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Differences in the physiological characteristics of bladders of toads from different geographical sources

The urinary bladder of the toad, *Bufo marinus*, has been used for many studies *in vitro* of transport of water and electrolytes across an epithelial membrane. Using this preparation many features of osmotic movement of water and transport of Na^+, and of the effects of vasopressin and aldosterone have been studied.

Results of such studies by different workers in recent years have been at variance in small but important details. Two of these concern the transport of Na^+ and Cl^-. The toad bladder has been thought to transport actively only Na^+ (ref. 1). However, FINN, HANDLER AND ORLOFF[2,3] have found that when they exposed toad bladders to K^+-free solutions, the membrane potential and short-circuit current not only fell rapidly to a low value but became negative with mucosa positive to serosa. They also showed that this reversal of potential and current was due to active transport of Cl^- which could be abolished by the addition of dinitrophenol and cyanide. Previous studies in this laboratory have shown that, under these K^+-free conditions, Na^+ transport is essentially abolished, but they did not show, however, any reversal of potential or short-circuit current[4,5]. On inquiry, we found that the toads used in these different studies come from different sources. All are nominally *Bufo marinus*. This laboratory has used toads originating in the Dominican Republic (National Reagents, Inc., 2161 Main St., Bridgeport 6, Conn.) whereas FINN, HANDLER AND ORLOFF[2] used toads originating in the area around Baranquilla in Northern Colombia (The Pet Farm, 3310 N.W. South River Drive, Miami 42, Fla.). Because of their different origins a study of the behavior of bladders from both Colombian and Dominican toads has been carried out simultaneously and under identical conditions.

Bladders were removed from the toads, after double pithing, and mounted between lucite chambers. Measurements of potential and short-circuit current across the toad bladder were made by the method of USSING AND ZERAHN[6]. The bladders were incubated, initially, in a frog-Ringer's solution containing 113 mM NaCl, 3.5 mM KCl, 2.4 mM $NaHCO_3$ and 0.89 mM $CaCl_2$. pH in air of this solution was 8.0 and the total solute concentration, 220 milliosmoles/kg of water. After an initial stabilization period this solution was changed to a K^+-free solution of the following compo-

TABLE I

POTENTIAL DIFFERENCE AND SHORT-CIRCUIT CURRENT IN TOAD BLADDERS EXPOSED TO K^+-FREE BATHING SOLUTIONS

	Number	*Colombian*	*Number*	*Dominican*
Mean potential (mV)	8	−7.9	14	+1.5
Range		+1.0 to −17.0		0.0 to 3.6
Mean current (μA)	6	−30	14	+7
Range		−19 to −49		1 to 26
Mean duration of K^+-free conditions (min)	8	138	14	153
Range		70 to 260		65 to 255

sition: 116.5 mM NaCl, 2.4 mM $NaHCO_3$ and 0.89 mM $CaCl_2$. pH in air of this solution was 8.2 and the total solute concentration, 220 milliosmoles/kg of water. The chambers and bladders were rinsed 3 times in this solution to remove residual K^+ as completely as possible. In some experiments only potential, and in others, potential and short-circuit current, were measured before and after the change to K^+-free bathing solutions. In four experiments with Dominican toads a "high-bicarbonate" Ringer's solution of the following composition was used: 92 mM NaCl, 25 mM $NaHCO_3$, 0.89 mM $CaCl_2$. No difference in response was observed.

The results of these experiments are shown in Table I. Removal of K^+ from the bathing medium quickly lowered both potential and short-circuit current. In the case of bladders from Dominican toads the potential stabilized at a few mV, serosa positive, and did not reverse. Bladders from Colombian toads, on the other hand, reversed their potential, the serosa becoming negative to the mucosa, thus confirming the findings of FINN, HANDLER AND ORLOFF[2,3]. Such bladders maintained a reversed short-circuit current for up to 6 h; the reversal was rapidly eliminated by anaerobiosis. Two experiments illustrating these effects and showing the rapidity of the reversal of current in the Colombian toads are shown in Fig. 1.

This finding, that bladders from toads of the same species can give such different experimental results under similar conditions, led us to consider another experimental situation where conflicting results have been obtained.

