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Effect of Cyclic Aromatics on Sodium Active Transport in Frog Skin

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Isolated abdominal frog skin, when mounted in an in vitro chamber, actively transports sodium from the pond-side bathing solution to the serosal-side when the skin is chamber-mounted. Active transport of sodium causes an electrical potential difference (PD), up to 140 mV, that is proportional to the log of the pond-side sodium concentration (Koefoed-Johnsen and Ussing 1958). When the solution bathing each side of the skin is identical and enough external current is applied to change the PD to zero, the skin is "short-circuited". Under short-circuit conditions, no driving force for ions or water exists across the skin. Thus, any net flux of ions (or water) must be due to processes internal to the skin and equal to the short-circuit current (ISC). Ussing and Zerahn (1950) showed that the ISC correlated well with the measured net sodium flux. Diuretics lower the ISC whereas anti-diuretics increase ISC (and sodium transport). Amiloride, a potent diuretic in all vertebrates, causes the ISC to decrease to zero whereas arginine vasopressin, the amphibian anti-diuretic hormone, causes ISC to increase.

In a previous study, Blankemeyer and Hefler (1990) showed that naphthalene increases Na^+ active transport of the frog skin with an EC50 of 4.4 mg/L. They also showed that the probable site of action of naphthalene was at the pond-side membrane of the frog skin. We have investigated the effect of other cyclic organics on the active transport of sodium by the frog skin.

MATERIALS AND METHODS

Frogs, Rana pipiens, were purchased from Wm. Lemberger (Oshkosh, Wisconsin) and kept unfed in tap water (pH 7, 20° C, 146 mg/L hardness) until use. Generally the

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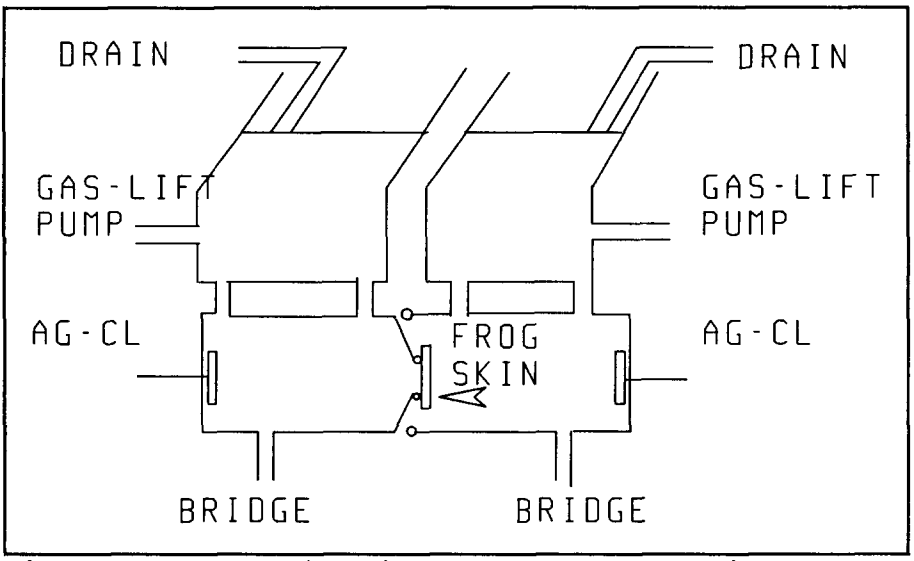

Figure 1. Schematic Diagram of glass Ussing-chamber illustrating placement of frog skin relative to the gas-lift pumps, salt-bridges, and silver chloride wires.

frogs were used within 2 wk of arrival. Frogs were anesthetized by injection of 10% urethane into the dorsal lymph sac, then euthanized by decapitation. All animal procedures were performed in strict concordance with the AVMA accepted methods for euthanasia (Smith et al 1986). The frog's abdominal skin was excised and placed in frog Ringer's solution that contained 110 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, 2.5 mM TRIS buffer, adjusted to pH 8.3. Solutions containing organics were freshly prepared for each experiment. Organics were dissolved in DMSO. DMSO and the organic were added (1% DMSO v/v) to the frog Ringer's. The control Ringer's contained 1% DMSO.

A modified glass Ussing-chamber was used to mount the skin (Figure 1). The PD was measured by two 3% agar-frog Ringer's bridges. Current (i.e. ISC) was passed by Ag-AgCl electrodes placed so that current density was uniform across the skin. Ringer's solution, bathing each side of the frog skin, was stirred and aerated by gas-lift pumps. Drains were connected to vacuum lines. The frog skin was tied onto the chamber lip with surgical thread. An automatic voltage clamp, outputting the ISC required, maintained the PD of the frog skin at zero mV. ISC was recorded from a digital panel meter on the voltage clamp.

