TABLE 2

Response of Dogfish Dorsal Aortic Pressure to Bolus Injections of Three Angiotensin II Antagonists and [$Ile^8$] AII

| TREATMENT | DORSAL AORTIC PRESSURE* | | | |
|---|---|---|---|---|
| | Control | | Response | |
| | Systolic | Diastolic | Systolic | Diastolic |
| [$Sar^1$-$Ile^8$] AII 40 μg** n = 4 | 27.7 ± 4.4 | 21.7 ± 3.8 | 35.5 ± 3.7 ($p < 0.025$) | 27.5 ± 2.1 ($p < 0.01$) |
| [$Sar^1$-$Thr^C$] AII 40 μg** n = 5 | 27.2 ± 4.4 | 21.4 ± 5.1 | 28.2 ± 5.4 (N.S.) | 22.4 ± 5.0 (N.S.) |
| [$Ile^8$] AI 40 μg** n = 7 | 26.2 ± 4.7 | 20.4 ± 4.3 | 27.6 ± 4.7 (N.S.) | 21.3 ± 4.8 (N.S.) |
| [$Ile^8$] AII 20 μg*** n = 4 | 26.5 ± 5.0 | 18.7 ± 4.2 | 35.0 ± 7.0 ($p < 0.005$) | 22.2 ± 5.0 ($p < 0.05$) |

* Pressure in Torr ± 1 SE; ** Larger fish (6-8 kg) received 80 μg; *** Larger fish received 40 μg

for catecholamine release in dogfish is different from that of mammals, or, that dogfish have a unique complement of adrenergic catecholamines and/or adrenergic catecholamine receptors. Supported by Grant No. 18868, National Institutes of Health.

## GLUCOSE OXIDATION: THE RESPONSE TO DIAMIDE IN THE SCULPIN AND DOGFISH CORNEA AND LENS

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Corneal transparency in vertebrates is preserved by the expenditure of metabolic energy. This energy is utilized in keeping the hydrophilic stroma at a minimum of physiological hydration. Swelling of this transparent tissue results in opacification. It has previously been shown that the limiting layers of the cornea (epithelium and endothelium) are sites of active ion transport, effecting the osmotic movement of water out of the hydrophilic stroma. In aquatic animals the environmental osmotic stress further complicates the maintenance of corneal transparency, and various anatomical adaptations have evolved to prevent hydration. In cyclostomes a primary spectacle containing sutural fibers protects the cornea (Van Horn et al., J. Ultrastruct. Res. 26:454, 1969). In sharks a thick epithelium is present and the stroma is nonswelling as a result of sutural fibers (Smelser, Invest. Ophth. 1:1, 1962; and Goldman and Benedick, Invest. Ophth. 6:574, 1968). These sutural fibers are lost in the teleosts. The corneas of marine species have been shown to be divided into an inner and outer layer capable of becoming hydrated (Fischer and Zadunaisky, Exp. Eye Res. 25:149, 1977). By comparison in fresh water teleosts the cornea is completely fused and possesses a thick epithelium which essentially makes it impermeable (Edelhauser et al., Invest. Ophth. 4:290, 1965). Regardless of structural adaptations that have evolved, the corneas of all species contain an epithelium, endothelium and stroma which depend upon metabolic energy for the maintenance of their pump function, cell division, collagen secretion and ultimately corneal transparency. We have previously reported the $QO_2$ and $Q_{10}$ values of marine fish corneas (Bull. MDIBL 17:6, 10, 1977) and have recently measured the hexose monophosphate shunt (HMS) in the component layers of the rabbit cornea in the normal and after diamide treatment

treatment (Geroski et al., Exp. Eye Res. 26:611, 1978). Diamide [diazenedicarboxylic acid bis (N,N-dimethylamide)] has been shown to oxidize intracellular gluthathione and stimulate HMS activity (Kosower et al., Biochem. Biophys. Res. Comm. 37:593, 1969).

This series of experiments was undertaken to evaluate the glucose oxidation (HMS activity) in the component layers of the sculpin and dogfish cornea and lens, and to study the response of this pathway to the oxidative attack of diamide.

