

SHORT COMMUNICATION

INTESTINAL SALT ABSORPTION IN THE
STENOTHERMIC ANTARCTIC FISH, *NOTOTHENIA ROSSII*

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All Antarctic fish studied so far show two adaptive strategies to life at sub-zero water temperatures; antifreeze glycoproteins, which act non-colligatively to inhibit ice propagation (DeVries, 1983), and elevated plasma electrolyte levels (150–200 mosmol⁻¹ higher than that of temperate marine teleosts: Holmes & Donaldson, 1969; Dobbs & DeVries, 1975; O'Grady, Ellory & DeVries, 1983), equivalent to a freezing point reduction of 0.4°C. However, Prosser, Mackay & Kato (1970) have argued that the elevated plasma Na⁺ concentration is an energy-saving device, resulting in a smaller concentration gradient between fish plasma and salt water, which allows the composition of the former to be maintained at a lower metabolic cost.

Osmoregulation in marine teleosts involves replenishment of branchial water loss by intestinal absorption of salt and water with subsequent Na⁺ excretion by the gills (reviewed by Ellory & Gibson, 1983). It is, therefore, of interest to determine the rate of salt transport in the anterior intestine (as a major site of osmoregulation) of an Antarctic fish species, *Notothenia rossii*, at 4°C and compare it with that of temperate marine species at their ambient temperature. Transport across fish intestine follows the classical Prechtian pattern of temperature adaptation (Precht, 1958; Smith, 1976; Gibson, Ellory & Cossins, 1985), making this comparison with temperate species a test of the energy-saving theory.

It is also pertinent to ask if Antarctic fish intestine is a Cl⁻-transporting epithelium in the Na⁺, K⁺, Cl⁻-coupled pattern present in other marine teleosts such as plaice and flounder (Field *et al.* 1978; Ramos & Ellory, 1981; Gibson, 1985). Early work by House & Green (1965) on *Cottus*, a Northern hemisphere species which could be regarded as closer to Antarctic fish than the flat fishes, indicated possible differences in the mechanism of epithelial transport with small positive values for transepithelial potential difference (PD).

Key words: Antarctic, intestine, transport, temperature.

In the present paper, therefore, we have measured electrical parameters and transepithelial Cl^- fluxes in the anterior intestine of *Notothenia rossii*. Cl^- absorption was followed in preference to Na^+ absorption because of its more central role in osmoregulation in other teleosts (e.g. Hirano, 1974; Lau, 1982). The effects of mucosal application of bumetanide, to inhibit the apical cell membrane $\text{Na}^+, \text{K}^+, \text{Cl}^-$ cotransporter (Field *et al.* 1978; Ramos & Ellory, 1981), and amphotericin B, to reveal electrogenic basolateral cell membrane Na^+ pumps mediated through its effect on increasing mucosal Na^+ permeability (Reuss, 1981; Ellory, Lahlou & Ramos, 1981), were also tested.

Antarctic fish, *Notothenia rossii*, collected in South Georgia, were obtained on the day of use from British Antarctic Survey, Madingley Road, Cambridge, where they were kept in aerated recirculating sea water at 4°C and fed daily on shrimps. Pieces of stripped intestine were mounted in conventional Ussing chambers (Ramos & Ellory, 1981) 0.7 cm^2 in area under voltage-clamp conditions. Construction of electrodes and circuits has been described previously (Ramos & Ellory, 1981), except that calomel electrodes were found to give better stability if made and kept at 4°C .

Saline for Antarctic fish was modified from that described by O'Grady *et al.* (1983) and had the following composition (in mmol l^{-1}): NaCl , 250; KCl , 5; MgSO_4 , 3; NaHCO_3 , 2; CaCl_2 , 2.5; L-alanine, 2.5; D-glucose, 5; buffered with Mops, 5; and Tris, 10; and adjusted with Tris base to give a pH of 7.8 at 4°C . It was bubbled with O_2 during the experiments. Bumetanide (Leo Pharmaceutical Products, Aylesbury, Bucks) was used at $40\text{ }\mu\text{mol l}^{-1}$ final concentration in saline. Amphotericin B (Fungizone: E. R. Squibb & Sons Inc., Princeton, NJ) was used as a saturated suspension at a nominal concentration of $80\text{ }\mu\text{g ml}^{-1}$.

