To determine the underlying reasons for the observed differences in the organic anion secretory abilities of the two species, we examined the PAH transport characteristics of two components of the Jonah crab renal system (bladder and labyrinth) and compared the findings to those for rock crab. Unidirectional fluxes of labeled PAH across excised Jonah crab bladder tissues were measured in the same dual flux chamber that had been used for rock crab bladders (Holliday and Miller op. cit.). Paired bladder sheets were excised from pithed Jonah crabs and mounted between pairs of cover slips with centered, beveled-edge holes which exposed 0.283 cm^2 of bladder. Thus mounted, tissues were clamped between the halves of the flux chamber. Separate, 1.0 ml aliquots of CR bathed each side of the two tissues and were aerated and stirred (4 ml/min) by a four-channel peristaltic pump at 18-20°C. Labeled and unlabeled PAH (10 μM final concentration) was added to the CR bathing the luminal side of one tissue and to the serosal side of the other tissue. Unidirectional PAH fluxes were calculated from the rate of appearance of label in the two chambers which were originally isotope-free. Under these conditions Jonah crab bladder showed a significant, net secretory (S → L) flux of PAH (Fig. 2). Addition of 1 mM bromocresol green (BCG, a competitor organic anion, which, at 1 mM, blocks concentrative PAH uptake in bladder slices) to both the serosal and luminal baths reduced the S → L flux to the same level as the control L → S flux (BCG data not shown). Note, that because of this net secretory flux, the Jonah crab bladder may be a useful model for the vertebrate proximal renal tubule. In contrast, net reabsorption was found in intermoult rock crab bladder (Holliday and Miller, op. cit.). Thus, the difference in degree of PAH secretion in these two crabs is related to a difference in the transport function of the bladders. Further, our preliminary micropuncture experiments with Jonah crabs indicate that the labyrinth in this crab secretes PAH less strongly than that in the rock crab (PAH ratios labyrinth fluid: Plasma in lower labyrinths were 4.2 ± 0.3, n = 3 in one Jonah crab, and 14.8 ± 2.2, n = 16 in four rock crabs).

In conclusion, our results show that PAH is handled differently by the renal systems of two closely related crabs. The rock crab strongly secretes PAH in the labyrinth and partially reabsorbs it in the bladder, resulting in PAH/PEG clearance ratios of 2-3. The Jonah crab shows lower PAH secretion in the labyrinth and strong secretion in the bladder, resulting in PAH/PEG clearance ratios of 30-100. Based on the present findings, one might expect that these two crabs would exhibit greatly different abilities to excrete anionic pollutants, such as the herbicides 2,4-D and 2,4,5-T, i.e., under similar exposure conditions, rock crabs would exhibit a tendency to retain such toxicants for longer times than Jonah crabs. Such retention could result in an increased sensitivity to anionic toxicants for the former species. Supported by N.I.H. grants ES-00920, AM-15973 and RR 05764 and N.S.F. grant PCM-77-26790.

FURTHER STUDIES ON CELL VOLUME REGULATION IN SLICES OF DOGFISH (Squalus acanthias) RECTAL GLAND

A. Kleinzeller, J. Goldstein, C.J. Cha, and J. Forrest, University of Pennsylvania, Department of Physiology, Philadelphia, Pa., Brown University, Providence, Rhode Island, and Department of Medicine, Yale University, New Haven, Conn.

Our previous studies on cell volume regulation in slices of the rectal gland of the dogfish shark (Squalus acanthias) have been extended with the aim of elucidating the role of cell anions and external K^+ in cellular swelling. Most experimental conditions and analytical procedures were described by Booz, et al., Bulletin, MDIBL 18: 26, 1978; and by Goldstein, et al., Bulletin, MDIBL 19: 3, 1979. The determination of tissue urea and amino acids: 50-100 mg. tissue slices (fresh tissue or after aerobic incubation (air) for 60 min at 15°C) were first blotted, weighed, and then extracted with 2 ml ice cold 5% trichloroacetic acid. The deproteinizing agent was removed by extraction with ethyl ether, and the solutes were then determined in an amino acid analyzer.

1. The intracellular non-diffusible anions. Under conditions of aerobic incubation for 60 min at 15°C the ionic distribution in the tissue approaches a steady state (Booz et al., Bulletin, MDIBL 18: 44, 1978). Tissue Cl^- then represents not more than 64% of the bulk cations $Na^+ + K^+$. This observation (c.f. also data of Shuttleworth and Thompson, Bulletin, MDIBL 19: 3, 1979) raised the question whether acidic amino acids, e.g., taurine, might represent a portion of the intracellular, effectively non-diffusible anions (see Forster & Goldstein, Yale J. Biol. Med. 52: 497, 1979), involved in Donnan forces. Table 1 shows that the tissue contains high concentrations of taurine and of glutamic acid. Since little loss of these amino acids occurred during incubation of the tissue, these

Table 1.--Water and solutes in slices of dogfish rectal gland

	TISSUE	
	Fresh	Incubated
H_2O. kg/kg D.W.	2.78 ± 0.02	3.32 ± 0.03
Extracell. space, kg/kg W.W.	--	0.20 ± 0.01
Na^+, mequiv./kg W.W.	57.4 ± 1.1	83.7 ± 1.4
K^+, mequiv./kg W.W.	102.1 ± 1.3	97.2 ± 1.0
Cl^-, mequiv./kg W.W.	68.9 ± 0.6	105.1 ± 2.6
Urea, mmole/kg W.W.	296 ± 3.3	258 ± 2.6
Taurine, mmole/kg W.W.	27.1 ± 0.5	23.4 ± 0.3
Glutamic a., mmole/kg W.W.	4.3 ± 0.06	2.7 ± 0.01

