EVIDENCE OF GLUCOSE STEREOSPECIFICITY CROSSING OCULAR BARRIERS IN THE SPINY DOGFISH, *Squalus acanthias*

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Experiments were performed to test glucose entry into the ocular fluids in order to determine if the facilitated diffusion for sugars shows stereospecificity. The results reveal that the ocular barriers in t shark discriminate and influence glucose transport on the basis of molecular specificity. In this study 3-O-methyl glucose, d-glucose, l-glucose and urea were used.

Dogfish weighing from 1.3 to 2.6 Kg were restrained on a wooden rack, head submerged nearly flat in a tank of fresh flowing native sea water. The dorsal aorta was canulated with P.E. 60 tubing and the cannula was fitted with a 2-way stopcock allowing free access to the dogfish plasma (the fish was heparinized to avoid clotting). At zero time a bolus injection of two compounds was performed, one labeled with ^{3}H and th other with ^{14}C. At times 2, 3, 5, 7, 10, 13, 15, 20, 25 and 30 min, 0.3 ml of whole blood was withdrawn an centrifuged. At the preset end of the experiment, a final sample of blood was removed, the animal sacrificed and both eyes quickly removed. The dogfish eyes were frozen in dry ice for subsequent dissection, performed twenty minutes later. The vitreous humor was divided into sections termed "anterior vitreous" an "peripheral vitreous" and a composite wedge called "mixed vitreous" (Figure 1).

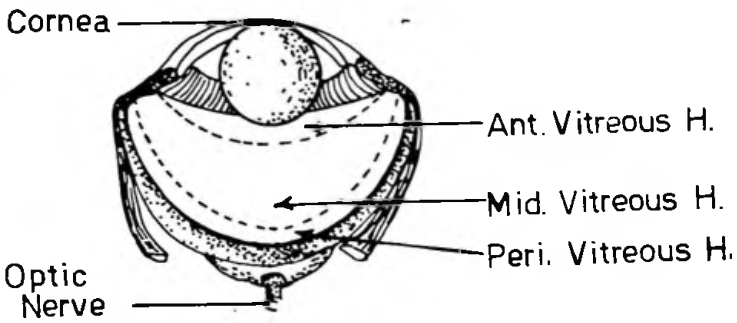

Figure 1. Diagram of the dogfish eye illustrating the sectioning of the vitreous humor.

After thawing the samples of vitreous body, 100 µl of ocular fluids and plasma dissolved in 10 ml of TT21 (Yorktown Research, Hackensack, N.J.) were counted for ^{14}C and ^{3}H by liquid scintillation spectrometry

The plasma data were plotted on semi-log paper and a best fit function determined as explained below. From these data, rate constants were calculated giving a measure of the rate of entry of specific substance urea, 3-O-methyl-d-glucose, d-glucose, and l-glucose into regions of ocular fluids.

A relatively simple model of transport into an eye compartment introduced by H. Davson (Davson and Matchett, 1953) was used. The model, shown in Figure 2, assumes that transport to the ocular compartment can occur either by passive diffusion, active secretion or bulk removal. The resulting system equation is:

$$\frac{dCa}{dt} = K_d C_p - K_d C_a + K_f n C_p - K_f C_a \qquad (1)$$

where C_a and C_p are concentration variables for plasma (P) and ocular humor (A); K_d is the diffusion rate constant; K_f is the secretion constant which is assumed to be equal to the bulk absorption constant since t eye compartment volume remains relatively constant. The concentration of newly secreted fluid is taken to be $n C_p$, which is a simple linear function of plasma concentration.

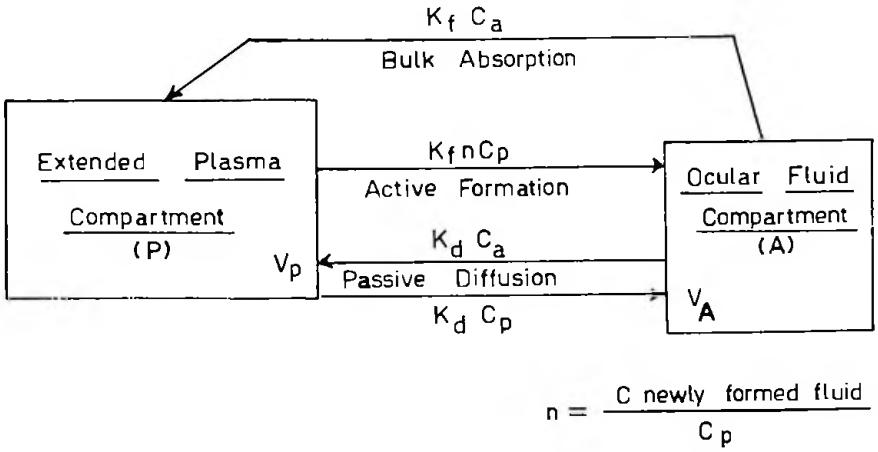

Figure 2. Ocular compartmental model utilized in the calculation of rate constants.