The effect of toxicants on the ISC was determined by using the 15 min prior to toxicant administration as a control period, then calculating the change in ISC

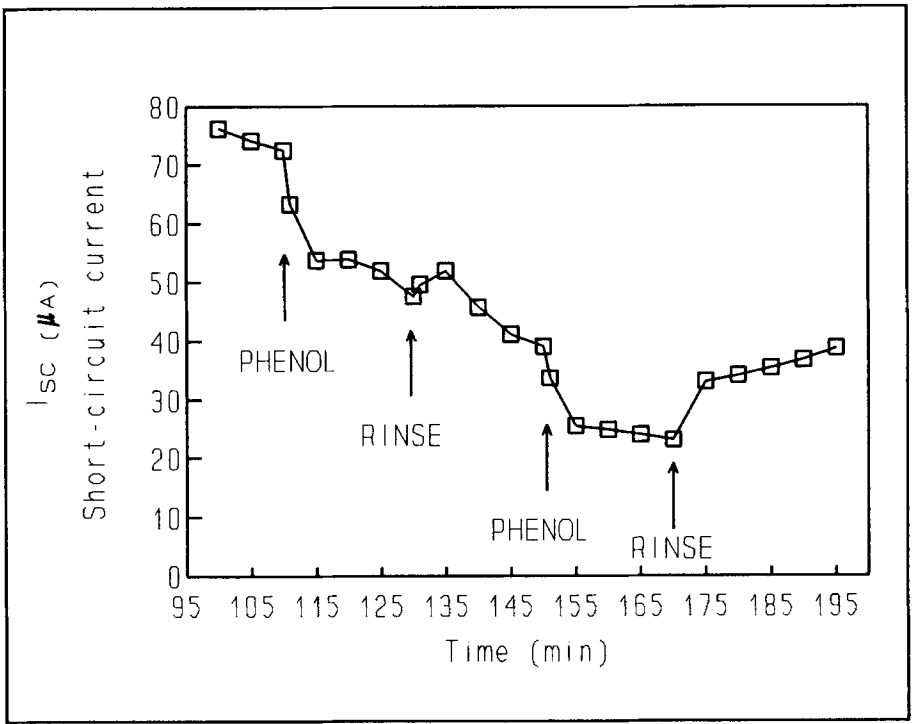

Figure 2. Time-course of an in vitro frog skin experiment plotting ISC vs time. Phenol Ringers replaced control Ringer's at 110 min and 150 min(PHENOL). Control Ringer's replaced phenol Ringer's at 130 min and 170 min (RINSE).

during the toxicant period as a percent of the control ISC. Data were collected from at least four skins for each toxicant concentration. Statistical significance was determined by calculating 't' values and evaluating at the 95% level.

RESULTS AND DISCUSSION

Phenol comprises a large component of oilfield waste (Anderson et al 1974). We investigated the effect of phenol on Na^+ active transport by the frog skin. As described in Methods the skin was maintained at zero mV PD and the ISC recorded. Figure 2 describes an experiment in which 0.1% (v/v) phenol Ringer's (on the external or pond-side of the frog skin) replaced the control Ringer's after an equilibration period of 110 min. The ISC decreased 36% during the first 20 min after the change to 0.1% phenol Ringer's. At 130 min the phenol solution was replaced by control frog Ringer's to test whether the ISC would recover to pre-phenol levels. As can be observed in the period from 130 to 150 min there was an incomplete recovery (i.e.

