintroduced into the bathing solutions.

The fluxes of $^{36}Cl^-$ and $^{22}Na^+$ across the isolated, short-circuited opercular epithelium of 100 % seawater-adapted F. heteroclitus

The results of paired isotope flux experiments across short-circuited opercular epithelia from SW-adapted *F. heteroclitus*, when bathed on both sides with Ringer and gassed with either 100 % O_2 or 95 % O_2/5 % CO_2,

TABLE 1. The $^{36}Cl^-$ and $^{22}Na^+$ fluxes and the mean electrical properties across isolated, short-circuited opercular epithelia of SW-adapted *F. heteroclitus* bathed on both sides with Ringer and gassed with either 100% O_2 or 95% O_2/5% CO_2. All experiments were perfomed as influx and efflux pairs on pieces of epithelia from the same fish. The results are expressed as mean ± S.E. of mean and the number of paired experiments given in parentheses. J_i = flux of the isotope in units of mass; I_i flux of the isotope in units of current; I_{sc} = the short-circuit current; p.d. = the transepithelial potential difference; R = the transepithelial d.c. resistance

Flux direction	J_i (μequiv. $cm^{-2}.hr^{-1}$)	I_i ($\mu A/cm^2$)	I_{sc} ($\mu A/cm^2$)	P.d. (mV)	R ($\Omega.cm^2$)	$[HCO_3^-]$ (mM)	Gassing
$^{36}Cl^-$ efflux (10)	7·90 ± 1·00	211·7 ± 27·1	166·7 ± 24·7	20·8 ± 2·8	136·6 ± 15·3	16	100% O_2
$^{36}Cl^-$ influx (10)	1·83 ± 0·37	48·9 ± 10·0	150·5 ± 22·2	18·0 ± 2·5	131·9 ± 14·9	16	100% O_2
$^{36}Cl^-$ net flux	6·07 ± 1·15	162·8 ± 30·9					
Mean electrical properties (20)			158·6 ± 16·3	19·4 ± 1·9	134·2 ± 10·4		
$^{22}Na^+$ efflux (10)	1·20 ± 0·12	32·2 ± 3·3	61·8 ± 13·2	11·8 ± 1·6	257·2 ± 44·0	16	100% O_2
$^{22}Na^+$ influx (10)	1·30 ± 0·15	34·8 ± 4·1	52·0 ± 9·4	12·2 ± 2·3	244·9 ± 27·2	16	100% O_2
$^{22}Na^+$ net flux	−0·10 ± 0·17	−2·6 ± 4·6					
Mean electrical properties (20)			56·9 ± 8·0	12·0 ± 1·4	251·0 ± 25·2		
$^{36}Cl^-$ efflux (8)	7·32 ± 2·13	196·3 ± 57·2	130·1 ± 24·4	14·1 ± 1·5	192·6 ± 49·0	16	95% O_2/5% CO_2
$^{36}Cl^-$ influx (8)	2·86 ± 1·13	76·6 ± 30·2	108·4 ± 40·3	11·1 ± 1·9	147·9 ± 31·5	16	95% O_2/5% CO_2
$^{36}Cl^-$ net flux	4·46 ± 1·09	119·6 ± 29·3					
Mean electrical properties (16)			119·2 ± 22·9	12·6 ± 1·2	170·2 ± 28·7		
$^{22}Na^+$ efflux (8)	2·63 ± 0·45	70·4 ± 12·0	76·4 ± 17·8	10·3 ± 1·5	156·1 ± 14·2	16	95% O_2/5% CO_2
$^{22}Na^+$ influx (8)	2·95 ± 0·26	79·0 ± 7·0	72·4 ± 11·8	9·9 ± 1·6	147·4 ± 18·5	16	95% O_2/5% CO_2
$^{22}Na^+$ net flux	−0·32 ± 0·62	−8·6 ± 16·5					
Mean electrical properties (16)			74·4 ± 10·3	10·1 ± 1·5	151·8 ± 11·3		

are presented in Table 1. When gassed with 100 % O_2 the Cl^- effluxes ranged from 3·91 to 14·50 μequiv.cm^{-2}.hr^{-1} and the Cl^- influxes ranged from 0·63 to 3·90 μequiv.cm^{-2}.hr^{-1}. The mean Cl^- efflux was 4·3 times greater than the mean Cl^- influx resulting in a net Cl^- efflux of 6·07 μequiv.cm^{-2}.hr^{-1} (162·8 μA/cm^2) which was not statistically different ($P > 0·70$) than the mean I_{sc} of 158·6 μA/cm^2 for these $^{36}Cl^-$ flux experiments. The mean I_{sc} accounted for 97·4 % of the net Cl^- efflux indicating that the I_{sc} almost completely reflected the electrogenic Cl^- transport across these epithelia. The Na^+ effluxes ranged from 0·35 to 1·65 μequiv.cm^{-2}.hr^{-1} and the Na^+ influxes ranged from 0·65 to 2·08 μequiv.cm^{-2}.hr^{-1}. The mean unidirectional Na^+ fluxes of 1·20 and 1·30 μequiv.cm^{-2}.hr^{-1} were not statistically different from each other ($P > 0·20$), indicating no net movement of this cation across these epithelia. The differences in the mean electrical properties of the epithelia for the $^{36}Cl^-$ and $^{22}Na^+$ flux studies resulted from the fact that most of the $^{36}Cl^-$ experiments were performed in May while most of the $^{22}Na^+$ experiments were performed in July.

When gassed with 95 % O_2/5 % CO_2 the Cl^- effluxes ranged from 2·50 to 21·23 μequiv.cm^{-2}.hr^{-1} and the Cl^- influxes ranged from 0·36 to 9·71 μequiv.cm^{-2}.hr^{-1}. The mean Cl^- efflux was 2·6 times greater than the mean Cl^- influx, resulting in a net Cl^- efflux of 4·46 μequiv.cm^{-2}.hr^{-1} (119·6 μA/cm^2) which was not statistically different ($P > 0·80$) from the mean I_{sc} of 119·2 μA/cm^2 for these $^{36}Cl^-$ flux experiments. The Na^+ effluxes ranged from 1·52 to 5·31 μequiv.cm^{-2}.hr^{-1} and the Na^+ influxes from 1·85 to 4·01 μequiv.cm^{-2}.hr^{-1} when these epithelia were gassed with 95 % O_2/5 % CO_2. The mean unidirectional Na^+ fluxes of 2·63 and 2·95 μequiv.cm^{-2}.hr^{-1} were not statistically different from each other ($P > 0·30$), indicating that there was no net movement of this cation across these epithelia.

As can be readily seen from Table 1 the isolated, short-circuited opercular epithelium of SW-adapted *F. heteroclitus* when bathed on both sides with Ringer and gassed with either 100 % O_2 or 95 % O_2/5 % CO_2, exhibited a net Cl^- secretion equivalent to the I_{sc} and there was no measurable net movement of Na^+. The mean unidirectional Cl^- influx was 56·7 % higher when these epithelia were gassed with 95 % O_2/5 % CO_2 than when gassed with 100 % O_2 but the mean unidirectional Cl^- effluxes were approximately the same. The mean unidirectional Na^+ efflux and influx were 118·6 and 127·0 % greater respectively when these epithelia were gassed with 95 % O_2/5 % CO_2 than when gassed with 100 % O_2. With both experimental conditions, however, the net Cl^- secretion and the I_{sc} were equivalent, indicating that the active transport of Cl^- was electrogenic and responsible for the measured net movement of charge across the epithelia. This ruled

out the presence of a neutral transepithelial Cl^-/HCO_3^- exchange but not the possibility of a Cl^-/HCO_3^- exchange across one membrane.

The effects of anoxia and some pharmacological agents on the electrical properties across the isolated opercular epithelium of 100 % seawater-adapted F. heteroclitus

The effects of anoxia and certain pharmacological agents, known to influence Cl^- transport, on the electrical properties of the opercular epithelia of SW-adapted *F. heteroclitus* are illustrated in Text-fig. 5 and the combined results for all such experiments are presented in Table 2. As shown in Text-fig. 5*A* substituting 100 % N_2 for the 100 % O_2 gassing both sides of the chamber caused the I_{sc} and potential difference across the epithelium to decline to lower steady-state levels within 30 min which were readily restimulated with the resumption of 100 % O_2 gassing. This effect of anoxia on the potential difference across the opercular epithelium resembled those observed with the isolated, perfused gill of the flounder, *Platichthys flesus*, when bathed on both sides with Ringer (Shuttleworth, Potts & Harris, 1974). These anoxia results on opercular epithelia and flounder gill suggested that the potential difference across these epithelia were dependent on metabolic energy and therefore may not be purely diffusion potentials when bathed on both sides with Ringer.