Bidirectional Cl^- fluxes were measured using $^{77}\text{Br}^-$ (Medical Research Council Cyclotron Unit, Hammersmith Hospital, London) and $^{36}\text{Cl}^-$ (Amersham International, Amersham, Bucks), see Ramos & Ellory (1981). Preliminary experiments showed that $^{77}\text{Br}^-$ behaved as a Cl^- tracer in nototheniids, as observed in other teleosts (Ramos & Ellory, 1981; Gibson, 1985).

Samples of anterior intestine from *N. rossii* gave consistent results for electrical parameters with negative short-circuit currents (SCCs) and PDs of $-19.5 \pm 1.9\text{ }\mu\text{A cm}^{-2}$ ($\pm\text{S.E.M.}$, $N=28$) and $-1.8 \pm 0.2\text{ mV}$ ($N=28$), respectively, with a transepithelial resistance of $94 \pm 10\text{ }\Omega\text{cm}^2$ ($N=28$). The tissues proved to be very stable maintaining these values over at least 2 h following the initial equilibration period, during which SCC and PD usually drifted to more negative values by up to 30%.

From the tracer experiments, there was a net chloride absorption of $2.91 \pm 0.51\text{ }\mu\text{mol cm}^{-2}\text{ h}^{-1}$ ($N=16$) with J_{ms} and J_{sm} being 6.57 ± 0.62 ($N=16$) and 3.66 ± 0.48 ($N=16$), respectively.

The application of bumetanide ($40\text{ }\mu\text{mol l}^{-1}$) in the mucosal solution produced an immediate fall in SCC and PD to less than 20% of control values within 60 min and with a half-time of about 20 min (Fig. 1). Transepithelial resistance did not change significantly, the control value was $90 \pm 13\text{ }\Omega\text{cm}^2$ ($N=16$) and that after bumetanide was $93 \pm 20\text{ }\Omega\text{cm}^2$ ($N=15$).

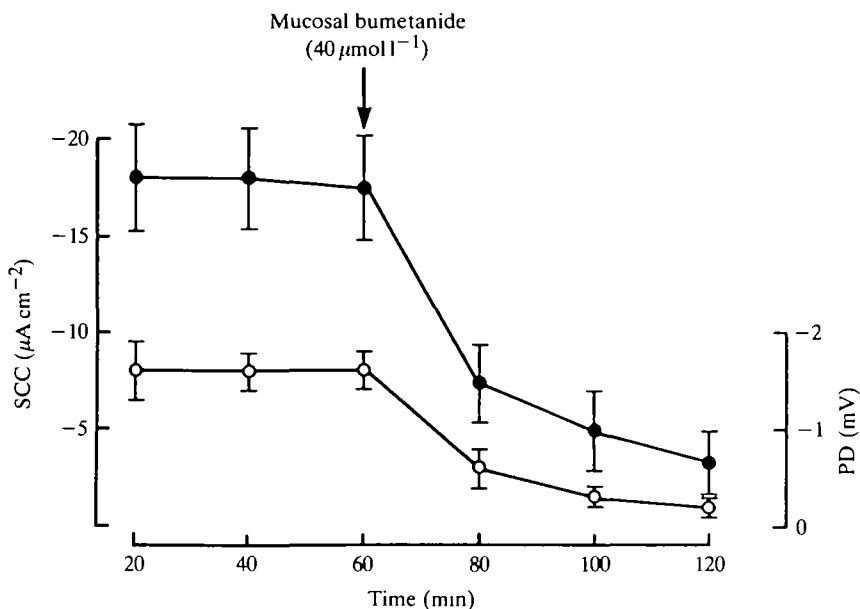


Fig. 1. The effect of mucosal application of bumetanide ($40 \mu\text{mol l}^{-1}$) on short-circuit current (SCC) ($\mu\text{A cm}^{-2}$, ●) and potential difference (PD) (mV, ○) of intestines taken from *Notothenia rossii* mounted at 4°C *in vitro*. Each point gives mean \pm S.E.M. ($N = 16$).