Samples taken: fresh tissue and slices incubated aerobically (air) for 60 min at 15°C in standard dogfish saline. Mean values, ± S.E., or 4 analyses are given, expressed per kg tissue dry wt. (D.W.) or per kg tissue wet wt (W.W.).

solutes are in effect non-diffusible anions and as such are involved in the determination of forces governing cell volume regulation. From the data in Table 1 the apparent intracellular concentrations (subscript i) of the pertinent solutes may be derived: The total 'non-diffusible anion' is then given by the algebraic sum of 41.7 mequiv. Na_i + 168.7 mequiv. K^+ - 77.9 mequiv. Cl^- = 132.5 mequiv. other anions/kg intracellular H_2O. Of this amount, 41.2 mequiv. is represented by taurine and some 4.7 mequiv. by glutamate. It should be pointed out here that the remaining value of 87 mequiv./kg cell H_2O is close to that found in some other tissues, e.g., mammalian renal cortex (Kleinzeller, Biochim. Biophys. Acta 43: 41, 1960).

2. Effect of high external K^+. Boyle and Conway, J. Physiol. 100: 1, 1941), have demonstrated that increased external K^+ produces cellular swelling related to its depolarizing action on the cell membrane with a consequent shift of electrolytes (and H_2O). Table 2, exp. 1 shows a marked swelling effect when the external K^+ for the incubated tissue was increased from 6 mM (control) to 100 mM. It will be noted that this swelling was also associated with a marked reduction of the extracellular space E. A membrane depolarization was indicated by a decrease of the apparent Donnan ratio for K^+ from 19.5 (control) to 1.5 (experimental); the Donnan ratio of Cl^- also significantly

Table 2.--Effect of K^+ on cell volume regulation in slices of dogfish rectal gland

Exptl. conditions	H_2O kg/kg D.W.	Na^+ mequiv/kg D.W.	K^+ mequiv/kg D.W.	Cl^- mequiv/kg D.W.	E kg H_2O/kg W.W.
Exp. 1					
Fresh tissue	2.57 ± 0.01	172 ± 10	261 ± 8	--	
Incubation: Medium					
Control (6 mM K^+)	3.35 ± 0.08	295 ± 28	316 ± 22	--	0.16 ± 0.017
100 mM K^+	3.89 ± 0.03	107 ± 4	573 ± 11	--	0.09 ± 0.008
Exp. 2					
Fresh tissue	2.85 ± 0.03	333 ± 19	34 ± 2	384 ± 18	--
Incubation: Medium					
Control (6 mM K^+)	3.31 ± 0.04	426 ± 12	335 ± 6	506 ± 14	0.21 ± 0.004
K^+-free	3.18 ± 0.07	481 ± 22	210 ± 2	509 ± 24	0.26 ± 0.003
Urea-free (6 mM K^+)	3.60 ± 0.04	483 ± 16	372 ± 4	582 ± 25	0.22 ± 0.004
Urea and K^+-free	3.37 ± 0.02	492 ± 9	216 ± 5	490 ± 10	0.23 ± 0.006

Tissue was incubated aerobically (air) for 60 min at 15°C in standard dogfish saline (6 mM K^+) or in modified salines by an equimolecular substitution of NaCl and KCl. A portion of expt. #2 was carried out in urea-free (hypotonic) saline, with and without K^+. Data are mean values ± S.E. (n=6), expressed in kg H_2O or mequiv. electrolytes per kg tissue dry wt (D.W.). The extracellular (polyethylene glycol) space E is given in kg H_2O per kg tissue wet wt.

dropped from 1.98 ± 0.29 to 1.28 ± 0.05. The major discrepancy between the Donnan ratios of K^+ and Cl^- is noteworthy.

The swelling effect of 100 mM K^+ does not appear to be commensurate with a practically complete (by 92%) depolarization of the membrane. Under similar conditions, other cells practically double their volume (Kleinzeller, 1960). Such result implies that in the rectal gland the cellular swelling is restrained by structural elements of the tissue.

3. The role of K^+ on cellular swelling produced by hypotonic (urea-free) conditions. As compared with controls, placing tissue into a hypotonic medium (urea-free saline without further modification) produces an irreversible tissue swelling which is associated with a net influx of KCl into the cells while the Donnan ratio of K^+ (and of Cl^-) remains constant (Goldstein et al., 1979). It was postulated that the apparent coupling between urea efflux and K influx is indirect, reflecting a) commensurate, relatively low permeabilities of the cells for urea and K^+; b) a high permeability for H_2O; and c) a tendency to maintain the membrane potential constant. Such model would permit the prediction that the swelling effect should be reduced on restriction of K^+ influx. Table 2, Expt. 2 demonstrates that this is indeed the case: The absence of added external K^+ (final K^+ in the medium was 1.5 mM, owing to K^+ efflux from the tissue) abolished the swelling effect. This investigation was supported by a grant from the Whitehall Foundation and NIH grant AM 12619 to Dr. A. Kleinzeller.