Ouabain (Sigma Chem. Co., St Louis, Mo.), the classical inhibitor of Na^+, K^+-activated ATPase, has been shown to inhibit active Cl^- transport in a variety of epithelia (Cooperstein, 1959; Zadunaisky, Candia & Charandini, 1963; Candia, 1972; Burg & Green, 1973) and a coupling between Na^+/K^+ exchange and Cl^- transport in the teleost gill has been suggested (Epstein, Maetz & de Renzis, 1973; Pic, Mayer-Gostan & Maetz, 1975). At 10^{-5} M in both bathing solutions (Text-fig. 5*B* and Table 2), ouabain produced a steady and irreversible decline in the I_{sc} and potential difference across the epithelium which reached lower steady-state levels around 5–10 % of their control levels in 60–90 min. Furosemide (Hoechst Pharm. Inc., Somerville, N.J.), a specific inhibitor of Cl^- transport in the kidney (Burg, Stoner, Cardinal & Green, 1973), cornea (Candia, 1973), and red blood cell (Brazy & Gunn, 1976) caused the I_{sc} and potential difference to decline to near zero levels in about 30 min as illustrated in Text-fig. 5*C*. The combined results of six furosemide experiments at 10^{-3} M are presented in Table 2. These inhibitory effects of furosemide were not readily reversed by rinsing the chambers several times with fresh Ringer.

Thiocyanate (Fisher Scientific Co., Fairlawn, N.J.), a known inhibitor of Cl^- transport (Davenport, 1940; Durbin, 1964; Zadunaisky, Lande & Hafner, 1971), had no effect at 10^{-3} M in both bathing solutions. At 10^{-2} M, however, thiocyanate (SCN^-) caused a decline in the I_{sc} and potential

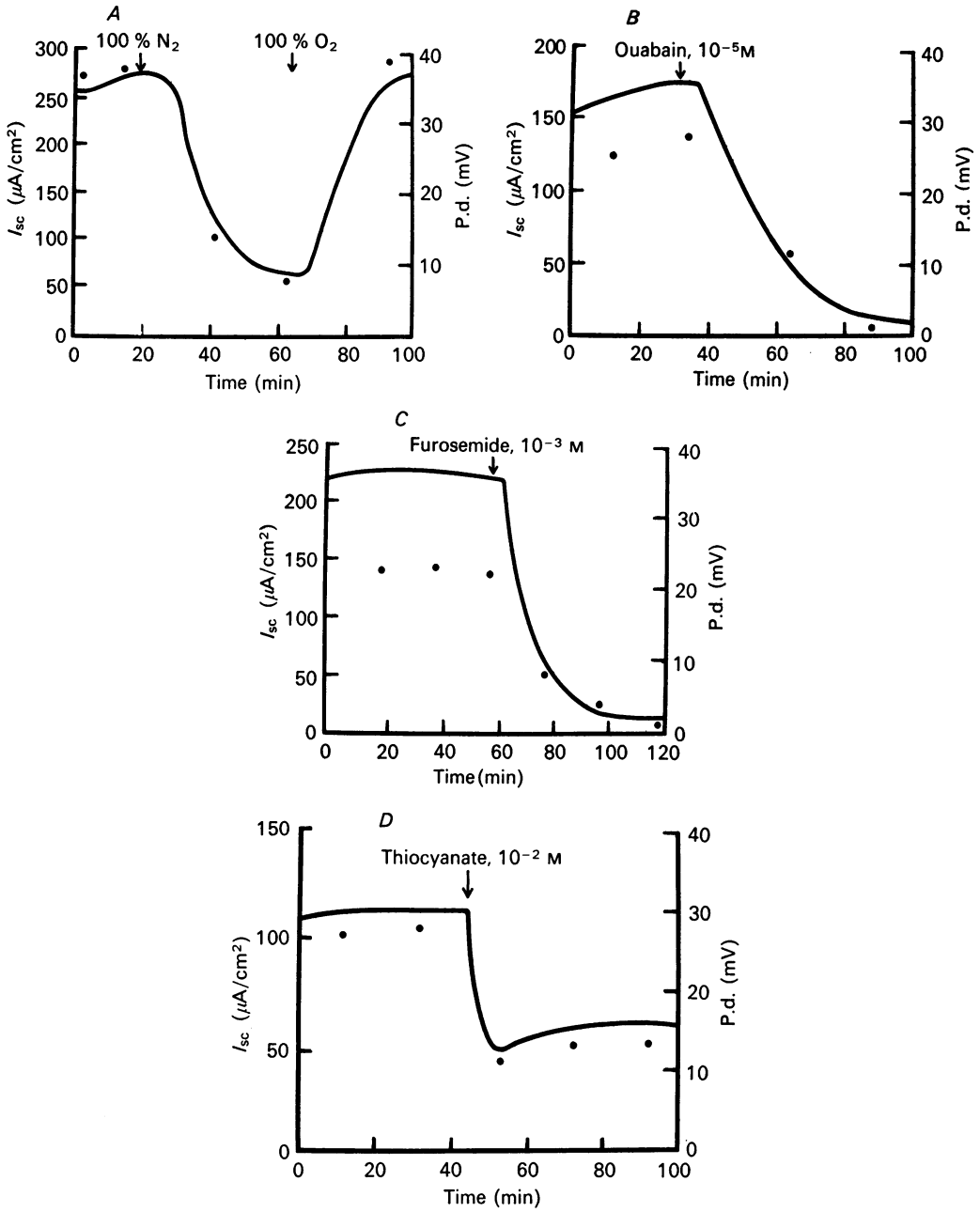
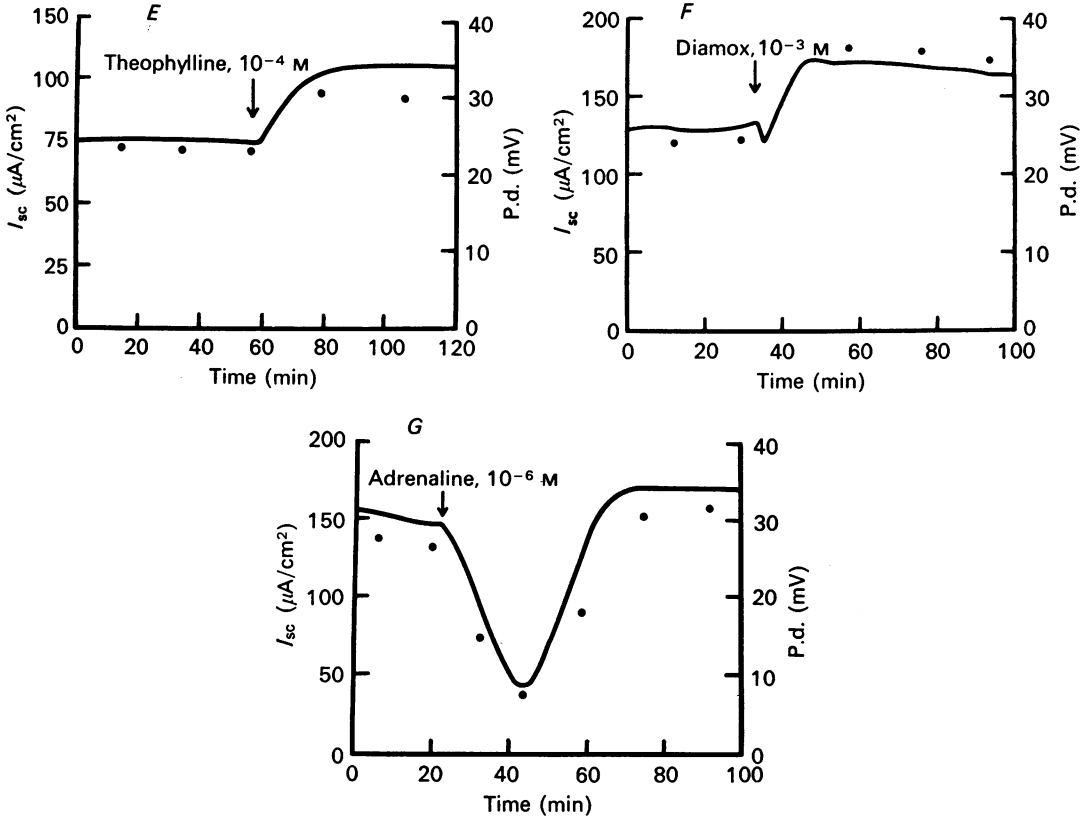


Fig. 5*A-D*. For legend see opposite.