Net chloride absorption was also inhibited, falling to $1.65 \pm 0.31 \mu\text{mol cm}^{-2} \text{h}^{-1}$ after 60 min. Inhibition was achieved by a fall in J_{ms} from $6.57 \pm 0.62 \mu\text{mol cm}^{-2} \text{h}^{-1}$ ($N = 16$) in the control period to $4.88 \pm 0.56 \mu\text{mol cm}^{-2} \text{h}^{-1}$ ($N = 16$) after 60 min with bumetanide, without J_{sm} changing significantly, 3.66 ± 0.48 ($N = 16$) and $3.25 \pm 0.37 \mu\text{mol cm}^{-2} \text{h}^{-1}$ ($N = 16$), respectively.

In 12 tissues, amphotericin B ($80 \mu\text{g ml}^{-1}$) was applied to the mucosal solution, following previous addition of bumetanide. SCC and PD were stimulated to positive values, rising from $-5.9 \pm 4.0 \mu\text{A}$ ($N = 12$) and $-0.3 \pm 0.2 \text{mV}$ ($N = 12$) following bumetanide to $+12.0 \pm 3.1 \mu\text{A cm}^{-2}$ ($N = 12$) and $+1.1 \pm 0.2 \text{mV}$ ($N = 12$) after 60 min in amphotericin. Again transepithelial resistance was unaffected, with values of $154 \pm 30 \Omega\text{cm}^2$ ($N = 12$) before, and $140 \pm 24 \Omega\text{cm}^2$ ($N = 12$) after, amphotericin B.

Intestinal Cl^- transport of Antarctic nototheniids at 4°C would thus appear to be similar to that of temperate marine teleosts measured at room temperature, samples of stripped anterior intestine mounted in Ussing chambers showing a negative SCC and PD with a net Cl^- absorption in excess of the current, which indicates a significant net movement of other ions, presumably net Na^+ absorption.

PD, SCC and Cl^- absorption were all inhibited by mucosal application of the loop-diuretic, bumetanide, the effect on Cl^- transport being mediated *via* a reduction in J_{ms} , without a change in J_{sm} , as observed in plaice for piretanide (Ramos & Ellory, 1981). The ionophore amphotericin B stimulated SCC and PD to positive values, consistent with the presence of an electrogenic Na^+ pump in the basolateral cell membrane (Graf & Giebisch, 1979; Reuss, 1981). Furthermore, it is known

from isolated intestinal sac experiments that fluid absorption in the Antarctic fish *Pagothenia borchgrevinki* (Boulenger) is reduced by serosal application of ouabain (O'Grady *et al.* 1983).

In other marine teleosts (Field *et al.* 1978; Ramos & Ellory, 1981; Ellory *et al.* 1981; Gibson, 1985) similar findings have been attributed to the function of a basolateral cell membrane Na^+, K^+ -ATPase coupled to a bumetanide-sensitive $\text{Na}^+, \text{K}^+, \text{Cl}^-$ cotransporter at the apical cell membrane.

Fig. 2 compares nototheniid transport parameters measured at 4°C with those of temperate marine teleosts measured at 22°C. The magnitudes of the fluxes, at the respective experimental temperatures, are similar, and since one would expect fluxes in temperate fish to be considerably reduced at 4°C compared with that at room temperature (Smith, 1976; Gibson *et al.* 1985) or with respect to nototheniids, Antarctic fish must, therefore, possess cellular adaptations to account for the relatively high Cl^- transport rates at 4°C. Conventional models of epithelial transport suggest that the main regulatory sites include the permeability of the apical and basolateral cell membranes, the intercellular junctions, and the basolateral cell membrane Na^+ pumping capacity (e.g. Field *et al.* 1978). Previous work on temperature acclimation on teleost intestine has ascribed a principal role to the activity of the Na^+, K^+ -ATPase (Smith & Ellory, 1971; Gibson *et al.* 1985) resulting from changes in turnover rate, membrane fluidity or ATP supply (e.g. Smith & Ellory, 1971; Cossins, 1983; Wodtke, 1981). Metabolic compensations do occur in

