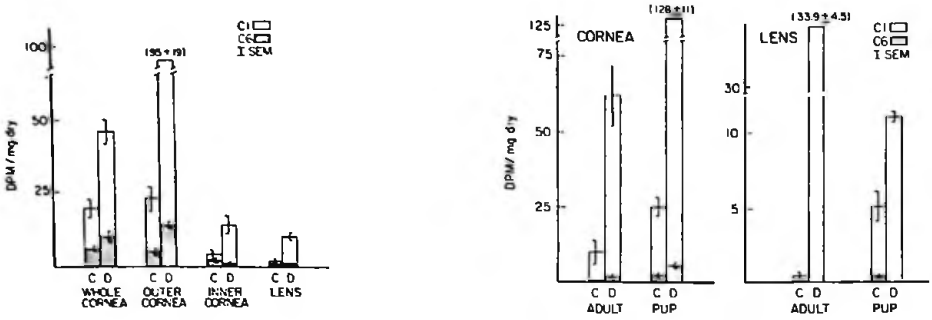

Figure 1. DPM (per mg dry wt) as $^{14}CO_2$ from ^{14}C-1 and ^{14}C-6 glucose for cornea and lens of sculpin incubated with (D) and without (C) 10^{-4} M diamide. Each bar graph represents the mean ± SEM of 5-6 measurements.

Figure 2. DPM (per mg dry wt) as $^{14}CO_2$ from ^{14}C-1 and ^{14}C-6 glucose for cornea and lens of dogfish incubated with (D) and without (C) 10^{-4} M diamide. Each bar graph represents the mean ± SEM of 4-6 measurements.

The addition of 10^{-4} M diamide to the incubation medium caused a marked stimulation in C-1 glucose metabolism in all tissues of the sculpin and dogfish (Figures 1 and 2). This reflects increased activity of the hexose-monophosphate shunt.

The results of this study indicate that the HMS is a prominent pathway of glucose oxidation in ocular tissues of marine teleosts and elasmobranchs. The greatest shunt activity was measured in the dogfish pup tissue. Since the two major functions of the HMS are the metabolism of pentoses and the generation of reducing power in the form of NADPH, the high HMS activity seen in the dogfish pup is consistent with the metabolic requirements of this actively dividing tissue. It is interesting to note that in the cornea of the sculpin, proportionately more glucose is oxidized by the TCA pathway. The ATP thus generated could be involved with the maintenance of normal hydration of this tissue.

The diamide induced increase in HMS activity is in agreement with the findings of other investigators (Fukui et al., Documenta Ophth. Proc. Ser. 8:161, 1976; Geroski et al., Exp. Eye Res. 26:611, 1978) who reported a similar increase in C-1 glucose metabolism for rabbit cornea and lens.

The dominance of the HMS in the glucose oxidation of marine fish ocular tissue is undoubtedly of importance in the maintenance of corneal hydration and transparency. The reserve HMS capacity that is present in these tissues as measured by the use of diamide could provide the cornea and lens with the means of minimizing the effects of oxidative stress. Indeed this pathway most likely has played an important role in the adaptation of these tissues to the osmotic stress of the environment. This work was supported in part by the National Eye Institute grant EY 00933.

AUTORADIOGRAPHIC STUDIES OF THE TRANSCELLULAR MOVEMENT OF UREA ACROSS THE BLADDER OF THE TOAD (*Bufo Marinus*)

Richard M. Hays, Denise E. Georgopoulis and Nicholas Franki, Dept. of Medicine, Albert Einstein College of Medicine, Bronx, New York

In tissues such as toad urinary bladder, vasopressin elicits a broad permeability response, in which water, urea, sodium and other small solutes cross the luminal cell membrane at accelerated rates. There appear to be separate pathways for water and urea, since a number of inhibitory agents block urea or water flow selectively. Among the possibilities to be considered in relation to "separate pathways" are specialized epithelial cells: the granular cell for water transport (Di Bona et al., J. Memb. Biol. 1:79, 1969) and a second for urea transport (for example, the mitochondria-rich cell).

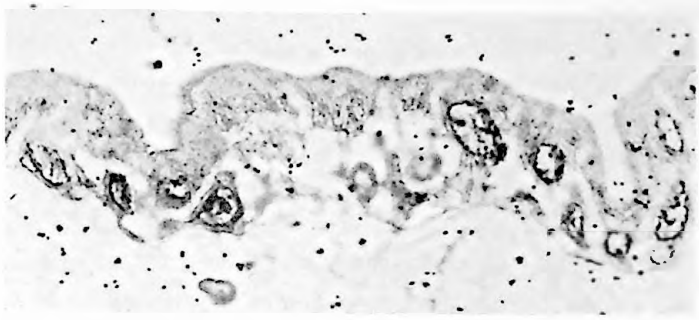

Figure 1. ^{14}C-sucrose autoradiograph of toad bladder x 1000.

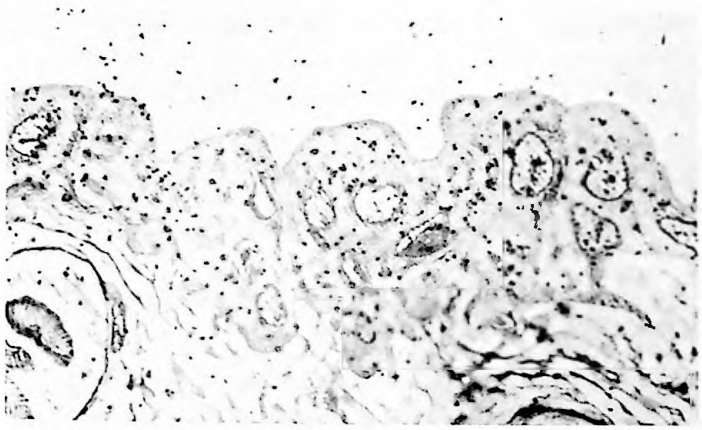

Figure 2. ^{14}C urea autoradiograph; vasopressin absent x 1000.