Text-fig. 5. The effects of anoxia and some pharmacological agents on the electrical properties across the isolated opercular epithelium of SW-adapted *F. heteroclitus* bathed on both sides with Ringer and gassed with 100% O_2. The continuous lines are the continuous I_{sc} recordings and the dots are the spontaneous open-circuited potential difference measurements. The drugs were tested by addition to both sides of the epithelia. *A*, anoxia; *B*, 10^{-5} M ouabain; *C*, 10^{-3} M furosemide; *D*, 10^{-2} M thiocyanate; *E*, 10^{-4} M theophylline; *F*, 10^{-3} M Diamox; *G*, 10^{-6} M adrenaline.

difference to about 50% of their control levels within 10 min which was always followed by spontaneous tendencies to return to the control levels. In three experiments only slight recoveries after maximum inhibition were observed as illustrated in Text-fig. 5*D* while in three other experiments these electrical parameters recovered to within 10–20% of their initial control levels within 30–60 min after maximum inhibition. Table 2 lists the maximum inhibition achieved in these six SCN^- experiments. Durbin (1964) demonstrated competitive kinetics between SCN^- and Cl^- for the transport system in the gastric mucosa and SCN^- has been shown to inhibit

TABLE 2. The effects of anoxia and some pharmacological agents on the electrical properties of isolated opercular epithelia of SW-adapted *F. heteroclitus* when bathed on both sides with Ringer. Data are from epithelia gassed with 100 % O_2 except those marked with an asterisk (*) which represent combined data from preparations gassed with 100 % O_2 and with 95 % O_2/5 % CO_2. Results are expressed as the mean ± S.E. of mean and the number of experiments is given in parentheses. Statistical significance was taken at $P < 0.01$

	I_{sc} (μA/cm^2)	P.d. (mV)	R(Ω.cm^2)
Control (6)	234·5 ± 30·5	26·1 ± 4·0	122·7 ± 20·5
Cl-free Ringer	8·8 ± 3·9	1·9 ± 0·6	58·5 ± 40·0
% change	96·3 %	92·7 %	52·3 %
P	< 0·001	< 0·001	> 0·02
Cl-rich Ringer	224·5 ± 27·8	23·5 ± 2·5	121·3 ± 28·9
Control (4)	236·6 ± 4·7	26·3 ± 2·5	110·7 ± 9·8
Anoxia (100 % N_2)	39·6 ± 10·4	8·6 ± 1·7	269·2 ± 74·7
% change	83·3 %	67·3 %	143·2 %
P	< 0·001	< 0·001	> 0·02
Oxygenation (100 % O_2)	233·0 ± 14·7	30·8 ± 2·6	131·9 ± 5·3
Control (7)	126·7 ± 23·6	17·9 ± 2·6	151·9 ± 30·1
Ouabain, 10^{-5} M	9·3 ± 3·4	0·9 ± 0·3	136·6 ± 8·4
% change	92·7 %	95·0 %	10·1 %
P	< 0·01	< 0·005	> 0·01
Control (6)	224·3 ± 39·9	28·1 ± 3·3	149·6 ± 38·2
Furosemide, 10^{-3} M	33·7 ± 11·7	3·7 ± 0·9	157·5 ± 47·5
% change	85·0 %	86·8 %	5·3 %
P	< 0·005	< 0·001	> 0·40
Control (6)	120·6 ± 12·2	21·0 ± 2·3	143·4 ± 21·5
Thiocyanate, 10^{-2} M	66·0 ± 10·4	11·8 ± 1·6	145·4 ± 22·3
% change	45·3 %	43·8 %	1·4 %
P	> 0·05	> 0·30	> 0·60
Control (12)*	79·9 ± 25·8	9·6 ± 1·2	198·0 ± 29·6
Theophylline, 10^{-4} M	123·8 ± 27·6	16·8 ± 1·8	162·6 ± 22·4
% change	54·9 %	75·0 %	17·9 %
P	< 0·001	< 0·001	< 0·005
Control (12)*	77·2 ± 13·2	11·1 ± 1·7	166·9 ± 26·1
Diamox, 10^{-3} M	97·3 ± 13·5	15·2 ± 2·2	185·2 ± 26·1
% change	26·0 %	36·9 %	11·0 %
P	< 0·001	< 0·001	< 0·005
Control (10)*	118·1 ± 9·4	16·7 ± 1·1	150·7 ± 9·3
Adrenaline, 10^{-6} M	44·2 ± 6·5	6·3 ± 1·0	145·8 ± 11·0
% change	62·6 %	62·3 %	3·3 %
P	< 0·01	< 0·01	> 0·50

Cl^- efflux in the eel gill (Epstein *et al.* 1973) and the dogfish rectal gland (Siegel, Silva, Epstein, Maren & Hayslett, 1975). This suggested that SCN^- inhibited the Cl^- efflux across the opercular epithelium and that the spontaneous recoveries of the I_{sc} and potential difference may have resulted from active SCN^- transport by this epithelium. Contrary to these effects of SCN^- on the isolated opercular epithelium, I.P. injections of SCN^- did not inhibit the Cl^- efflux in intact *F. heteroclitus* whereas similar injections reduced the Cl^- efflux by 50% in eels and flounders (Epstein, Silva, Forrest & Solomon, 1975). It was also noted that $^{35}SCN^-$ was secreted against an electrochemical gradient by the eel gill, suggesting active SCN^- transport.

Theophylline (Sigma Chem. Co., St Louis, Mo.), known to stimulate Cl^- transport in the cornea (Chalfie, Neufeld & Zadunaisky, 1972) and the isolated rectal gland of the dogfish (Silva, Stoff, Field, Stevens, Forrest & Epstein, 1975), caused variable stimulations in the I_{sc} and potential difference which reached maximum levels within 30 min as illustrated in Text-fig. 5*E*. These stimulatory effects of theophylline were variable, ranging from very slight to a doubling of these parameters, and in every instance there was a lowering of the d.c. resistance (Table 2). Diamox (Lederle Labs., Pearl River, N.Y.), an inhibitor of carbonic anhydrase, at 10^{-3} M in both bathing solutions produced slight stimulations in the I_{sc} and potential difference as illustrated in Text-fig. 5*F*. These changes were significant ($P < 0{\cdot}001$; Table 2) but subject to question since such high doses of this inhibitor may have produced generalized cellular effects unrelated to carbonic anhydrase activity. A carbonic anhydrase-mediated Cl^-/HCO_3^- exchange operating across the basal-lateral membranes of the gill chloride cell of SW-adapted fish has been proposed by Maetz (1971). In the present opercular epithelium studies, stock solutions of Diamox were prepared in Ringer and the pH adjusted with H_2SO_4 to avoid any stimulation of the I_{sc} by an increased Cl^- concentration. No inhibition was observed even with a few experiments at 10^{-2} M Diamox, indicating that this proposed Cl^-/HCO_3^- exchange was probably not involved in the electrogenic Cl^- transport. There was no explanation for the slight stimulatory effects of Diamox but the point to emphasize was that there was no inhibition with this carbonic anhydrase inhibitor.

Adrenaline (Sigma Chem. Co., St Louis, Mo.) at a concentration of 10^{-6} M in both bathing solutions produced rapid and transitory decreases in the I_{sc} and potential difference as illustrated in Text-fig. 5*G*. At 10^{-5} M and higher concentrations adrenaline rapidly abolished the I_{sc} and potential difference which remained at near zero levels, with no reversal, for varying times depending on the initial dose of this adrenergic agent. Table 2 lists the maximum inhibition produced in 10 experiments with 10^{-6} M adrenaline.

In all instances the I_{sc} and potential difference returned to control levels slightly greater (10–20 %) than their initial control levels. These inhibitory effects of adrenaline were contrary to that seen on Cl^- transport in the cornea (Chalfie *et al.* 1972) but similar to that seen on Cl^- secretion across the gill of the SW-adapted mullet, *Mugil capito* (Pic *et al.* 1975).

Amiloride (Merck, Sharp & Dohme, West Point, Pa.) and amphotericin B (E. R. Squibb & Sons, Princeton, N.J.) were also tested on the opercular epithelium by addition to both sides of the chamber. Amiloride at 10^{-5} M ($n = 4$) and 10^{-4} M ($n = 2$) produced very small ($< 10\ \mu A/cm^2$) decreases in the I_{sc} which were insignificant when compared to the 100–200 $\mu A/cm^2$ across these epithelia. Amphotericin B, at 10^{-5} M ($n = 5$) appeared to have no effect on the I_{sc}. Amiloride is a potent inhibitor of active Na^+ transport (Bentley, 1968) with no apparent influence on active Cl^- transport (Kirschner, Greenwald & Kerstetter, 1973; Degnan & Zadunaisky, 1977), and amphotericin B is a stimulant of Na^+ transport (Lichtenstein & Leaf, 1965). The small decrease in the I_{sc} caused by amiloride could have resulted from a reduction in the Na^+ influx similar to the effect of amiloride on the isolated, perfused gill of the FW-adapted trout (Greenwald & Kirschner, 1976) or the intact SW-adapted sailfin molly (Evans, 1975). An increase in the Na^+ efflux, observed when amiloride was applied to SW-adapted eels (Cuthbert & Maetz, 1972), could also account for the small reduction in the I_{sc} across the opercular epithelium.

The effects of certain pharmacological agents on the $^{36}Cl^-$ and $^{22}Na^+$ fluxes across the isolated, short-circuited opercular epithelium of 100 % seawater-adapted F. heteroclitus

A few of the pharmacological agents listed in Table 2 were tested on the isotope fluxes across the opercular epithelium of SW-adapted *F. heteroclitus* when bathed on both sides with Ringer, short-circuited, and gassed with 100 % O_2. The results of these experiments are listed in Table 3. 10^{-4} M theophylline stimulated the Cl^- efflux 48·5 % while causing a 49·4 % decrease in the Cl^- influx. The overall effect of theophylline was a 104·0 % increase in the net Cl^- secretion and a 60·5 % increase in the I_{sc}. Theophylline also produced a 32·8 % stimulation of the Na^+ efflux while having no apparent effect on the Na^+ influx. It appeared from these experiments that theophylline stimulated primarily the Cl^- efflux and that the increased I_{sc} resulted from this stimulation. In numerous other experiments with theophylline large fluxes of both $^{36}Cl^-$ and $^{22}Na^+$ in both directions across the epithelia were noted while a stimulation of the I_{sc} was always produced. This was interpreted as theophylline causing large passive leaks across the epithelia. The data presented in Table 3 are from those experiments in which these leaks were relatively small.