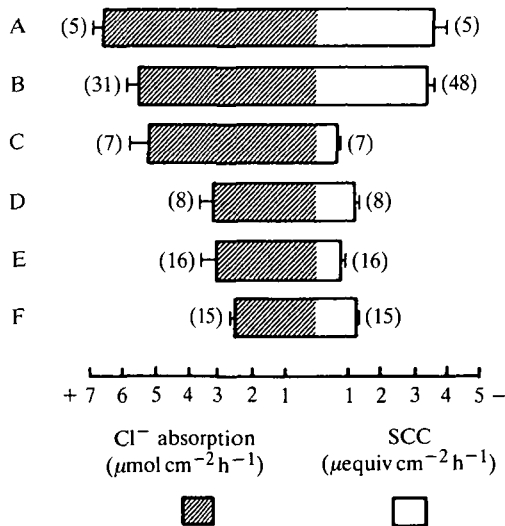


Fig. 2. Comparison of Cl^- absorption ($\mu\text{mol cm}^{-2} \text{h}^{-1}$) and short-circuit current (SCC) ($\mu\text{equiv cm}^{-2} \text{h}^{-1}$) of *Notothenia rossii* intestines mounted at 4°C with those of temperate marine teleosts at room temperature (about 22°C): (a) *Pseudopleuronectes americanus* (Field, Smith & Bolton, 1980), (b) *Pleuronectes platessa* (Ramos & Ellory, 1981), (c) *Gillichthys mirabilis* (Loretz, 1983), (d) *Anguilla japonica* (Ando, Utida & Nagahama, 1975), (e) *Notothenia rossii* (present paper), (f) *Platichthys flesus* (Gibson, 1985). Histograms show fluxes and SCC as mean \pm S.E.M. (N).

Antarctic fish (Scholander *et al.* 1957; Holeton, 1974) but further studies are required to elucidate the adaptive strategy of Antarctic fish intestine.

In conclusion, the intestinal component of osmoregulation in nototheniids would appear to be uncompromised. The present results suggest that intestinal NaCl transport is no different, either qualitatively or quantitatively, from that of other temperate marine teleosts except that it is very well adapted to function at low temperatures. The mechanism of this adaptation deserves further study.

