The carbonic anhydrase activities in the homogenate, membranes and brush border were 50% inhibited with 10^{-8} M methazolamide or acetazolamide and fully inhibited at 10^{-6} M, suggesting the presence of a single "high activity" isozyme in all the fractions.

In epithelia which are capable of acidifying their mucosal fluid, e.g., the gastric mucosa and kidney, the serosal fluid is simultaneously alkalinized. This process is equivalent to the splitting of a water molecule and a transfer of H^+ ions to the mucosal side of the epithelial cell and a concomitant transfer of OH^- ions to the serosal side. This process has been shown to occur in the proximal tubules of rats (Filho and Malnic, Pflugers Arch., 363:211, 1976). Contraluminal carbonic anhydrase catalyzes the reaction $OH^- + CO_2$, buffering rapidly the high OH^- and providing HCO_3^- as a counter ion to sodium for reabsorption. At brush border there is no analogous reaction and there appears to be a lower concentration of enzyme here than in total membrane. The function of carbonic anhydrase at brush border has been supposed to be the rapid dehydration of luminal H_2CO_3 (Rector, in The Kidney, ed. by B. M. Brenner and F. C. Rector, Vol. I, 1976, Saunders); although if H_2CO_3 is diffusible, there is no compelling reason why this reaction cannot be mediated by the very high concentration of enzyme in the cytosol (Maren, Can. J. Physiol. Pharm., 52:104, 1974).

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THE TRANSPORT OF 3-O-METHYL-D-GLUCOSE FROM BLOOD TO BRAIN AND OCULAR FLUIDS IN THE DOGFISH

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The movements of various monosaccharides in many biological systems are facilitated by so-called carrier-mediated processes. There is strong evidence that such mechanisms operate across brain capillary endothelium (e.g., Crone, J. Physiol., 181:103, 1965) and choroid plexus epithelium (e.g., Fishman, Am. J. Physiol., 206:836, 1964) of mammals. As part of an ongoing study of blood-brain-cerebrospinal fluid (CSF) transfer mechanisms in *Squalus acanthias*, an examination of 3-O-methyl-D-glucose (3 MG) transport from blood to brain and CSF was begun. In these same experiments, the distribution of this sugar from blood to aqueous and vitreous humors and skeletal muscle was also studied.

Method. Dogfish weighing from 1.5-3.0 kg were used. Solutions containing both an ^3H-labeled material (3 MG) and ^{14}C-labeled compounds (3 MG or urea) were injected intramuscularly in the same fish. Sometimes the ^3H- and ^{14}C-compounds were injected simultaneously; in other cases, the two labeled materials were administered at different times (the latter procedure allowed the study of 3-O-methyl-D-glucose's distribution for two different time periods in the same animal). The experimental durations were 5, 10, 15, 30, and 60 minutes plus 2, 3, and 4 hours. At 10-12 times during the course of each experiment, samples of blood were obtained. Except for the few seconds when blood samples were drawn, the dogfish swam freely in large sea water filled tanks.

At the end of the experimental period, the animals were removed from the water and quickly decapitated. Immediately thereafter a sample of CSF was taken, and both eyes plus the brain removed. Samples of aqueous humor were aspirated from each eye; subsequently both eyes were frozen in preparation for regional sampling of the vitreous humor. Pieces of brain (medulla) and skeletal muscle were obtained for analysis. Samples of fluid from the anterior, central, and posterior regions of the

vitreous body were taken from the frozen globes; plasma was gotten from the blood by centrifugation. Duplicate samples of all fluids and tissues obtained were prepared for liquid scintillation spectroscopy and counted in the customary double-label manner. From the raw data, corrected concentrations of radioactivity (either as cpm/μl for fluids or cpm/mg for tissues), and tissue:plasma concentration ratios were calculated.

In one set of experiments a test of the saturability of 3 MG's transfer system was made. The experimental procedure was the same as the preceding except that 11 ml of a 1.6 M 3 MG solution was intravenously infused over a one minute period at the beginning of the test period.

Results. The early experiments demonstrated that the distribution kinetics of ^{3}H- and ^{14}C-3-O-methyl-D-glucose were similar, and thus both tracer forms of this sugar were used in the remainder of the study.

In nearly all experiments the plasma concentrations of the labeled sugar remained fairly constant after the first five minutes; therefore, the plasma values from five minutes to the end of the experiment were averaged and a "mean" plasma concentration determined for each animal. The mean plasma concentration was used for the calculation of the fluid:plasma and tissue:plasma ratios.