TABLE 3. The effect of a few pharmacological agents on the $^{36}Cl^-$ and $^{22}Na^+$ undirectional fluxes across isolated short-circuited opercular epithelia of SW-adapted *F. heteroclitus* when bathed on both sides with Ringer and gassed with 100% O_2. The drugs were tested by addition to both sides of the epithelia. Results are expressed as mean ± S.E. of mean and the number of experiments is given in parentheses.

	Efflux (μequiv.cm^{-2}.hr^{-1})	Influx (μequiv.cm^{-2}.hr^{-1})	Net flux (μequiv.cm^{-2}.hr^{-1})	Net flux (μA/cm^2)	Average I_{sc} (μA/cm^2)
			$^{36}Cl^-$ fluxes		
Control (3)	4·83 ± 0·69	1·75 ± 0·26	3·08 ± 0·73	82·6 ± 19·6	93·5 ± 9·7
Theophylline, 10^{-4} M	7·17 ± 0·29	0·88 ± 0·37	6·29 ± 0·31	168·8 ± 8·1	150·1 ± 7·2
% change	48·5%	49·4%	104·0%	104·4%	60·5%
Control (3)	4·05 ± 0·85	1·05 ± 0·60	3·00 ± 0·59	80·4 ± 15·9	87·6 ± 15·6
Diamox, 10^{-3} M	4·46 ± 0·97	1·12 ± 0·38	3·34 ± 0·65	89·5 ± 17·4	101·7 ± 14·1
% change	10·3%	7·0%	11·4%	11·4%	16·1%
Control (3)	4·96 ± 0·53	0·47 ± 0·12	4·49 ± 0·57	120·3 ± 15·2	122·1 ± 14·3
Adrenaline, 10^{-6} M	3·04 ± 0·45	0·44 ± 0·17	2·60 ± 0·53	69·6 ± 14·1	67·6 ± 9·6
% change	38·8%	6·4%	42·2%	42·1%	43·0%
			$^{22}Na^+$ fluxes		
Control (4)	1·14 ± 0·35	1·26 ± 0·40	−0·12 ± 0·03	−3·5 ± 0·9	75·1 ± 12·0
Theophylline, 10^{-4} M	1·51 ± 0·33	1·25 ± 0·60	0·27 ± 0·06	7·2 ± 1·7	116·0 ± 11·9
% change	32·8%	1·2%	321·5%	305·7%	54·5%
Control (5)	2·41 ± 0·30	1·91 ± 0·15	0·50 ± 0·36	13·4 ± 9·8	121·9 ± 20·3
Ouabain, 10^{-6} M	1·50 ± 0·28	1·77 ± 0·12	−0·27 ± 0·18	−7·3 ± 5·0	77·1 ± 26·6
% change	37·7%	7·4%	154·0%	156·7%	36·8%

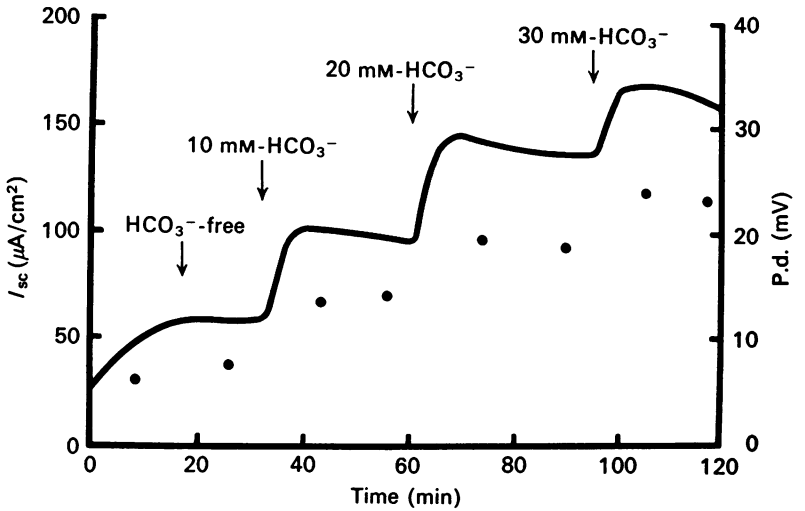
10^{-3} M Diamox produced a 10·3 % increase in the Cl^- efflux and a 7·0 % increase in the Cl^- influx. This resulted in an 11·4 % increase in the net Cl^- secretion which was close to the 16·1 % increase in the I_{sc}. These experiments were done to ascertain whether Diamox had any inhibitory effect on Cl^- transport. The negative results confirmed the suggestion that no carbonic anhydrase-mediated Cl^-/HCO_3^- exchange contributed to Cl^- secretion across this epithelium. 10^{-6} M adrenaline produced a 42·2 % decrease in the net Cl^- secretion while causing a 43·0 % inhibition in the I_{sc}. This resulted mostly from the 38·8 % decrease in the Cl^- efflux. There was essentially no change in the Cl^- influx during the periods of adrenaline inhibition.

Ouabain, at 10^{-5} M in both bathing solutions, produced large fluxes of isotope in both directions across the epithelia, indicating that this drug also produced transepithelial leaks. However, at 10^{-6} M, ouabain does not produce leaks for at least 2 hr after introduction into the bathing solutions while it does produce a slow and gradual decrease in the I_{sc}. At 10^{-6} M, ouabain caused a 37·7 % inhibition in the Na^+ efflux while having no substantial effect on the Na^+ influx. In these particular experiments this inhibition resulted in a reversal of the small net Na^+ movement. It is interesting to note that the 37·7 % inhibition in the Na^+ efflux was similar to the 36·8 % inhibition in the I_{sc} caused by ouabain. These results suggested that at least part of the Na^+ efflux across these epithelia was dependent on Na^+, K^+-activated ATPase while the Na^+ influx was not dependent on this enzyme and most likely passive.

The influence of HCO_3^- on the electrical properties across the isolated opercular epithelium of 100 % seawater-adapted F. heteroclitus

During these studies it was noted that HCO_3^- had a stimulatory effect on the I_{sc} and potential difference across the opercular epithelium of SW-adapted *F. heteroclitus* when bathed on both sides with Ringer and gassed with either 100 % O_2 or 95 % O_2/5 % CO_2. The stepwise titration of HCO_3^-, as $NaHCO_3$, into the Ringer bathing both sides of the epithelium produced corresponding increases in the I_{sc} and potential difference as illustrated in Text-fig. 6. In the absence of exogenous HCO_3^- the I_{sc} and potential difference were comparatively low. The titration of HCO_3^- to both sides of the epithelium, in 10 mM increments, produced fast and long lasting stimulations in the I_{sc} and potential difference which were independent of any Na^+ or pH changes. The introduction of $NaHCO_3$ into the bathing solutions produced small increases in the Na^+ concentration and pH but similar changes produced by the titration of NaOH into the solutions had no stimulatory effects on the I_{sc} or the potential difference. Small increases in the transepithelial d.c. resistance was also observed with

increasing HCO_3^- concentrations but these were usually indistinguishable from the usual increase in resistance with time across these *in vitro* epithelia. The fact that Diamox had no inhibitory effect on Cl^- secretion across these epithelia suggested that HCO_3^- acted in some other way than the possible stimulation of a Cl^-/HCO_3^- exchange. One alternative may be the stimulation of a HCO_3^- activated ATPase (Kasbekar & Durbin, 1965) which has been identified in a number of anion transporting epithelia.



Text-fig. 6. The typical stimulatory effect of HCO_3^- on the I_{sc} and potential difference across the opercular epithelium of SW-adapted *F. heteroclitus* bathed on both sides with Ringer and gassed with 100 % O_2. The continuous line is the continuous I_{sc} recording and the dots are the open-circuited potential difference measurements. The preparation was bathed initially with a HCO_3^--free Ringer on both sides and then HCO_3^- was titrated to both sides in 10 mM increments.