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REFERENCES

- ANDO, M., UTIDA, S. & NAGAHAMA, H. (1975). Active transport of chloride in eel intestine with special reference to sea water adaptation. *Comp. Biochem. Physiol.* **51A**, 27–32.
- COSSINS, A. R. (1983). The adaptation of membrane structure and function to changes in temperature. In *Cellular Acclimatisation to Environmental Change* (ed. A. R. Cossins & P. Shterline), pp. 3–32. Cambridge: Cambridge University Press.
- DEVRIES, A. L. (1983). Antifreeze peptides and glycopeptides in cold-water fishes. *A. Rev. Physiol.* **45**, 245–260.
- DOBBS, G. H. & DEVRIES, A. L. (1975). Renal function in the Antarctic teleost fishes: serum and urine composition. *Mar. Biol.* **29**, 59–70.
- ELLORY, J. C. & GIBSON, J. S. (1983). Cellular aspects of salinity adaptation in teleosts. In *Cellular Acclimatisation to Environmental Change* (ed. A. R. Cossins & P. Shterline), pp. 197–216. Cambridge: Cambridge University Press.
- ELLORY, J. C., LAHLOU, B. & RAMOS, M. M. P. (1981). The effect of amphotericin B on NaCl transport in plaice intestine. *J. Physiol., Lond.* **320**, 135P–136P.
- FIELD, M., KARNAKY, K. J. JR, SMITH, P. L., BOLTON, J. E. & KINTER, W. B. (1978). Ion transport across the isolated mucosa of the winter flounder, *Pseudopleuronectes americanus*. I. Functional and structural properties of cellular and paracellular pathways for Na and Cl. *J. Membr. Biol.* **41**, 265–293.
- FIELD, M., SMITH, P. L. & BOLTON, J. E. (1980). Ion transport across the isolated intestinal mucosa of the winter flounder, *Pseudopleuronectes americanus*. II. Effects of cyclic AMP. *J. Membr. Biol.* **55**, 157–163.
- GIBSON, J. S. (1985). Adaptive strategies in transepithelial salt transport. Ph.D. thesis, Cambridge University, UK.
- GIBSON, J. S., ELLORY, J. C. & COSSINS, A. R. (1985). Temperature acclimation of intestinal Na transport in the carp (*Cyprinus carpio* L.). *J. exp. Biol.* **114**, 355–364.
- GRAF, J. & GIEBISCH, G. (1979). Intracellular sodium activity and sodium transport in *Necturus* gallbladder epithelia. *J. Membr. Biol.* **47**, 327–355.
- HIRANO, T. (1974). Some factors regulating water intake by the eel, *Anguilla japonica*. *J. exp. Biol.* **61**, 737–747.
- HOLETON, G. F. (1974). Metabolic cold adaptation of polar fish: fact or artifact? *Physiol. Zool.* **47**, 137–152.
- HOLMES, W. N. & DONALDSON, E. M. (1969). The body compartments and distribution of electrolytes. In *Fish Physiology*, vol. 1 (ed. W. S. Hoar & D. J. Randall), pp. 1–89. New York: Academic Press.
- HOUSE, C. R. & GREEN, K. (1965). Ion and water transport in isolated intestine of the marine teleost, *Cottus scorpius*. *J. exp. Biol.* **42**, 177–189.
- LAU, K. R. (1982). Active chloride transport in fish. Ph.D. thesis, Cambridge University, UK.
- LORETZ, C. A. (1983). Ion transport by the intestine of the Goby, *Gillichthys mirabilis*. *Comp. Biochem. Physiol.* **75A**, 205–210.

- O'GRADY, S. M., ELLORY, J. C. & DEVRIES, A. L. (1983). The role of low molecular weight antifreeze glycoproteins in the bile and intestinal fluid of antarctic fish. *J. exp. Biol.* **104**, 149–162.
- PRECHT, H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of coldblooded animals. In *Physiological Adaptations* (ed. C. L. Prosser), pp. 50–77. Washington: American Physiological Society.
- PROSSER, C. L., MACKAY, W. & KATO, K. (1970). Osmotic and ionic concentrations in some Alaskan fish and goldfish from different temperatures. *Physiol. Zool.* **43**, 81–89.
- RAMOS, M. M. P. & ELLORY, J. C. (1981). Na and Cl transport across the isolated anterior intestine of the plaice *Pleuronectes platessa*. *J. exp. Biol.* **90**, 123–142.
- REUSS, L. (1981). Antibiotics as tools for studying the electrical properties of tight epithelia. *Fedn Proc. Fedn Am. Socs exp. Biol.* **40**, 2202–2205.
- SCHOLANDER, P. F., VANDAM, L., KANWISHER, J. W., HAMMEO, H. T. & GORDON, M. S. (1957). Supercooling and osmoregulation in Arctic fish. *J. cell. comp. Physiol.* **49**, 5–29.
- SMITH, M. W. (1976). Temperature adaptation in fish. *Biochem. Soc. Symp.* **41**, 43–60.
- SMITH, M. W. & ELLORY, J. C. (1971). Temperature-induced changes in sodium transport and Na⁺/K⁺-adenosine triphosphatase activity in the intestine of goldfish (*Carassius auratus* L.). *Comp. Biochem. Physiol.* **39A**, 209–218.
- WODTKE, E. (1981). Temperature adaptation of biological membranes. Compensation of the molar activity of cytochrome *c* oxidase in the mitochondrial energy-transducing membrane during thermal acclimation of the carp (*Cyprinus carpio* L.). *Biochim. biophys. Acta* **640**, 710–720.