Eyes were enucleated, corneas excised, and lenses removed from the sculpin, *Myoxocephalus scorpious*, and adult and intrauterine pups of the spiny dogfish, *Squalus acanthius*. The adult dogfish were fitted with protective head harness to prevent abrasion of the corneal epithelium (Fisher and Zadunaisky, Exp. Eye Res. 25:149, 1977). The sculpin cornea was separated into its inner and outer layers, which were studied in addition to the whole tissue. The corneas and lenses of the sculpin were incubated in Forster's flounder medium (Science 108:65, 1948) containing 5 mM glucose and 16 mM $HCO_3^-$, while tissue of the dogfish was incubated in Forster's Elasmobranch Ringer's (Comp. Biochem. Physiol., 43A:3, 1972) with 5 mM glucose. Diamide, $10^{-4}$ M, was added to the incubation medium in half of the experiments. Each tissue sample was incubated in 3.2 ml of medium containing 0.25 µCi of selectively labelled ($^{14}$C-1 or $^{14}$C-6) glucose. After a 2-hour incubation at 15°C, the medium was acidified and Hyamine, a $CO_2$ trap, was added to the center well. The samples were incubated for an additional hour at 37°C. At this time, the center wells containing the trapped $^{14}CO_2$ were withdrawn, placed into scintillation vials, and counted.

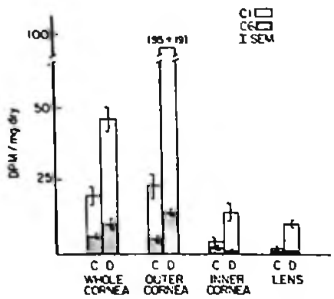
Table 1. DPM (per mg dry wt) as $^{14}CO_2$ from $^{14}$C-1 and $^{14}$C-6 glucose for cornea and lens of sculpin and dogfish.

| | n | C-1 DPM | C-6 DPM | % HMS |
|---|---|---|---|---|
| Cornea | | | | |
| 1) Sculpin | | | | |
| a) whole | 6 | 19.5 ± 2.7* | 5.7 ± 1.1 | 56 ± 6** |
| b) outer | 6 | 23.0 ± 3.7 | 4.9 ± 1.3 | 66 ± 1 |
| c) inner | 5 | 4.3 ± 1.5 | 2.0 ± 0.5 | 32 ± 11 |
| 2) Dogfish Adult | 6 | 10.4 ± 3.6 | 0.38 ± 0.12 | 92 ± 3 |
| 3) Dogfish Pup | 6 | 25.3 ± 2.8 | 2.4 ± 0.3 | 81 ± 3 |
| Lens | | | | |
| 1) Sculpin | 6 | 1.2 + 0.20 | 0.45 ± 0.20 | 51 ± 14 |
| 2) Dogfish Adult | 6 | 0.44 ± 0.19 | 0.013 ± 0.004 | 91 ± 3 |
| 3) Dogfish Pup | 4 | 4.96 ± 1.14 | 0.212 ± 0.059 | 90 ± 6 |

* Mean ± SEM

** % HMS calculated as $\frac{(\text{C-1 DPM} - \text{C-6 DPM})}{\text{Total DPM}} \times 100$

Glucose is oxidized symmetrically by the tricarboxylic acid (TCA) pathway, with $CO_2$ being evolved equally from the C-1 and C-6 positions (Science 122:72-73, 1955). By comparison, there is a preferential oxidation of the C-1 atom of glucose by the HMS. Hence, $CO_2$ generated from the C-1 position in excess of that generated from the C-6 position is related to HMS activity. The control data for corneas and lenses are shown in Table 1. All of the ocular tissues in this study show preferential utilization of the C-1 labelled glucose. The HMS is a major pathway of glucose oxidation for all of the ocular tissues studied, accounting for 56 to 92 percent of glucose oxidation in the corneas and from 51 to 91 percent of glucose oxidation in the lens group. In the dogfish, a greater percentage of glucose is diverted into the HMS compared to the sculpin--both in cornea and lens.



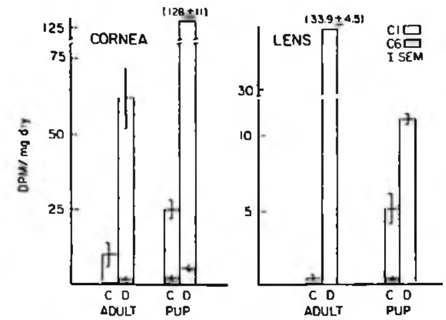




Figure 1. DPM (per mg dry wt) as $^{14}CO_2$ from $^{14}$C-1 and $^{14}$C-6 glucose for cornea and lens of sculpin incubated with (D) and without (C) $10^{-4}$ M diamide. Each bar graph represents the mean ± SEM of 5-6 measurements.

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*)

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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).