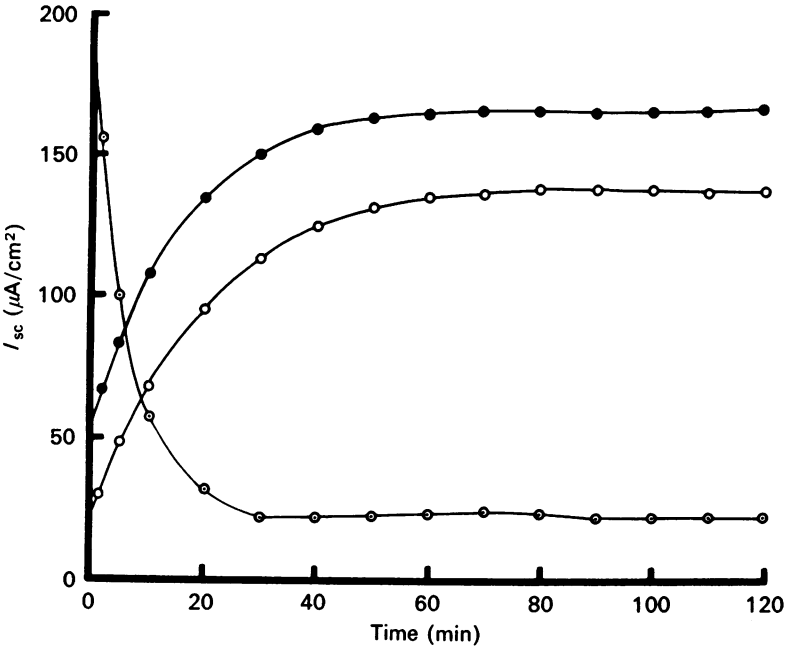
The electrical properties across the isolated opercular epithelia of freshwater-adapted and 200 % seawater-adapted F. heteroclitus

The mean electrical properties across isolated opercular epithelia of FW- and 2SW-adapted *F. heteroclitus*, when bathed on both sides with Ringer and gassed with 100 % O_2, are presented in Table 4 along with those of SW-adapted fish for comparison. The mean I_{sc}s of 94·1 μA/cm² and 33·5 μA/cm² for epithelia of FW- and 2SW-adapted fish respectively were 68·9 and 24·5 % respectively of the mean I_{sc} of 136·5 μA/cm² for epithelia from SW-adapted fish. The mean potential differences of 14·8 and 10·5 mV for epithelia of FW-and 2SW-adapted fish respectively were 79·1 and 56·2 % respectively of the mean potential difference of 18·7 mV for

epithelia from SW-adapted fish. For all adaptations the transepithelial difference displayed the same orientation of blood side positive when the epithelia were bathed on both sides with Ringer. The transepithelial d.c. resistances across epithelia of FW- and SW-adapted fish were similar and about one third that of 440·7 $\Omega.cm^2$ calculated for the epithelia of 2SW-adapted fish. The electrical properties of the opercular epithelia of SW- and FW-adapted fish were similar and these epithelia displayed similar I_{sc} characteristics when mounted in the chambers as shown in Text-fig. 7.

TABLE 4. Comparison of the mean electrical properties across epithelia of *F. heteroclitus*, adapted to different external salinities, when isolated and bathed on both sides with Ringer and gassed with 100% O_2. Results are expressed as mean ± S.E. of mean and the number of experiments given in parentheses. SW = seawater; FW = freshwater; 2SW = 200% seawater

Mean electrical properties	SW-adapted	FW-adapted	2 SW-adapted
I_{sc} ($\mu A/cm^2$)	136·5 ± 11·1 (64)	94·1 ± 10·4 (20)	33·5 ± 8·5 (18)
P.d. (mV)	18·7 ± 1·2 (64)	14·8 ± 1·9 (20)	10·5 ± 2·5 (18)
$R(\Omega.cm^2)$	173·7 ± 12·1 (64)	169·0 ± 14·0 (20)	440·7 ± 62·6 (18)



Text-fig. 7. The characteristic I_{sc} patterns across the opercular epithelia of *F. heteroclitus* adapted to different salinites. All epithelia were bathed on both sides with Ringer and gassed with 100% O_2. ●—●, SW-adapted; ○—○, FW-adapted; ⊙—⊙, 2SW-adapted.

The I_{sc}s across these epithelia were initially relatively low and steadily increased to some higher steady-state levels within 60 min where they remained relatively constant.

The unusual feature of the epithelia of 2SW-adapted fish was their comparatively high d.c. resistance. These epithelia also displayed a different I_{sc} characteristic when mounted in the chamber. The I_{sc}s were initially very high and rapidly decayed to lower steady-state levels below those usually displayed by epithelia from FW- and SW-adapted fish as shown in Text-fig. 7. In a few instances the I_{sc} across these epithelia displayed tendencies to increase after a few hours at low steady-state levels. Numerous adaptive changes occur in teleosts when faced with different environmental salinities. The effector organs involved in these adaptations have been reviewed by Maetz (1970) and appear to involve primarily the kidneys, gut, and the gills and hormonal influences have been implicated. It is possible that these characteristic I_{sc} patterns observed *in vitro* reflect the waning influences of hormones which were circulating in the intact fish as adaptive responses to the different salinities. Since these epithelia were usually mounted in the chambers within 20 min from pithing, it might have been possible to observe these waning hormonal influences. It should be noted that these epithelia were bathed on both sides with Ringer, which in itself may have triggered changes in the properties of these epithelia.

$^{36}Cl^-$ and $^{22}Na^+$ fluxes across the isolated, short-circuited opercular epithelia of freshwater-adapted and 200% seawater-adapted F. heteroclitus

The $^{36}Cl^-$ and $^{22}Na^+$ fluxes across the isolated, short-circuited opercular epithelia of FW- and 2SW-adapted *F. heteroclitus*, when bathed on both sides with Ringer and gassed with 100% O_2, are presented in Table 5. Epithelia from FW-adapted fish demonstrated a mean Cl^- efflux 3 times greater than the mean Cl^- influx which resulted in a net Cl^- efflux of 3·55 μequiv.cm^{-2}.hr^{-1} (95·2 μA/cm^2) which was not statistically different ($P > 0{\cdot}30$) from the mean I_{sc} of 87·4 μA/cm^2 for these $^{36}Cl^-$ flux experiments. The difference in the mean unidirectional Na^+ fluxes across these epithelia was not statistically significant ($P > 0{\cdot}40$), indicating that there was no net movement of this cation across these epithelia from FW-adapted fish. The epithelia from 2SW-adapted fish demonstrated a mean Cl^- efflux 11·7 times greater than the mean Cl^- influx which resulted in a net Cl^- efflux of 3·59 μequiv.cm^{-2}.hr^{-1} (96·2 μA/cm^2). This was approximately twice the mean I_{sc} of 48·9 μA/cm^2 for these $^{36}Cl^-$ flux experiments. The mean unidirectional Na^+ fluxes across these epithelia were not equivalent. The mean Na^+ efflux was approximately 6 times the mean Na^+ influx which resulted in a net Na^+ efflux of 2·43 μequiv.cm^{-2}.

TABLE 5. The $^{36}Cl^-$ and $^{22}Na^+$ fluxes and the mean electrical properties across opercular epithelia of FW- and 2SW-adapted *F. heteroclitus* when isolated, bathed on both sides with Ringer, and gassed with 100% O_2. Paired influx and efflux experiments were performed on pieces of epithelia from the same fish. The results are expressed as mean ±S.E. of mean and the number of paired experiments given in parentheses. Abbreviations as in Table 1

Adaptation	Flux Direction	J_i (μequiv. $cm^{-2}.hr^{-1}$)	I_i ($\mu A/cm^2$)	I_{sc} ($\mu A/cm^2$)	P.d. (mV)	R ($\Omega.cm^2$)
Freshwater	$^{36}Cl^-$ efflux (5)	5·36 ± 0·71	143·7 ± 19·0	92·0 ± 22·1	16·8 ± 5·7	181·6 ± 30·1
	$^{36}Cl^-$ influx (5)	1·81 ± 0·29	48·5 ± 7·8	82·7 ± 22·8	16·2 ± 4·2	206·4 ± 27·3
	$^{36}Cl^-$ net flux	3·55 ± 0·60	95·2 ± 16·6			
	Mean electrical properties (10)			87·4 ± 22·2	16·5 ± 3·3	194·0 ± 19·0
	$^{22}Na^+$ efflux (5)	3·49 ± 0·90	93·5 ± 24·4	88·4 ± 14·9	13·0 ± 2·8	161·2 ± 29·7
	$^{22}Na^+$ influx (5)	3·09 ± 0·64	82·7 ± 17·5	113·4 ± 27·7	13·0 ± 3·1	126·6 ± 20·6
	$^{22}Na^+$ net flux	0·40 ± 0·45	10·8 ± 12·3			
	Mean electrical properties (10)			100·9 ± 15·0	13·0 ± 1·9	143·9 ± 17·5
200% seawater	$^{36}Cl^-$ efflux (5)	3·92 ± 1·86	105·2 ± 49·9	35·4 ± 13·6	9·7 ± 3·7	347·2 ± 84·7
	$^{36}Cl^-$ influx (5)	0·34 ± 0·20	9·0 ± 5·4	62·4 ± 19·9	15·3 ± 7·2	385·3 ± 61·7
	$^{36}Cl^-$ net flux	3·59 ± 1·92	96·2 ± 51·5			
	Mean electrical properties (10)			48·9 ± 17·3	12·5 ± 3·9	366·2 ± 49·0
	$^{22}Na^+$ efflux (4)	2·93 ± 1·20	78·4 ± 32·1	13·6 ± 4·3	7·5 ± 5·2	447·2 ± 213·4
	$^{22}Na^+$ influx (4)	0·49 ± 0·18	13·2 ± 4·9	12·2 ± 2·0	8·4 ± 3·1	632·7 ± 157·6
	$^{22}Na^+$ net flux	2·43 ± 1·07	65·3 ± 28·6			
	Mean electrical properties (8)			12·9 ± 2·1	8·0 ± 2·7	539·9 ± 123·6

hr^{-1} (65·3 $\mu A/cm^2$). This net secretion of Na^+ was in the proper direction and of the appropriate magnitude to account for most of the discrepancy between the net Cl^- secretion and the I_{sc} across these epithelia from 2SW-adapted fish.