$$\text{At steady state } (t = \infty), \frac{dC_a}{dt} = 0 \text{ and}$$

$$\frac{C_a}{C_p}(\infty) = \frac{K_f n + K_d}{K_f + K_d} = \frac{K_i}{K_o} \tag{2}$$

Overall rate of entering and exiting is defined by

$$K_i = K_f n + K_d \text{ and } K_o = K_f + K_d \tag{3}$$

The plasma data for each experiment fit graphically to an equation of the form:

$$C_p = A + Be^{-b_1 t} + Ce^{-b_2 t} \tag{4}$$

The coefficients and exponential constants were determined.

Figure 3 shows typical plasma functions for d- and l-glucose. The values have been normalized to C_{po} (initial plasma concentration). It can be seen that the experimental points follow the solid line generated from the computer analysis of the plasma data for each experiment.

It can be demonstrated that the ocular concentration function resulting from equation (4) is of the form:

$$C_a = \frac{K_i}{K_o}\left[A + \left(\frac{K_o B}{b_1 - K_o} + \frac{K_o C}{b_2 - K_o} - A\right)e^{-K_o t} + \left(\frac{K_o B}{K_o - b_1}\right)e^{-b_1 t} + \left(\frac{K_o C}{K_o - b_2}\right)e^{-b_2 t}\right] \tag{5}$$

From an experimental determination of $\left(\frac{C_a}{C_p}\right)$ at steady state and the necessary plasma constants the above equation can be solved with the aid of a computer to yield K_o and K_i overall rate constants. Thus, in a given experiment we determine the overall rate of entering and leaving the ocular compartment for a given test substance.

Table 1 lists the results of the A/P ratios and the rate constants for exit and entry of the molecules studied into compartments of the dogfish eye. The A/P ratio is the experimentally determined ratio of ocular to plasma concentration obtained for a set of double label experiments at 30 minutes. Thus at 30 minutes in the aqueous humor, the A/P ratio for d-glucose is 0.243 ± .018 S.E. for 8 experiments whereas it is 0.103 ± .008 S.E. in a mixed wedge of vitreous humor indicating that penetration is significantly faster in

TABLE 1

Analysis of ocular humors in the dogfish (*Squalus acanthias*)

	D-Glucose (8)*		L-Glucose (8)		3-O-Methyl-D-Glucose (6)		Urea (6)	
	A/P ratio+	$K_i x 10^{+3}$ (min^{-1})	A/P ratio	$K_i x 10^{+3}$ (min^{-1})	A/P ratio	$K_i x 10^{+3}$ (min^{-1})	A/P ratio	$K_i x 10^{+3}$ (min^{-1})
Aqueous H. (mix)	0.243 ±.018	3.70 ±.23	.0205 ±.0040	0.44 ±.016	0.207±.016	3.62±.21	0.306±.010	3.33±.09
Vitreous H. (mix)	0.103 ±.008	1.27 ±.26	.0051 ±.0008	0.10 ±.02	0.071±.005	1.19±.21	0.059±.006	0.60±.07
Anterior Vitreous H.	0.0496±.0223	0.80±0.30	.00453±.00082	0.091±.017	.037±.008	0.61±.15	.062±.007	0.64±.07
Middle Vitreous H.	0.0557±.0139	0.91±0.14	.00414±.00092	0.082±.017	.031±.002	0.51±.10	.036±.008	0.37±.05
Peripheral Vitreous H.	0.1651±.0154	2.47 ± .24	.00518±.00092	0.103±.018	.157±.020	2.70±.25	.119±.030	1.24±.10

*Number of experiments.

+A/P ratio - ocular humor to plasma concentration ratio at 30 minutes.

