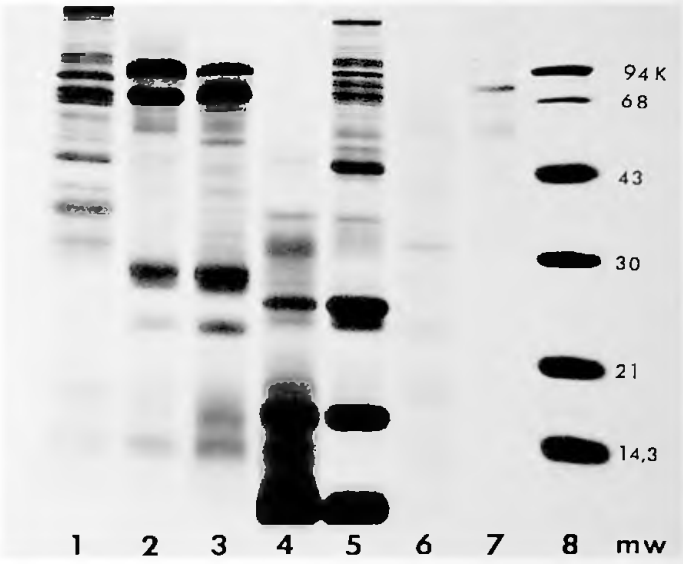
pup epithelia but absent in the adult dogfish. Since the sculpin and pup corneas are very active metabolically in comparison to the much less active dogfish adult cornea (MDIBL Bulletin 18:38-40, 1978), it is tempting to postulate that these prominent high molecular weight bands are related to the metabolic machinery of these active corneas. In the dogfish adult most soluble protein is of low molecular weight. All three epithelia share at least two major protein bands (51K and 41.6K). In the dogfish three very striking bands are shared by the adult and pup at 27.5K, 16.5K and 10.7K. These three bands alone account for nearly 30% of the total soluble protein in the dogfish epithelium.



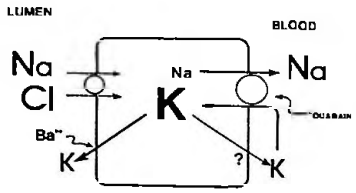
Stromal preparations are shown in lanes 2,3,6 and 7. The sculpin stroma, both inner and outer layers, is characterized by two very prominent bands at 79.4K and 69.9K. These two proteins account for one-third of total soluble protein in sculpin stroma. At least one major protein seems to be present both in sculpin and shark stroma. It has an apparent molecular weight of 25K.

Though none of the samples studied shows a major protein band at 54K, the presence of BCP54 cannot be ruled out in these corneas. The prominent band seen in sculpin and dogfish epithelia at 51K certainly could be the 54K or a closely related protein. Future studies using immunochemical methods will make the final determination.

BASOLATERAL POTASSIUM CONDUCTANCE IN FLOUNDER URINARY BLADDER

David C. Dawson and David Andrew, Department of Physiology, University of Michigan Medical School, Ann Arbor, Mi. and Cornell University, Ithaca, NY

We reported previously that the urinary bladder of the Winter Flounder actively secretes potassium and that net potassium flow can be measured directly as the short circuit current (I_{sc}) produced by the bladder in vitro (Dawson and Andrew, Bull. M.D.I.B.L. 20:89 1980). The results of previous experiments can be conveniently summarized by the simple model shown in Figure 1 in which potassium secretion is envisioned as consisting of two steps: potassium entry across the basolateral membrane via an electrogenic Na/K exchange pump and potassium exit from the cell via a barium-sensitive potassium channel in the apical membrane. Mucosal barium reversibly blocks I_{sc} and net K secretion, presumably by blocking an apical potassium channel. The present experiments were undertaken to investigate the possibility that barium might be used to identify a potassium conductance in the basolateral membrane.



If the basolateral membrane of the potassium secreting cells of the urinary bladder contains a barium-sensitive channel then the simple scheme depicted in Figure 1 would suggest that the addition of barium to the serosal bath might increase potassium secretion. In fact, however, serosal barium has little, if any, effect on I_{sc} (data now shown). This result could be interpreted as indicating the lack of barium-sensitive potassium channels on the basolateral membrane. Alternatively, the absence of a serosal Barium effect could reflect the fact that the ratio of the potassium conductances of the apical and basolateral membranes in a secreting bladder is already so high that a further decrease in the basolateral conductance does not augment secretion.

As a more direct test for a basolateral potassium conductance we attempted to induce "reverse" potassium flow through secreting cells by applying a transmural (M to S) potassium gradient. A transmural potassium gradient was produced by simply adding from 15 to 30 mM potassium to the mucosal bath in a small volume of 3M KCl. In bladders which did not exhibit a spontaneous I_{sc} this maneuver produced little or no response, suggesting that in such tissues the apical potassium conductance is reduced or absent. In bladders exhibiting a spontaneous I_{sc} (positive in the S to M direction) the response was typically a dramatic, transient reversal of I_{sc}. All of the responses elicited could be separated into two groups, representative examples of which are shown in Fig. 2.