TABLE 6. Comparison of the $^{36}Cl^-$ and $^{22}Na^+$ fluxes across opercular epithelia of *F. heteroclitus*, adapted to different salinities, when isolated and bathed on both sides with Ringer and gassed with 100 % O_2. Fluxes are presented in units of current for comparison to the I_{sc}. Results are expressed as mean ± S.E. of mean and the number of experiments given in parentheses

Flux ($\mu A/cm^2$)	SW-adapted	FW-adapted	2SW-adapted
$^{36}Cl^-$ efflux	211·7 ± 27·1 (10)	143·7 ± 19·0 (5)	105·2 ± 66·2 (5)
$^{36}Cl^-$ influx	48·9 ± 10·0 (10)	48·5 ± 7·8 (5)	9·0 ± 5·4 (5)
$^{36}Cl^-$ net flux	162·8 ± 30·9	95·2 ± 16·1	96·2 ± 51·5
Mean I_{sc} for $^{36}Cl^-$ flux studies	158·6 ± 16·3 (20)	87·4 ± 22·2 (10)	48·9 ± 17·3 (10)
$^{22}Na^+$ efflux	32·2 ± 3·3 (10)	93·5 ± 24·4 (5)	78·4 ± 31·5 (4)
$^{22}Na^+$ influx	34·8 ± 4·1 (10)	82·7 ± 17·5 (5)	13·2 ± 4·9 (4)
$^{22}Na^+$ net flux	− 2·6 ± 4·6	10·8 ± 12·2	65·3 ± 28·6
Mean I_{sc} for $^{22}Na^+$ flux studies	56·9 ± 8·0 (20)	100·9 ± 15·0 (10)	12·9 ± 2·1 (8)

Table 6 compares the Na^+ and Cl^- fluxes across the isolated, short-circuited opercular epithelia from SW-, FW- and 2SW-adapted *F. heteroclitus*. All these epithelia displayed substantial effluxes of Cl^-. The epithelia from FW- and 2SW-adapted fish had Cl^- effluxes of 67·9 and 49·7 % respectively of that of epithelia from SW-adapted fish. The Cl^- influxes across epithelia from SW- and FW-adapted fish were equivalent whereas that across epithelia from 2SW-adapted fish was approximately one fifth of that across the other two epithelia. The net Cl^- secretion across the epithelia from FW- and 2SW-adapted fish were approximately the same and 58·5 and 59·1% respectively of that across epithelia from SW-adapted fish. However, only with the epithelia from FW-adapted fish was the net Cl^- secretion and the mean I_{sc} equivalent. With epithelia from 2SW-adapted fish the net Cl^- secretion was approximately twice the mean I_{sc}. The unidirectional Na^+ fluxes across epithelia from FW-adapted fish were approximately 3 times greater than those across epithelia from SW-adapted fish but equal in both directions. The epithelia from 2SW-adapted fish displayed a net Na^+ efflux which appeared to result primarily from the reduced influx of this cation when compared to epithelia from SW- and FW-adapted fish.

The unusual feature of epithelia from 2SW-adapted *F. heteroclitus* was the reduced influx of both Cl^- and Na^+ and the high d.c. resistance when

compared to the epithelia from SW- and FW-adapted fish. In most of these influx studies with epithelia from 2SW-adapted fish there was no change in the activity from background in the Ringer on the blood side of these epithelia after hours of incubation with 5 μc of isotope in the Ringer on the seawater side. These flux data coupled with the relatively high d.c. resistance across these epithelia suggested that a tightening of these epithelia occurred as an adaptive response to the increased environmental salinity. However, no such adaptive responses were evident between epithelia from FW- and SW-adapted fish. The reduced influx of both Cl^- and Na^+ across these epithelia suggested that these fluxes were passive and that the leak pathway for these ions was non-selective, permitting the movement or restricting the movement of both anions and cations.

DISCUSSION

Comparison of the electrical data for isolated opercular epithelium with data from intact fish and isolated, perfused gills

The isolated opercular epithelium of SW-adapted *F. heteroclitus*, when bathed on both sides with Ringer, gave a mean I_{sc} of 136·5 μA/cm^2, a mean transepithelial potential difference of 18·7 mV (blood side positive), and a mean transepithelial d.c. resistance of 173·7 Ω.cm^2. Only the potential difference can be compared to data obtained from intact fish and isolated, perfused gill preparations. Maetz & Bornancin (1975) have compiled the *in vivo* potential difference measurements made by several investigators across the gills of five species of SW-adapted teleosts. The mean potential difference of 18·7 mV across the opercular epithelium is in good agreement with these *in vivo* values which ranged from 10·1 to 25·2 mV (blood side positive). It is also in good agreement with those potential difference measurements made in the intact flounder, *P. flesus*, and the isolated, perfused gill of this teleost when bathed externally with sea water, but 3–4 times greater when these two flounder preparations were bathed externally with Ringer (Shuttleworth *et al.* 1974). In both the intact SW-adapted flounder (Potts & Eddy, 1973) and the irrigated gill of the SW-adapted trout, *Salmo gairdneri* (Kirschner, Greenwald & Sanders, 1974), the gill potentials were shown to be primarily cation diffusion potentials. The potential difference across the isolated opercular epithelium of SW-adapted *F. heteroclitus* increased when bathed externally with sea water and reversed its orientation to blood side negative when bathed externally with 0·1 Ringer. This potential reversal across the opercular epithelium is similar to the potential reversal observed across the gill epithelia of *Dormitator maculatus* (Evans, Carrier & Bogan, 1974), *Anguilla anguilla* (House & Maetz, 1974), and *P. flesus* (Potts & Eddy, 1973) when transferred from

seawater to 0·1 seawater or freshwater. These observations suggest that the potential differences across these epithelia are primarily cation diffusion potentials resulting from the imposed concentration gradients. However, when bathed on both sides with Ringer, the potential differences across the flounder gill epithelium (Shuttleworth *et al.* 1974) and the *F. heteroclitus* opercular epithelium are considerably dependent on aerobic metabolism, suggesting that these potentials are, in part, electrogenic under these conditions. It appears that cation diffusion potentials dominate when these epithelia are bathed on opposite sides with different salt solutions and that these diffusion potentials are minimized when bathed on opposite sides with the same salt solutions, revealing the electrogenic potential. Further studies, however, are required to establish the role of Cl^- transport across the teleost gill and the opercular epithelium in the generation of the transepithelial potential difference. Although Cl^- transport across the short-circuited opercular epithelium is electrogenic, in the sense that it results in a net movement of charge, this transport may be only partially responsible for the generation of the potential difference in the intact fish.

Nature of the movement of Cl^- across the isolated opercular epithelium and the teleost gill

Numerous investigators have measured the isotopic Na^+ and Cl^- fluxes across the gills of intact SW-adapted teleosts and have demonstrated relatively large unidirectional fluxes of these ions which resulted in small net secretions of both Na^+ and Cl^- (data compiled by Maetz & Bornancin, 1975). Cl^- is secreted against an electrochemical gradient and the ratio of the unidirectional Cl^- fluxes does not correspond to that predicted by the flux-ratio equation (Ussing, 1960). These data suggest active Cl^- transport across the teleost gill. With the isolated, short-circuited opercular epithelium of SW-adapted *F. heteroclitus* active Cl^- secretion was clearly demonstrated which occurs at a rate equivalent to the short-circuit current. This equivalency makes it unlikely that other ions are actively transported by this epithelium but does not rule out the possibility of cation/cation or anion/anion exchanges. The lack of any inhibitory effect of Diamox on the I_{sc} and Cl^- secretion across the opercular epithelium suggests that no Cl^-/HCO_3^- exchange is involved in the electrogenic Cl^- secretion and that HCO_3^- does not enhance the Cl^- secretion via this exchange. Perhaps other carbonic anhydrase inhibitors may produce different results. The HCO_3^--activated, SCN^--inhibited, ouabain-insensitive ATPase, first described by Kasbekar & Durbin (1965) in the frog gastric mucosa, has subsequently been found in a variety of anion-transporting tissues including teleost gills (Kerstetter & Kirschner, 1974; Solomon, Silva, Bend

& Epstein, 1975; Maetz & Bornancin, 1975). This ATPase has an apparent K_m for HCO_3^- of 16 mM (Kerstetter & Kirschner, 1974) which was the concentration of HCO_3^- in the Ringer used to bathe the isolated opercular epithelium. Assuming that the intracellular chloride cell HCO_3^- concentration is in equilibrium with the extracellular HCO_3^- concentration, increasing the latter would lead to an increase in the intracellular HCO_3^- and an increased activity of the HCO_3^--activated ATPase.