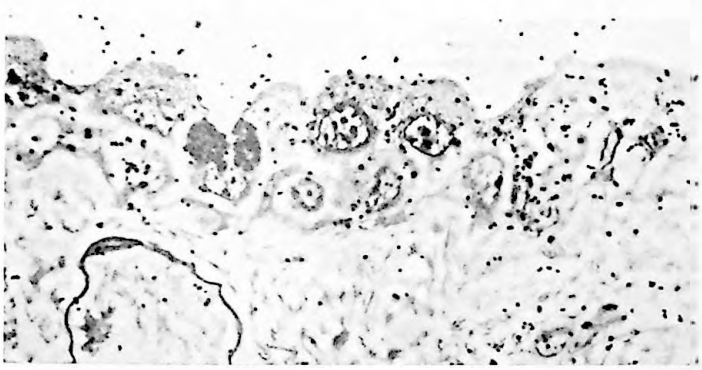

Figure 3. ^{14}C urea autoradiograph; vasopressin present x 1000.

Alternatively, water may traverse a transcellular, and urea a paracellular route; or, both water and urea may traverse the same cell. The experiments to be described are a preliminary attempt to distinguish between these possibilities by determining the pattern of ^{14}C urea traversing the epithelial layer of the toad bladder, using a modification of the technique of autoradiography developed by Sterling and Kinter (J. Cell Biol. 35:585, 1967).

Bladders were excised, removed from the toad, and portions were tied across the ends of rings cut from 1 cm diameter propylene test tubes. The luminal surface of the bladder faced out. 1 ml of amphibian Ringer's solution bathed the inner (serosal) surface of the bladder. Vasopressin (90 mU/ml) was added to the serosal medium of some bladders; others received no vasopressin. The plastic rings were then suspended by attached wires in Ringer's solution and incubated for 15 minutes. The rings were then carefully placed in 2 ml of Ringer's containing 50 µc/ml of ^{14}C urea, with the luminal surface of the bladder just below the surface of the Ringer's. Additional rings were similarly exposed to 50 µc/ml ^{14}C sucrose, a large nonpenetrating molecule serving as a control. After 5 minutes of exposure to the isotope, the rings were removed from the medium, the serosal fluid withdrawn, and the rings

rotated 90° for ease of freezing. The bladders, still tied to the rings, were frozen in liquid propane; freeze-dry plastic section autoradiography was then carried out.

Figures 1-3 show the bladder epithelial cells (largely granular cells) after 15 days of emulsion development. ^{14}C sucrose is almost completely excluded from the epithelial cells. ^{14}C urea, on the other hand, labels the cells in a relatively uniform manner both in the presence and absence of vasopressin. An unexpected finding is the failure to find more numerous grains in the presence of vasopressin.

The findings suggest that there are no specialized epithelial cells involved in urea transport, and that urea, like water, traverses the granular cell. The "independent pathways," then, would be separate sites in the granular cell membrane.

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EFFECTS OF TRIAMINOPYRIMIDINE (TAP) ON Na AND Cl TRANSPORT BY *Pseudopleuronectes americanus* INTESTINE

Raymond A. Frizzell,* Dennis C. Clayton* and Michael Field**
*Dept. of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania
**Dept. of Medicine, The University of Chicago, 950 East 59th Street, Chicago, Illinois

Field et al. (J. Memb. Biol. 41:265, 1978) proposed a model for Na and Cl transport by flounder intestine whose essential features are depicted in Figure 1. According to this model, neutral (one-

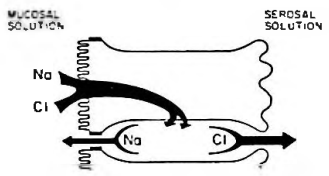

Figure 1. Model for Na and Cl transport by flounder intestine under short-circuit conditions.

for-one) transcellular NaCl transport is obscured by the permselective characteristics of the paracellular pathway, such that much of the Na transported into the lateral intercellular space recycles to the mucosal solution. Under open-circuit conditions, a diffusion potential develops across the tight junction, accounting for the spontaneous, serosa-negative electrical potential difference that is observed across this tissue, and under short-circuit conditions, a preponderance of Cl over Na absorption is observed (Field et al., J. Memb. Biol. 41:265, 1978).

Support for the notion of neutral transcellular NaCl transport was derived from studies by Frizzell et al. (J. Memb. Biol., in press) who identified an obligatory NaCl co-transport process at the brush border membranes of the absorptive cells. To address the question of whether the tight junctions represent the site of Na recycling, the effects of triaminopyrimidine (TAP) on NaCl transport by flounder intestine were evaluated. Moreno (J. Gen. Physiol. 66:97, 1975) demonstrated that addition of TAP to the solution bathing the mucosal surface of several epithelia elicited a reversible increase in tissue resistance that was due to a decrease in Na permeability of the paracellular pathway; the permselective properties of this pathway are dominated by those of the tight junction (Moreno and Diamond, In: Membranes--A Series of Advances (G. Eisenman, ed.), Dekker: New York, 1976). TAP is most active in this regard when present as the monovalent cation, which predominates at pHs below its pK_1 (6.7). However, flounder intestine requires the presence of HCO_3 in the bathing media for optimal rates of Na and Cl absorption. The results of studies reported elsewhere in this bulletin