In studies on the effects of aldosterone on Na^+ transport, dramatic responses to the addition of exogenous substrates have been observed[7,8]. In toad bladders which are depleted of endogenous substrate by prolonged incubation in substrate-free Ringer's solution the addition of glucose or pyruvate results in a prompt and sustained stimulation of Na^+ transport. In the absence of aldosterone different results have been reported. We have observed no stimulation of Na^+ transport upon addition of metabolic substrates in the absence of aldosterone. EDELMAN, BOGOROCH AND

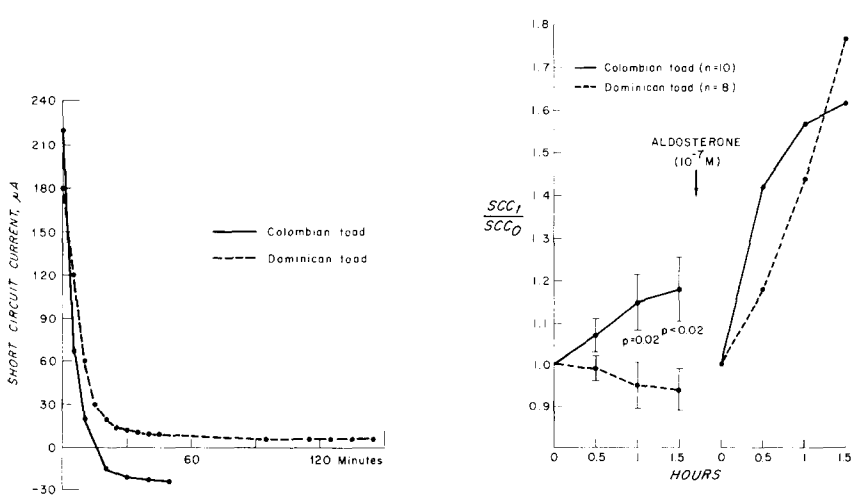

Fig. 1. The effect of K^+-free Ringer's solution on the short-circuit current in bladders of Colombian and Dominican toads.

Fig. 2. The effect of 5 mM sodium pyruvate on Na^+ transport in the absence and presence of aldosterone for Colombian and Dominican toads. SCC, short-circuit current.

PORTER[9], however, have reported that the addition of pyruvate after 15-h incubation in substrate-free media increases Na^+ transport by 40–50 %. Differences in technique and handling were found not to account for the different results. Consequently, a study of the response to substrates was carried out simultaneously and under identical conditions using both Colombian and Dominican toads.

Toads in this second study were maintained partially immersed in 0.6 % saline solution for 48 h prior to use so as to decrease the endogenous secretion of aldosterone. Bladders were mounted between lucite chambers using the technique described by PORTER AND EDELMAN[7]. After 15 h of incubation, 5 mM pyruvate was added to the Ringer's solution bathing both surfaces of the bladder and the short-circuit current recorded automatically for 1.5 h. The substrate was then removed from the chambers, by rinsing and refilling with fresh Ringer's solution, and (+)-aldosterone ($1 \cdot 10^{-7}$ M) added to the solution bathing the serosal surface of the bladder. 3 h later pyruvate was again added and the response monitored.

The results are shown in Fig. 2. In the absence of aldosterone, pyruvate elicits a stimulation of sodium transport only in bladders of toads originating in Colombia. No increase in transport occurred in bladders from Dominican toads. In the presence of aldosterone a typically large increase in the rate of Na^+ transport occurred in all bladders tested.

The results described here show that, under certain conditions, toad bladders have different characteristics depending upon the origin of the toads. In seeking the reasons for these differences we have considered both environmental and possible species differences. The Colombian toads are obtained from the area round Baranquilla and live in brackish swampland. The Dominican toads have been introduced into the island from mainland South America, as into many sugar-cane-growing countries to combat the sugar-cane beetle*. They were established in the Dominican Republic by 1938 and probably originated in the Amazon region of Northern Brazil. The toads, which we have used, are slightly different in appearance though still included in the species, *Bufo marinus*. Though it is not clear at this time whether the differences are due to environmental or hereditary factors it seems important to point out that toad bladders can no longer be considered a uniform biological model and that the origin of the toads must be considered.

In summary, urinary bladders of the toad, *Bufo marinus*, obtained from Colombia and from the Dominican Republic differ in their behavior under conditions *in vitro*. In K^+-free Ringer's solution bladders from Colombian toads, but not Dominican toads, quickly exhibit reversal of their membrane potential and short-circuit current due presumably to active chloride transport. Bladders of Colombian toads that have been depleted of endogenous substrate in the absence of aldosterone respond to the addition of pyruvate by an increase in Na^+ transport. Under identical conditions, bladders of Dominican toads do not respond to added substrate. In the presence of aldosterone the addition of pyruvate causes a marked increase in Na^+ transport in bladders from toads of both sources.

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