If this enzyme supplied the energy for the active Cl^- transport, a stimulation of its activity by HCO_3^- would explain the influence of this anion on Cl^- secretion across the opercular epithelium. The drawbacks to this hypothesis are that this enzyme has yet to be identified in this epithelium, no definite link between this HCO_3^--activated ATPase and active Cl^- transport has been established, this enzyme is not activated by Cl^-, and it appears to be located primarily in the mitochondrial fraction of gill homogenates (Kerstetter & Kirschner, 1974; Solomon *et al.* 1975) rather than in the membrane fraction. The chloride cells are rich in mitochondria and the opercular epithelium of *F. heteroclitus* is predominantly chloride cells, which suggests that this epithelium may be rich in this HCO_3^--activated ATPase.

The nature of Na^+ movement across the isolated opercular epithelium

The nature of Na^+ movements across the gills of SW-adapted teleosts is unsettled and represents a major controversy concerning ion movements across this tissue. Currently, the models for NaCl secretory mechanisms by the teleost gill Cl^- cell propose an important role for Na^+, K^+-activated ATPase, either directly (Maetz, 1969, 1971) or indirectly (Kirschner, 1977; Silva, Solomon, Spokes & Epstein, 1977). Preliminary observations (Miller, D. S. & Karnaky, K. J., unpublished) demonstrated that the opercular epithelium of *F. heteroclitus* is rich in Na^+, K^+-activated ATPase with a specific activity of about twice that of the gill in SW-adapted specimens. A Na^+, K^+-activated ATPase has been localized in the tubular membranes of the gill chloride cells of *F. heteroclitus* with high resolution [^{3}H]ouabain autoradiography (Karnaky *et al.* 1976). Since the gill and opercular epithelium chloride cells are identical in ultrastructure (Karnaky & Kinter, 1977), it is reasonable to assume that this ATPase is located in the tubular membranes of the opercular epithelium chloride cells. The effects of ouabain on the isolated opercular epithelium of *F. heteroclitus* demonstrates that Na^+, K^+-activated ATPase plays an important role in the Na^+ efflux, and suggests that the unidirectional Na^+ movements result from two different mechanisms. The Na^+ influx appears to be passive since it is unaffected by ouabain and substantially reduced in high resistance (2SW-adapted) epithelia. The Na^+ efflux, on the other hand, is ouabain-

sensitive, and unimpaired in high resistance (2SW-adapted) epithelia. The rates of Na^+ efflux and influx across opercular epithelia of SW-adapted *F. heteroclitus* were approximately equal, suggesting that this Na^+ extrusion is more compensatory than secretory in function, acting to balance the inward leak of Na^+, or simply serving as a K^+ conserving mechanism. With the present data there is no reason to postulate active Na^+ excretion across the opercular epithelium that serves as a substantial secretory mechanism. Na^+ could simply follow in the wake of the active Cl^- secretion as suggested by Kirschner (1977) and Silva *et al.* (1977).

The influence of drugs on the ion transport aross the opercular epithelium

Known inhibitors of Cl^- transport, furosemide and thiocyanate, inhibited the I_{sc}, and presumably the Cl^- transport, across the opercular epithelium. The effect of furosemide was relatively fast and was not readily reversed once a full inhibitory effect was accomplished. This irreversibility is contrary to that of furosemide in the kidney (Burg *et al.* 1973). Thiocyanate, on the other hand, initially inhibits the I_{sc} but spontaneous tendencies towards recovery after a full thiocyanate effect occur which may result from the active transport of thiocyanate ions by the Cl^- transporting mechanism. We can offer no explanation for the inconsistent observations that thiocyanate inhibits Cl^- transport in the isolated opercular epithelium but fails to inhibit Cl^- efflux in the intact *F. heteroclitus* (Epstein *et al.* 1975).

The administration of adrenaline to intact *F. heteroclitus* (Pickford, Srivastava, Slicher & Pang, 1971) results in an increase in plasma osmolarity, presumably from the osmotic loss of water and the inhibition of Na^+ and Cl^- secretion across the gills. Adrenaline causes an increase in the water permeability of the gill which is mediated via β-adrenergic receptors (Pic, Mayer-Gostan & Maetz, 1974) and an inhibition in the Cl^- secretion which is mediated through α-adrenergic receptors (Pic *et al.* 1975). It has been suggested (Pic, Mayer-Gostan & Maetz, 1973) that the chloride cells of the gill epithelium have the α-receptors while the β-receptors are located elsewhere in the gill. Adrenaline inhibits Na^+ and Cl^- secretion 40–60 % in the gill of the SW-adapted mullet, *Mugil capito* (Pic *et al.* 1975). Cuthbert & Pic (1973) showed that adrenaline increased the cyclic-AMP levels in the gill of the mullet via β-receptors since this effect of adrenaline was blocked by propranalol, a β-clocking agent, and unaffected by phentolamine, an α-blocking agent. These investigators suggested that activation of the α-adrenergic receptors may lead to a decrease in the chloride cell cyclic-AMP levels since the administration of adrenaline with phentolamine increased the gill cyclic-AMP levels slightly greater than when adrenaline

was administered alone. The effect of adrenaline on Cl^- secretion in the opercular epithelium is similar to that observed on the gills of the mullet (Pic *et al.* 1975). These two observations taken together support the suggestion that adrenaline acts directly on the chloride cells of the gill epithelium to inhibit Cl^- transport rather than indirectly by altering the gill haemodynamics. This effect of adrenaline on the opercular epithelium also supports the suggestion that adrenaline acts via the α-receptors to lower the cyclic AMP levels of the chloride cells because theophylline, which increases intracellular cyclic-AMP levels (Handler, Butcher, Sutherland & Orloff, 1965), has a stimulatory effect on Cl^- secretion across the opercular epithelium.

Nature of ion transport across the isolated opercular epithelium of fish adapted to low and high salinities

In *F. heteroclitus* gill, chloride cell size and number and specific activity of Na^+, K^+-activated ATPase increase with adaptation to increasing salinities from 10 % seawater to 200 % seawater (Karnaky *et al.* 1976). In striking contrast, the number of opercular epithelium chloride cells of this same species remains relatively constant with adaptations ranging from freshwater to 200 % seawater (Karnaky & Kinter, 1977). Moreover, the specific activity of Na^+, K^+-activated ATPase is similar in opercular epithelia of SW- and 2SW-adapted *F. heteroclitus* (Miller, D. S. & Karnaky, K. J., unpublished). This constancy in the chloride cell population would explain the net Cl^- secretion observed across epithelia of FW-adapted fish. In the freshwater teleost gill a net uptake of Na^+ and Cl^-, by independent mechanisms, has been reported (Maetz & Garcia-Romeu, 1964; de Renzis & Maetz, 1973). A net Cl^- secretion and no net movement of Na^+ was observed across opercular epithelia of FW-adapted *F. heteroclitus* which were similar to those across epithelia of SW-adapted fish. It should be emphasized that the application of the short-circuit current technique requires the isolated tissue to be bathed on both sides with the same solution. The observed Cl^- secretion across epithelia of FW-adapted fish may have resulted from the fact that these epithelia were bathed externally by a solution with a salinity much greater than freshwater. Hormonal influences (Maetz, 1970) must also be considered. In intact freshwater fish certain hormones, such as adrenaline, may be circulating in the blood and suppressing or minimizing Cl^- secretion across the opercular epithelia. The interruption of this hormonal influence(s) in the *in vitro* condition may lead to the resumption of Cl^- secretion. Such quick acting and transitory hormonal influences on Cl^- secretion, which adrenaline appears to have, would be an efficient mechanism for euryhaline fish, such as *F. heteroclitus*, to regulate their osmoregulatory functions on a daily basis. With regard to

the possible role of the chloride cell apical crypt in Cl^- secretion, it is interesting to note that these crypts, which are not present in the gill chloride cells of tapwater-adapted *F. heteroclitus*, begin to develop within 30 min after transfer of these fish into seawater (Copeland, 1950). It is possible that the apical crypts may serve as a morphological index of Cl^- secretion.

A net secretion of both Cl^- and Na^+ occurs across epithelia of 2SW-adapted *F. heteroclitus*. Bathing these epithelia externally with Ringer and the interruption of possible hormonal influences may be important factors on Cl^- secretion in these *in vitro* preparations. The net secretion of Na^+ observed across these epithelia resulted mostly from the reduced influx of this ion. The high resistance of these epithelia, together with the reduced influx of both Na^+ and Cl^-, suggests that these unidirectional fluxes are passive and that the pathway for these leaks is non-selective, permitting the movement of both anions and cations. The Na^+ efflux across epithelia of 2SW-adapted fish is not drastically different from that across epithelia of FW- and SW-adapted fish. Assuming this Na^+ efflux is ATPase-dependent, this finding is consistent with that showing no difference in ATPase activity in opercular epithelia of SW- and 2SW-adapted *F. heteroclitus* (Miller, D. S. & Karnaky, K. J., unpublished). This asymmetry in the unidirectional Na^+ fluxes across these epithelia argues against a Na^+/Na^+ transepithelial exchange proposed for the teleost gill (Motais, Garcia-Romeu & Maetz, 1966), as comprising a large part of the Na^+ movement across this tissue and possibly epithelia of FW- and SW-adapted fish. The increased resistance across the epithelia of 2SW-adapted fish may have been an adaptive response to the increased external salinity minimizing the osmotic loss of water and the passive entry of salts down their concentration gradients.

