interesting because it suggests that this nucleotide, apart from providing the necessary energy for active transport, also plays a role in promoting the cyclic changes in selectivity for these ions required by most active transport schemes.

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Na^+ transport across the isolated skin of *Ambystoma mexicanus*

In contrast to the numerous studies of electrolyte transport across anuran skin, little work has been done on the skin of urodeles[1,2]. We describe the Na^+ transport of isolated skins of adult *Ambystoma mexicanus*, a system with some important differences from the frog skin.

Metamorphosis of larval Ambystoma (60–120 g) was induced by the intramuscular injection of 100 μg of thyroxine. After metamorphosis their skins were mounted between two lucite half chambers (area 3.14 cm^2)[3]. Symmetrical calomel half cells, connected through 3 M KCl–agar bridges to the chambers, were used for potential recording and current delivery. The main solutions employed were Cl^- Ringer (115 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, 3 mM Tris–maleate buffer, pH 7.5) and SO_4^{2-} Ringer (77 mM Na_2SO_4, 1.25 mM K_2SO_4, 8 mM $CaSO_4$, 3 mM Tris–maleate buffer, pH 7.5).

The outer surface of the skin immersed in Cl^- Ringer was 59.5 ± 8.2 mV (± S.E., $n = 14$) negative with respect to the inner surface. Short circuit current (s.c.c.) mean value was 26.2 ± 9.7 μA/cm^2. To study the dependence of the potential on the cations bathing the skin, we tried to reduce the short circuiting effect of Cl^- by replacing it with SO_4^{2-}. Contrary to what has been observed in frog skin, SO_4^{2-} markedly depressed s.c.c. and potential. In 12 experiments, complete substitution of

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Abbreviation: s.c.c., short circuit current.

Cl^- by SO_4^{2-} on both sides resulted in a 78.3 % drop in s.c.c. and a 66.8 % reduction of potential. Fig. 1 shows that it was only necessary to replace Cl^- by SO_4^{2-} at the external surface of the skin to observe an effect. Of the other anions used to substitute Cl^-, glutamate and methyl sulphate were as effective as SO_4^{2-}, while Br^- and I^- had little or no effect.

To determine the ionic basis of the short circuit current, measurements were made of the $^{22}Na^+$ and $^{36}Cl^-$ fluxes. The data are summarized in Table I. Paired skins of the same animal were used to compare efflux and influx. The results show that Na^+ influx, both in Cl^- and SO_4^{2-}, exceeded the s.c.c. by 1–3 μA. In those experiments

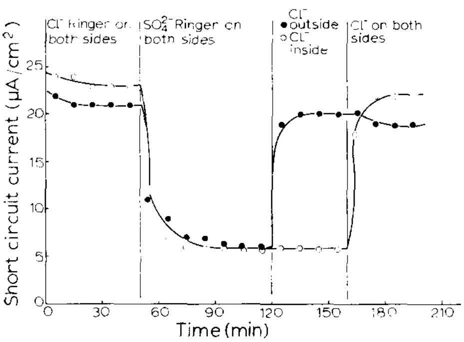

Fig. 1. The effects of Cl^--free solutions on s.c.c. across the skin of *A. mexicanus*. The effects of SO_4^{2-} on both sides on a pair of skins were first studied, then SO_4^{2-} was substituted by Cl^- at the external side of one skin and at the internal side of the other, finally both sides were again immersed in Cl^- Ringer.

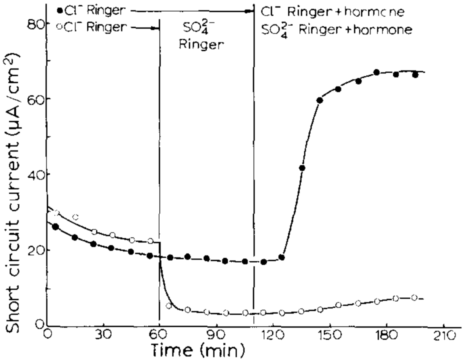

Fig. 2. The effects of arginine vasopressin (0.1 unit/ml) were tested on s.c.c. of a pair of skins, one of which had been previously immersed in SO_4^{2-} Ringer.

TABLE I

ION FLUXES IN SKINS OF ADULT *A. mexicanus* EXPRESSED IN μA/cm²

Every flux value is the average of measurements during 3 successive 30-min periods. S.c.c. differences between paired skins were never larger than 20 %.

Expt. No.		*Influx*	*Efflux*	*Net flux*	*S.c.c. (μA/cm²)*
1	$^{22}NaCl$	24.3	0.3	24.0	22.5
2		22.5	1.87	20.6	19.5
3		16.6	3.18	13.5	13.7
4		24.3	—	—	23.2
5		20.5	—	—	17.1
1	$^{22}Na_2SO_4$	4.5	—	—	3.1
2		4.3	—	—	2.4
3		3.2	—	—	2.2
4		2.8	—	—	1.2
1	$Na^{36}Cl$	2.75	2.1	0.6	21.6
2		2.8	1.3	1.5	28.0
3		1.8	1.0	0.8	26.3
4		3.5	1.1	2.4	36.6

where efflux was also measured, there was good agreement between s.c.c. and net Na^+-flux values. Table I also shows that the asymmetries in Cl^- fluxes were too small to contribute significantly to s.c.c.

Although BENTLEY AND HELLER[1] could not obtain a response to arginine vasopressin (112 μmoles/l) in 4 isolated skins of adult *Ambystoma tigrinum*, we tried the hormone on 4 paired skins of *A. mexicanus* (Fig. 2). In the skins immersed in Cl^- Ringer, 0.10 unit/ml (about 250 μmoles/l) of arginine vasopressin increased s.c.c. from 12.05 ± 6.4 μA/cm^2 ($\pm$ S.E.) to 47.7 ± 11.4 μA/cm^2 ($t < 0.01$). However, when pieces of skin of the same 4 animals were studied in SO_4^{2-} Ringer, the hormone only increased s.c.c. from 2.03 ± 2.2 μA/cm^2 to 4.8 ± 1.9 μA/cm^2 ($t < 0.2$).

We also found that the uptake of ^{22}Na (measured as in refs. 4 and 5) by skins in SO_4^{2-} Ringer is about 50% of the uptake in Cl^- Ringer*. If the trajectory of the Na^+ transported across the skin first involves the passage of Na^+ from the external surface into a transport compartment and then the active transport of Na^+ to the internal surface of the skin, this result suggests that SO_4^{2-} solutions interfere with the entry of Na^+ into the skin.

A Na^+ transport mechanism requiring Cl^- occurs in the gall bladder[6]. In this tissue the active transport of one Na^+ is directly coupled with the active transport of one Cl^-, *i.e.* the Na^+ transport does not generate a potential. The salt absorption mechanism of the skin of *A. mexicanus* is different, Cl^- is necessary for the independent transport of Na^+ and it seems that the entrance of Na^+ into the transport compartment is the Cl^--requiring step.

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* The term uptake refers to the amount of radioactive Na remaining in the tissue after exposure of the external surface of the skin to ^{22}Na for a given time. It should not be confused with the influx from the epidermal to the dermal side of the skin.