A summary of the transport parameters derived from the 5 minute to 4 hour studies with tracer levels of 3 MG is presented in Table 1 (the parameters are defined in the table's footnotes). The steady-state (CSF:plasma distribution ratio of 3 MG equals that of endogenous water. The water ratios for both of the ocular fluids are also around 1.05. In these fluids tracer 3 MG either seems to achieve a steady-state at a level much below that of water (aqueous humor) or has not reached a steady-state by four hours (vitreous humor). The 3 MG steady-state ratios in medulla and skeletal muscle also appear to be less than those of water (.80 and .78, respectively) but greater than those of sucrose (approximately .15 and .10, respectively), an extracellular marker.

Table 1. 3-O-Methyl-D-Glucose Transport Parameters Determined from Tracer Distribution Kinetics

	FLUID OR TISSUE SITE						
		Vitreous Humor					
Transport Parameter	Aqueous Humor	Anterior	Central	Posterior	Cerebrospinal Fluid	Medulla	Skeletal Muscle
Apparent Steady-State Ratio*	.51	> .40	> .35	> .35	1.05	.55	.26
Apparent Exchange $t_{1/2}$ +	60 min	> 4 hr	> 4 hr	> 4 hr	40 min	15 min	75 min

*The ratio is given by the fluid or tissue concentration of tracer at "infinite" time divided by the mean plasma concentration.

+The transport or exchange half-time ($t_{1/2}$) is the time taken for the tracer to achieve 50% of the steady-state ratio.

The rate of 3 MG entry was most rapid for the medulla ($t_{\frac{1}{2}}$ = 15 min) and slowest for the vitreous humor. The half-times for aqueous humor, cerebrospinal fluid, and skeletal muscle were all around 1 hr.

The data from the saturation experiments are given in Table 2. Statistical analysis (unpaired t-test) of these results indicated significant differences in the distribution ratios between normal and high plasma glucose groups for anterior vitreous humor ($p < .01$ for both 30 min and 2 hr), posterior vitreous humor ($p < .01$ for 2 hr only), CSF ($p < .01$ for both 30 min and 2 hr), and medulla ($.01 < p < .02$ for 30 min). Although the two hour 3 MG ratios for aqueous humor and skeletal muscle appeared to vary with the concentration of 3 MG, the scatter in the individual results was too great and

Table 2. Effects of High Plasma Concentrations of 3-O-Methyl-D-Glucose on the Tracer 3MG Distribution Ratios

Time	Total Plasma Glucose Concentration	DISTRIBUTION RATIO* FOR: Aqueous Humor	Vitreous Humor: Anterior	Vitreous Humor: Central	Vitreous Humor: Posterior	CSF	Medulla	Skeletal Muscle
30 min	Normal+ (N=4)	.101 ±.033	.122 ±.011	.059 ±.022	.064 ±.024	.452 ±.024	.344 ±.038	.076 ±.014
	High** (N=3)	.128 ±.020	.045 ±.001	.023 ±.000	.080 ±.004	.245 ±.029	.178 ±.036	.082 ±.020
2 hr	Normal+ (N=3)	.537 ±.079	.306 ±.021	.165 ±.026	.211 ±.001	1.005 ±.014	.375 ±.085	.284 ±.096
	High** (N=3)	.392 ±.020	.171 ±.007	.092 ±.005	.162 ±.012	.479 ±.010	.240 ±.141	.070 ±.019

*Values are mean fluid:plasma and tissue:plasma 3MG ratios ± 1 SE.

+Plasma concentration of glucose plus 3MG in normal animals was assumed to be 5 mM.

**Plasma concentration of glucose plus 3MG in this group averaged 42 mM.

these differences did not prove to be statistically significant.

The ^{14}C-urea data are more limited and have not been presented in tabular form. The results suggest that urea enters CSF more rapidly ($t_{\frac{1}{2}}$ = 10 min) than 3 MG but that the opposite holds for aqueous humor and medulla. No detectable differences in urea and 3 MG exchange were observed for vitreous humor and skeletal muscle.

Discussion. The transport of 3-O-methyl-D-glucose from blood into the brain and cerebrospinal fluid of mammals is stereospecific, saturable, and competitive but does not appear to be uphill; therefore these transport systems seem to be of the facilitated diffusion (equilibrating carrier) type. The present work indicates that similar transfer processes mediate the exchange of 3 MG between blood, brain, and CSF in the dogfish shark.

Carrier mediated transfer of sugars between blood and aqueous humor has not been clearly demonstrated in mammals. The extant evidence indicates weak stereospecificity and little or no competition or saturation for monosaccharide transport in this system. Our findings with 3 MG in *Squalus acanthias* seem to agree with this. In contrast, the vitreous humor data suggest that the blood-vitreous exchange of 3 MG in this species is partially saturable and possibly carrier mediated.