

shown by many hermit crabs for undamaged mollusc shells suggests a behavioral adaptation which also affords greater security against predators. The crabs in colonized shells occupied mollusc shells smaller than the preferred range suggests that there may be a selective advantage in maintaining possession of shells with *Hydractinia* colonies even if SAI values are low. The dominance characteristics displayed by hermit crabs in colonized shells supports the above view as such individuals although dominant appear to avoid direct combat. In these circumstances dominance plays essentially a defensive role by serving to reduce shell exchange between individuals inhabiting colonized and those occupying uncolonized shells.

This work was supported by NSF Grant GB-31548.

1973 #22

### THE UPTAKE OF 2-DEOXY-D-GLUCOSE BY THE CHOROID PLEXUS OF THE DOGFISH *Squalus acanthias*

Patricia M. Griffin, Megan Dethier, Joseph Fenstermacher and Arnost Kleinzeller. University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania and National Cancer Institute, Bethesda, Maryland

The epithelial cells of the dog choroid plexus actively accumulate D-glucose and D-galactose (Csáky and Rigor, Life Sci. 3:931, 1964). As part of a comprehensive project on the sugar transport in the choroid plexus of the dogfish (*Squalus acanthias*), the uptake of 2-deoxy-D-glucose by this tissue was investigated.

The choroid plexi of the third (including also the plexi of the lateral ventricles) and the fourth ventricle were rapidly removed from decapitated animals by cutting of the tissue close to its attachment. The tissues were separately placed into ice-cold dogfish Ringer solution for no longer than 15 minutes. The pooled plexi (of either the third or the fourth ventricles) of three animals were then placed into 2.5 ml dogfish saline containing usually 1 mM 2-deoxy-D-glucose-<sup>14</sup>C of the required specific activity (usually 0.2  $\mu$ Ci/ml) without or with other sugars. Aerobic incubation (air as gaseous phase) was then carried out at 15°C with occasional shaking. At the end of the incubation each piece of tissue was blotted, weighed, and placed into a homogenizing tube containing 2 ml water at 100°C for 10 minutes. The subsequent analytical procedure for the determination of tissue sugars was that described previously (Kleinzeller and McAvoy, J. Gen. Physiol. 62:169, 1973). In short the tissue was homogenized, the tubes were centrifuged and a portion of the supernatant was taken for the determination of the total (free plus phosphorylated) sugar. Free sugar was then determined after precipitation of the phosphorylated components by the  $ZnSO_4$ -Ba(OH)<sub>2</sub> procedure. Scintillation spectrometry was employed for the determination of radioactivity. The data are expressed as the tissue/medium (T/M) ratio of total sugar. Such an approach is justified on the assumption that the transport of free sugar across the membrane is the rate-limiting step, phosphorylation taking place within the cells. A considerable spread of T/M values for tissues of different animals was observed.

The time curve for the uptake of 2-deoxy-D-glucose showed that the choroid plexi of both ventricles took up the sugar at a rapid rate; even after two-hour incubation no steady state was

reached. 2-Deoxy-D-glucose was present in the tissue mostly in phosphorylated form; the apparent tissue concentration of the free sugar was well below the diffusion equilibrium level.

A reciprocal plot of the concentration dependence of total 2-deoxy-glucose uptake after one hour incubation is given in Figure 1. The sugar uptake by the tissue of the third ventricle appears somewhat faster than that of the fourth ventricle. The considerable spread of values for individual analyses did not permit an accurate determination of the transport  $K_m$ . The data are however com-