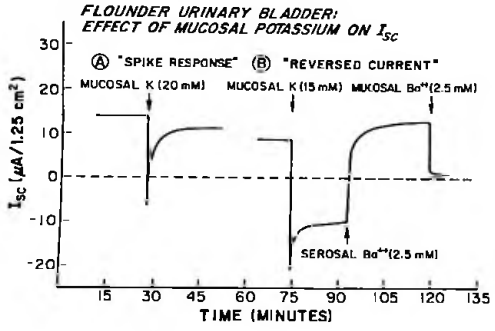


Figure 2

Most frequently the response of I_{sc} was similar to that shown as trace "A", a "Spike" of reversed I_{sc} which returned relatively rapidly to its original orientation at a reduced magnitude. In about 30% of the bladders tested, however, the response was as shown in trace "B"; an initial spike and subsequent decline to a new steady state in which the direction of current flow was from M to S. This "reversed" I_{sc} was abolished by mucosal barium (data not shown) as expected if the reversed I_{sc} represents net potassium flow from M to S through the cells. Serosal barium also had a dramatic effect on the reversed current, causing it to reverse a second time and assume a new steady-state in which the I_{sc} was again consistent with net potassium secretion. This current, now in the secretory direction despite the M to S potassium emf of about 40 mV, was abolished by mucosal barium (Fig. 2) and also by serosal ouabain (data not shown). These results suggest that in some

bladders it is possible to induce net potassium flow from M to S through the same cells which are the normal route of active potassium secretion. The effects of serosal and mucosal barium ions suggest a simple model for these cells in which the rate and direction of net potassium movement is determined by the balance between metabolic and electrochemical driving forces as well as the ratio of the apical and basolateral potassium conductances.

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90 CARBONIC ANHYDRASE IN URODELES

Thomas H. Maren and Christopher G. Azar, University of Florida College of Medicine, Gainesville, Fla., and Cornell University, Ithaca, N.Y.

Red cell and renal carbonic anhydrase in Class Amphibia generally has been held analagous in amount and function to that in birds and mammals (Physiological Reviews, Vol. 47 p. 595, 1967), although the red cell enzyme in frogs is quite different in type from those of man (Bundy and Cheng, Comp. Biochem. Physiol., Vol. 55 B, p. 265, 1976). However, little attention has been given to the class Urodela (= Caudata), with the exception of the work of Toews (Comp. Biochem. Physiol., Vol. 59 A, p. 211, 1978) who showed an enormous range of enzyme activity in Amphibia, with very low values in red cells of urodeles, and kidney enzyme present in reasonable amounts in all species studied.

We looked for carbonic anhydrase in red cells and other organs of Plethodon cinereus in connection with attempts to elicit defects in the regenerating limb of this salamander (Bull. MDIBL, Vol. 20, p. 24, 1980). Our micromethod was used (Journal of Pharmacology and Exp. Ther., Vol. 130, p. 26, 1960) with barbital buffer; in this assay human red cells have 20,000 enzyme units/ml. In P. cinereus, whole blood ranged from the limit of the method (about 20 units/ml) to 120 units/ml. Mean $\pm$ S.E. was 39 $\pm$ 30 (n = 14). Although these small amounts of enzyme made detection difficult, the findings were validated because 10^{-6} M methazolamide inhibited the activity. Another species of the family Plethodontidae, which is characterized by the absence of both lungs and gills in the adult stage, was examined (Pseudotriton ruber) and found to have about 2000 units/ml. Two species of the family Ambystomidae also had small but definite amounts of enzyme. Most surprising, was the absence of blood enzyme in Necturus maculatus, in agreement with Toews (vide supra). This is the sole vertebrate ever to show such a finding. Data are shown in Table 1, along with comparison to representatives of other classes of vertebrates.

TABLE 1

Organism		Carbonic Anhydrase in red cells, units/g
Man and Other Mammals		~ 20,000
Birds (chicken, gull)		~ 14,000
Reptiles:		
Turtle:	Chrysemys and Chelydra sp.	18,000
Alligator:	A. mississipiensis	2,000
	C. laitrostris	
Amphibia:		
Anura:	R. clamata and R. catesbiana	4,000
Urodela:	P. cinereus	39
	P. ruber	2,000
	A. tigrinum	500
	A. maculatum	2,500
	N. maculosus	< 20
Osteichthyes		5,000 - 20,000
Chondrichthyes, S. acanthias		7,000
Agnatha, M. glutinosa		1,000