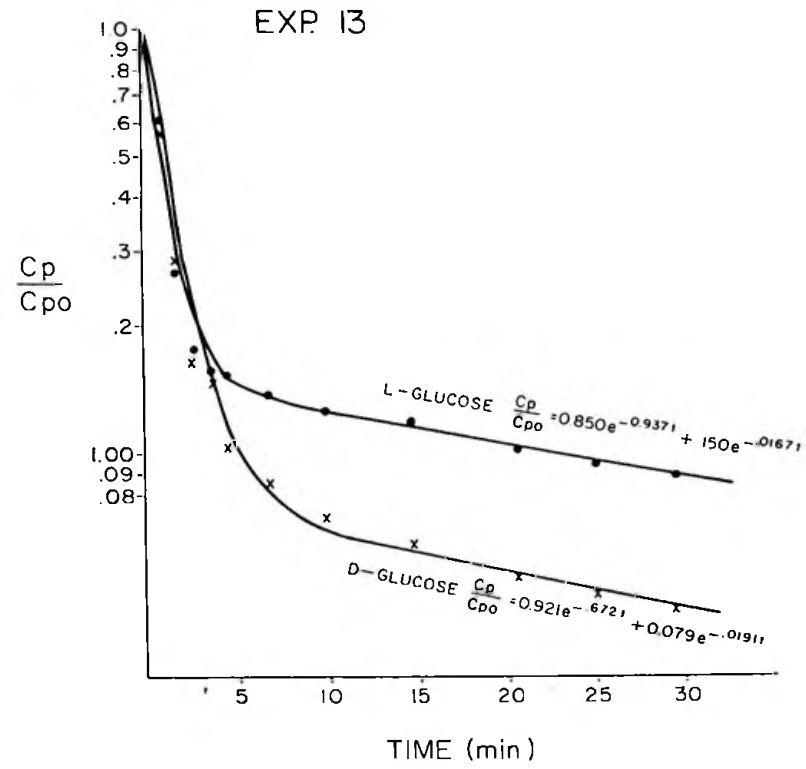

Figure 3. Plasma concentration curves for one experiment after an initial bolus injection of (^{3}H) l-glucose and (^{14}C) d-glucose. The solid circles and crosses represent experimental kinetic data; whereas, the solid lines and accompanying equations are determined from computer analysis.

the aqueous humor. The rate constant K_o is calculated on the basis that newly formed fluid is similar to to plasma in concentration; thus n = 1.0 and $\frac{C_a}{C_{p^\infty}}$ at steady state is equal to 1.0. $\frac{C_a}{C_p}$ is assumed equal to 1.0, thereby making $K_i = K_o$. These are good assumptions for urea, which is usually considered to enter passively, but an uncertain one for the sugars. We are assuming that d- and l-glucose as well as 3-O-methyl-d-glucose are not secreted against a concentration gradient but move downhill and at steady state would equal the plasma concentration if metabolism by intraocular tissues (lens and cornea) did not deplete sugar content. Table 2 lists the A/P ratio at 30, 60 and 120 minutes for l- and d-glucose and demonstrates that the d-glucose $\frac{C_a}{C_p}$ is increasing with time and has reached 0.605 in the aqueous in two hours and could reach 1.0 at steady state. A similar but slower situation is encountered in the vitreous humor. L-glucose enters both the aqueous and vitreous compartments of the eye much more slowly; it is therefore difficult to estimate the steady state value. However, if we assume that l-glucose enters solely by passive diffusion then $\frac{C_a}{C_p}$ at steady state equal to 1.0 is valid.

Table 1 reveals that although the A/P ratio for urea is greater than for d-glucose, the rate constant for d-glucose and 3-O-methyl-d-glucose demonstrate the reverse, d-glucose enters the eye compartments faster than urea. This is in contrast to what would be expected in that urea, being a much smaller molecule and having greater solubility than glucose, should be expected to cross membrane barriers with much greater ease.

TABLE 2

Comparison between L- and D-glucose penetration in ocular fluids of the dogfish

Time (min)	Aqueous humor			
	d-glucose		l-glucose	
	A/P	K_i (min^{-1})	A/P	K_i (min^{-1})
30 (6)*	0.243 ± .018	.00370 ± .00023	0.0205 ± .004	.00044 ± .00006
60 (1)	0.492	.00371	0.045	.00046
120 (1)	0.605	.0038	0.055	.00043
	Vitreous humor			
	d-glucose		l-glucose	
	V/P	K_i (min^{-1})	V/P	K_i (min^{-1})
30 (6)	0.103 ± .008	0.00127 ± .00026	0.0051	.00010 ± .00002
60 (1)	0.325	0.0023	0.0063	---
120 (1)	0.438	0.0021	0.0081	---

*Number of experiments.

One very significant advantage in using the dogfish is the size of the eye. With the large eye of the dogfish we have been able to analyze the vitreous humor by subdivisions which are also listed in Table 1. We note that concentrations of all substances studied are greatest in the peripheral section near the retinal pigment epithelium. This indicates that the materials can enter the vitreous humor by crossing the retinal pigment epithelium barrier and not diffusing back from the aqueous humor.

A most significant finding is the dramatic difference in rates of penetration between l-glucose and d-glucose; in each category of ocular humor both the A/P ratio and K_o and K_i rate constants for d-glucose are more than 10 times that of l-glucose. The difference between the means for the A/P ratios and rate constants are significant to $p > .001$ level for l- and d-glucose. This clearly demonstrates the stereo-specific nature of the plasma-aqueous and plasma-vitreous barriers in the dogfish.

One final point of possible significance is that the ratio of aqueous to vitreous rate constants for l- was 5.2 while this same ratio for d-glucose was only 2.4. This may reflect a relatively tighter barrier a the level of the retinal pigment epithelium.

These investigations were supported by NIH Research Grant # EY 01340 and Training Grant # EY 08009. T technical assistance of Ms Emily LaGreca is gratefully acknowledged.