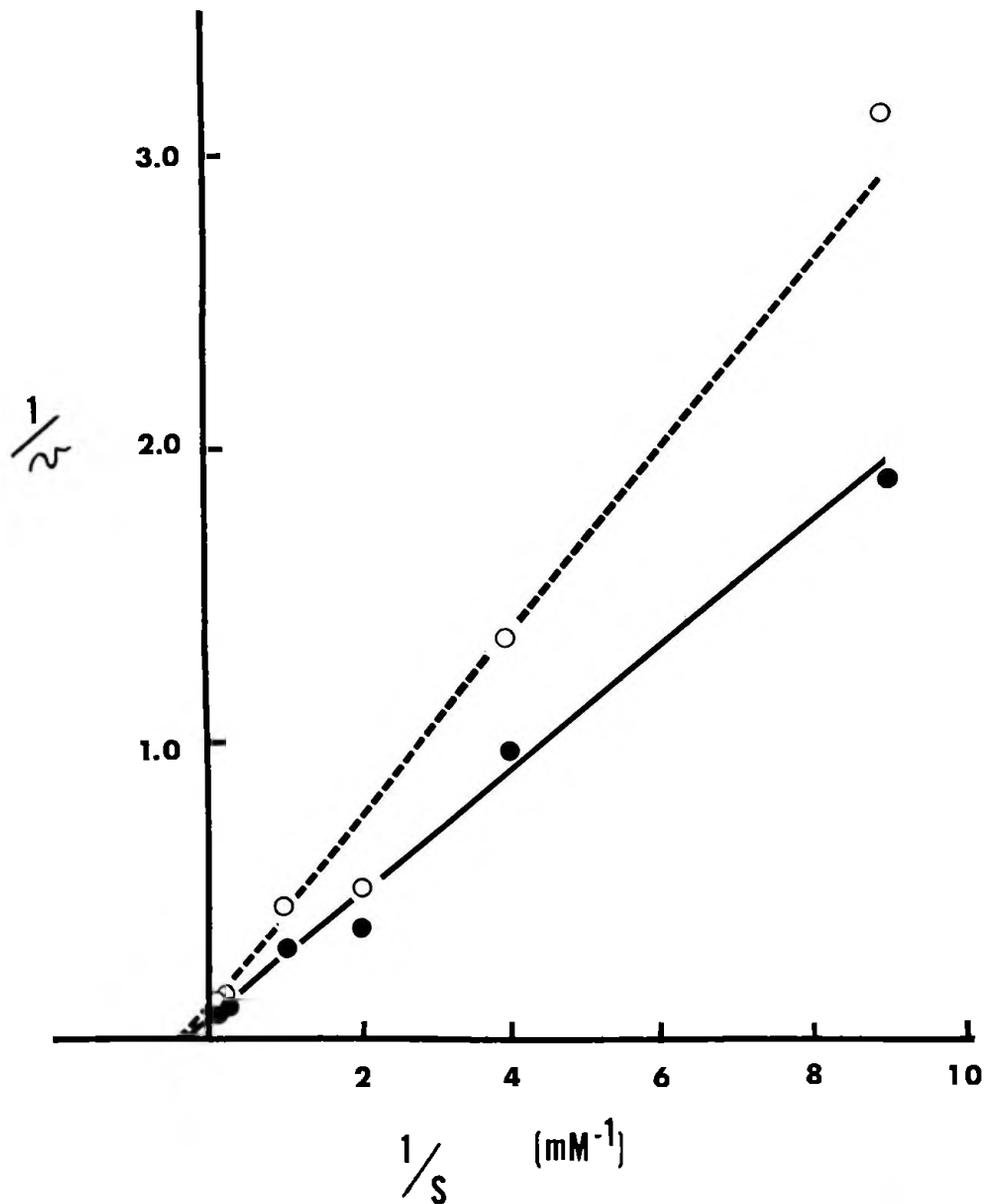


Figure 1. Effect of substrate concentration on the uptake of 2-deoxy-D-glucose by the choroid plexi of the dogfish. The plexi of the IIIrd (●) and IVth (○) ventricles were incubated aerobically for 1 hour at 15°C in salines containing varying concentrations of 2-deoxy-D-glucose- $^{14}\text{C}$ . Ordinate:  $1/v$  ( $v = \mu\text{mole total [free plus phosphorylated] sugar/g tissue wet wt./h}$ ). Each point is the mean of three analyses.

patible with the view that 2-deoxy-glucose is transported into the tissue by a saturable process.

The specificity pattern of the transport system for 2-deoxy-D-glucose was investigated, Table 1 shows that the uptake of 1 mM 2-deoxy-glucose was markedly inhibited by 10 mM D-glucose and

TABLE 1

Uptake of 2-deoxy-D-glucose by the choroid plexus  
of the dogfish: inhibition by sugars.

Tissue of at least six animals was incubated aerobically (air) for 60 min. at 15° C in standard saline containing 1 mM 2-deoxy-D-glucose-<sup>14</sup>C, without (control) or with 10 mM other sugars. Mean values of the T/M for total sugar are presented, ±S.E.

Inhibitor	Ventricle	
	III	IV
None (control)	3.22 + 0.59	3.31 + 0.32
D-Glucose, 10 mM	0.81 + 0.03	0.82 + 0.01
L-Glucose, 10 mM	3.79 + 0.18	3.28 + 0.42
D-Mannose, 10 mM	0.99 + 0.05	1.13 + 0.03
D-Galactose, 10 mM	4.52 + 0.24	3.88 + 0.22
α-Methyl-D-glucoside, 10mM	5.05 + 0.16	4.16 + 0.16
3-O-Methyl-D-glucose, 10mM	3.51 + 0.17	2.90 + 0.21

D-mannose whereas L-glucose, D-galactose, α-methyl-D-glucoside and 3-O-methyl-D-glucose were ineffective. Thus D-glucose and D-mannose appear to share the transport carrier for 2-deoxy-D-glucose. The data suggest the following structural requirement for the transport pathway shared by these three hexoses: C<sub>1</sub>-OH, C<sub>3</sub>-OH and C<sub>4</sub>-OH (the latter in the position found in D-glucose). It should be noted that this structural requirement coincides with that found for the system mediating the transport of D-glucose, D-mannose and 2-deoxy-D-glucose in the renal cells of the flounder *Pseudopleuronectes americanus* (see report #29).

This investigation was supported in part by USPHS Grant Number AM-12619. The cooperation of Miss Leslie Hogben was greatly appreciated.