The isolated opercular epithelium of F. heteroclitus as a third alternative approach to the study of teleost osmoregulation

It is clear from the observations on the isolated opercular epithelium of *F. heteroclitus*, reported in this paper, that this preparation shares several important morphological and physiological characteristics with the teleost gill. In contrast to the intact fish and the isolated, perfused gill, the opercular epithelium of *F. heteroclitus* can be mounted as a membrane in a chamber and studied under well-defined conditions. The study of this gill-like epithelium with the short-circuit current technique therefore offers an improved biophysical approach to details of the salt transport mechanisms which are key to the teleost's ability to survive in hypo- and hyperosmotic environments. The utility of this membrane preparation is considerably extended because this euryhaline species, *F. heteroclitus*, can adapt to a

wide range of salinities. It remains to be seen whether the opercular epithelia of other teleosts, stenohaline and euryhaline, can also be isolated and mounted in chambers to reveal details of teleost osmoregulation.

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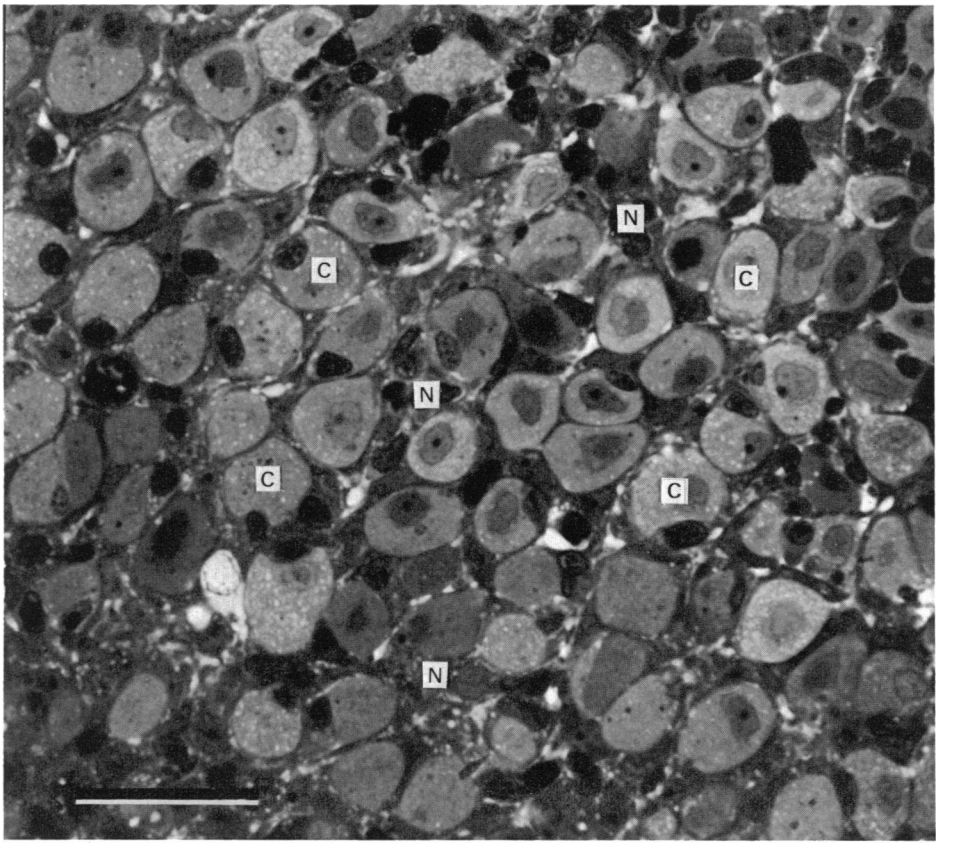
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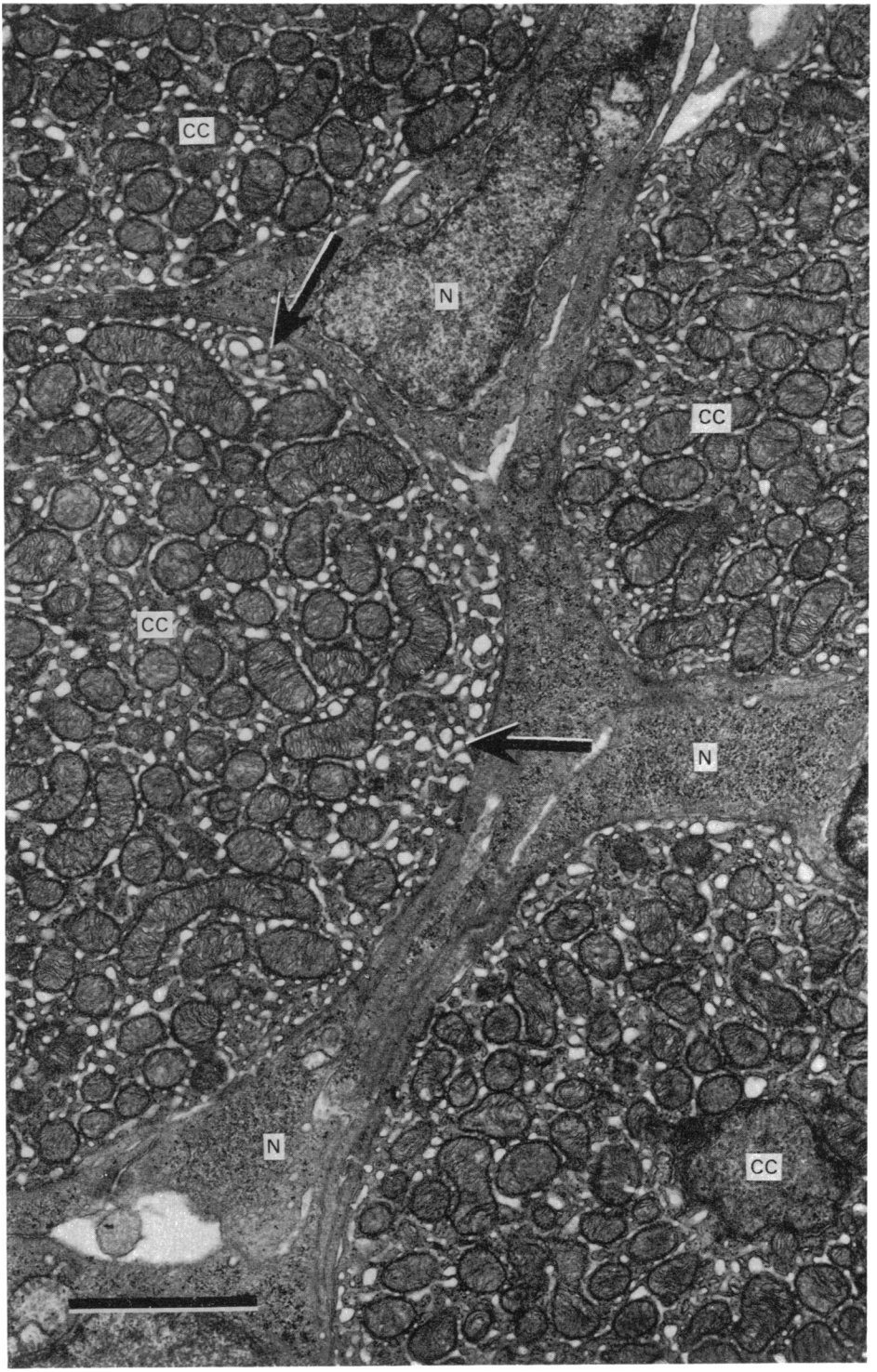
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EXPLANATION OF PLATES

PLATE 1

Tangential section through the epithelium of a piece of opercular skin dissected from a SW-adapted *F. heteroclitus*, mounted in the Lucite chamber, bathed on both sides with Ringer, short-circuited, and fixed with glutaraldehyde as described in the Methods section. At the time of fixation this epithelium had a potential difference of 21·5 mV, an I_{sc} of 214·3 μA/cm^2, and a d.c. resistance of 107·3 Ω.cm^2. The non-differentiated cells (N) and the large population of chloride cells (C) are clearly visible. Scale, 30 μm.

PLATE 2

Electron micrograph view of the tissue shown in Plate 1. Portions of four Cl cells (CC) are seen surrounded by several non-differentiated cells (N). Note the rich population of mitochondria. Elements of the branching tubular system are labelled (arrows) in the chloride cell at the left of the micrograph. Scale, 2 μm.