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

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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

Since the role of carbonic anhydrase is to assure the rapid dissimilation of the substrates $HCO_3^- = CO_2$ across small gradients, it is tempting to associate the near-absence of enzyme in many of the small skin respiring urodeles with the fact that the gradient of CO_2 from the blood to outside air is very large, about 40 mm Hg. The equivalent gradient in mammals is about 6 mm Hg, that from pulmonary capillary to alveolus. In fish, the gradient may also be small, particularly in deep waters. In birds and reptiles, the air capillaries, although an open system unlike alveoli, still may exchange CO_2 from HCO_3^- more effectively if the enzyme is present. Tentatively, we may say that the larger the gradient and the relative surface area, the lower the metabolism, the less need for the enzyme. The latter factor may dominate in the special case of Necturus, which does have gills.

The enzyme activities given in Table 1 also reflect different isozymes, a problem discussed elsewhere (Comp. Biochem. Physiol., Vol. 67 B, p. 69, 1980). Our present studies on the teleost enzyme also show the progression to a 'high activity' enzyme in Osteichthyes (Sanyal, Pessah and Tashian, In Preparation), and it is clear that birds and frogs also have carbonic anhydrases with high turnover numbers (Bundy, Comp. Biochem. Physiol. 57 B, p. 1, 1977). Turtles also have a high activity enzyme (Hall and Schraer, Comp. Biochem. Physiol., Vol. 63 B, p. 561, 1979). It is likely that alligators, urodeles, sharks, and hagfish have low specific activity carbonic anhydrases: this remains to be worked out, although the direction seems clear from Table 1.

Urodeles have renal carbonic anhydrase in small concentrations, mean $\pm$ S.E. = 36 enzyme units/g $\pm$ 9 (n = 11) in P. cinereus, 100 e.u/g in A. tigrinum and 45 e.u/g in Necturus. All were inhibited by 10^{-6} M methazolamide. Renal enzyme is present in vertebrates above the level of sea-going fish; it is absent from kidneys of the salt water types of the last 3 classes of the Table. The general idea developed by Homer Smith and the senior author seems to hold: that renal carbonic anhydrase arose in fresh water fish in association with lability of urinary pH. The enzyme in kidney then persists through all vertebrate evolution to catalyze the reaction $CO_2 + OH \rightleftharpoons HCO_3^-$ and its ultimate consequence, the excretion of protons or bicarbonate.

We found carbonic anhydrase in testes of P. cinereus, 25 e.u/g $\pm$ 3 (n = 6). Mammals have the enzyme in several segments of the male reproductive system (Leiter, Investigative Urology, Vol. 2, p. 58, 1964). The phylogeny and function of carbonic anhydrase in male sex organs remains to be worked out. Supported by NIH Grant HL-22258.

ULTRASTRUCTURE OF EXTRACELLULAR MATERIAL OF THE SINGLE CELL-LAYERED HEART OF BOLTENIA OVIFERA

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The tubular heart of the tunicate Boltenia ovifera is comprised of a single cell-layered myoepithelium (Morad, et al., MDIBL Bulletin 12, 1972). Its electrically inexcitable extraluminal surface faces the pericardial fluid and is lined with a cell coat and microvilli, whereas its luminal surface contains the electrically active membrane overlain with a cell coat and a basement membrane, 1-3μm thick (Nunzi and Morad, MDIBL Bulletin 18, 1978). We have enhanced the preservation and staining of the basement membrane and cell coat by including cationic dyes in the initial fixation solutions (Robinson, Cell Tiss. Res. 211, 353, 1980) and have then analyzed their structure and the topography of the luminal surface of the basement membrane by means of scanning and transmission electron microscopy.

Materials and Methods. The animal was placed in a dissecting tray, siphons facing down. The outer tunic was cut and peeled back to reveal the transparent, intact inner tunic closely apposed to the heart contained within the pericardial tube. Fixative, consisting of buffer (either sea water or 0.2M cacodylate buffer osmotically corrected with NaCl to approximately 950 mosmols), 6% glutaraldehyde, and a cationic dye (0.1% ruthenium