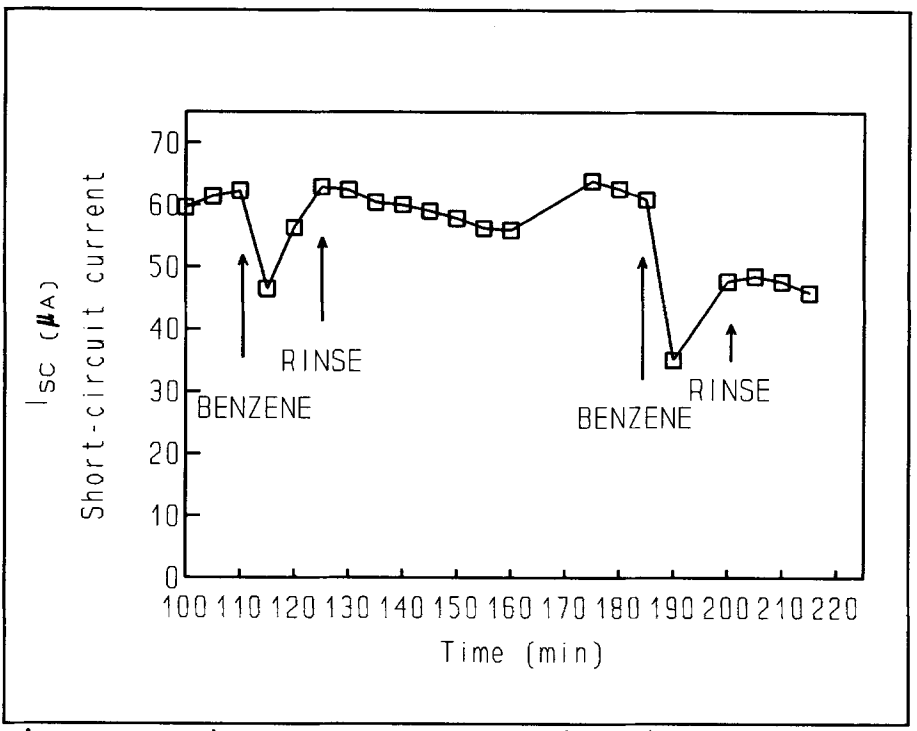

Figure 3. Time course of an *in vitro* frog skin experiment plotting ISC vs time. Benzene Ringer's replaced control Ringer's at 110 and 185 min(BENZENE). Control Ringer's replaced benzene Ringer's at 125 and 205 min (RINSE).

ISC did not recover to pre-phenol levels). At 150 min the control Ringer's solution was replaced again by 0.1% phenol Ringer's. In this second trial on the same skin, there was a greater response, a 45% decrease in ISC, and a partial recovery after the rinse with control frog Ringer's at 170 min. This experiment suggests that the effect of phenol is to reduce ISC and that the effect of phenol is complex and somewhat irreversible. Based on the agreement between ISC and net Na^+ transport, phenol decreased ISC and thus net Na^+ transport.

Another component of crude oil and crude oil waste is benzene. In the experiment depicted in Figure 3, a frog skin was equilibrated for 110 min in control Ringer's. At 110 min benzene Ringer's replaced the pond-side control Ringer's. The ISC decreased rapidly from 62 μA to 45 μA, a 29% decrease. ISC started to recover to pre-benzene values at 120 min, before the rinse with control Ringer's. At 125 min, the benzene Ringer's was replaced with control Ringer's. The recovery of the ISC to 60 μA was 99% of the

Table 1. Effect of Benzene, Phenol, and Phenanthrene on Short-Circuit Current (ISC) of Frog Skin.

Chemical	Concentration	Percent Change	p value
Benzene	0.1 % v/v	-29 ± 7.3	0.03
Phenol	0.1 % v/v	-37.2 ± 5.2	0.002
Naphthalene	30 mg/L	+7	*
Phenanthrene	100 mg/L	+2.6 ± 6	0.69

* Naphthalene data from Blankemeyer and Hefler (1990). Data is reported as percent change ± S.E.M. Probability 'p' value calculated using t tables. Each data point is the mean of at least four frog skins.

pre-benzene value of ISC suggesting that the benzene effect is reversible. A second trial was performed at 185 min again using 0.1% benzene. The percentage decrease in ISC was 48% and the recovery during the rinse at 200 min was incomplete. This experiment showed that benzene has a rapid and reversible effect on ISC. As in the case of phenol, benzene caused a decrease in ISC and net Na^+ transport.

Phenanthrene was tested for its effect on frog skin active transport of sodium using the same protocol as described for phenol and benzene. Phenanthrene had no statistically significant effect on ISC. Results obtained for benzene, phenol, and phenanthrene are summarized in Table 1. Only phenanthrene had no consistent effect on ISC. Naphthalene data (Blankemeyer and Hefler 1990), included for comparison, caused an increase in ISC. Benzene and phenol, as exemplified in Figures 2 and 3, caused statistically significant decreases in ISC and by inference sodium active transport. Phenanthrene had no statistically significant effect on ISC albeit an increase in ISC was suggested.

The experiments described here show that the effect of organics on sodium active transport of an epithelium is to alter the active transport of sodium ions. We speculate that epithelia and the animals exposed to cyclic organics would show some dysfunction in sodium and/or water transport.

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