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Molecular and Kinetic Parameters of Sugar Transport Across the Frog Choroid Plexus

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Summary. The nature of sugar transport across the "blood-cerebrospinal fluid barrier" has been investigated using an *in vitro* preparation of the frog posterior choroid plexus. The permeability of 41 sugars and related compounds was measured by the rapid osmotic procedure described previously. Sugar permeation was found to be stereospecific, inhibited by 1,5-difluoro-2,4-dinitrobenzene, insensitive to anoxia, and independent of the external alkali cation composition. In addition, the transport of a sugar was inhibited by structural analogues. Transport occurred equally well from the ventricular or serosal surface of the tissue, and the rate of transport could be described formally by Michaelis-Menten kinetics. The results were analyzed in terms of the conformation of the sugars in aqueous solution. Sugars which were transported have the D-glucose chair conformation. There is a good correlation between the affinity of the sugar for the transport system and the number of hydroxyl groups attached to the equatorial plane of the ring; D-glucose with five equatorial hydroxyl groups has the greatest affinity. It is concluded that sugar transport across the choroid plexus occurs by facilitated diffusion.

It is well established that the molecular forces which control the permeation of nonelectrolytes through cell membranes are mainly those which also control the distribution of nonelectrolytes between bulk phases of lipid and water. Thermodynamic analysis (Diamond & Wright, 1969) has shown that nonelectrolyte selectivity is largely due to differences in solute: water intermolecular forces; the greater these forces, the lower the permeability of the solute. The principal intermolecular forces between nonelectrolytes and water are hydrogen bonds. Consequently, the greater the hydrogen bonding between the solute and water, the lower the partition coefficient and the lower the permeability. Many important biological compounds, e.g., the sugars and amino acids, form hydrogen bonds to a high degree, and so it is expected that these solutes have low partition

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coefficients and permeate slowly across cell membranes. However, sugars pass through some cell membranes quite readily, e.g., sugar absorption from the gut, sugar reabsorption from the glomerular filtrate in the kidney, sugar entry into muscle, etc. Therefore, it is reasonable to suppose that cell membranes may possess special mechanisms for the transport of sugar.

In the accompanying paper (Wright & Prather, 1970), we have demonstrated that the choroid plexus provides a barrier to the distribution of solutes between blood and the cerebrospinal fluid (CSF). The permeability characteristics of the choroid plexus are very similar to those of other cell membranes; e.g. there is a good correlation between nonelectrolyte permeability and partition coefficients. In contrast, it was observed that the sugars D-glucose and L-arabinose permeate through the plexus more rapidly than expected from their partition coefficients, and they permeate more rapidly than their optical isomers. This suggested the presence of a sugar transport system.

The purpose of this paper is to describe sugar transport across an *in vitro* preparation of the frog choroid plexus. The parameter used to measure the permeability of the choroid plexus was the reflection coefficient which was determined by the osmotic procedure described in the accompanying paper. The results suggest that sugar is transported across the choroid plexus by facilitated diffusion.

The Principle of the Experiments

Sugar permeation through the choroid plexus was determined by measuring reflection coefficients. The thermodynamic principles and the experimental approach have been discussed in some detail by Wright and Diamond (1969) and by Wright and Prather (1970). Briefly, nonelectrolyte reflection coefficients are defined as the ratio of the osmotic flows produced by a given concentration gradient of a test solute to the flow produced by the same concentration gradient of an impermeable solute. The value of the reflection coefficient σ is related to the nature of both the membrane and the test solute; σ for an impermeable solute is 1 by definition, and for increasingly permeant solutes σ decreases progressively below 1. Reflection coefficients were determined by measuring the streaming potentials produced by equal concentration gradients of the sugar and the impermeable solute. As streaming potentials are directly proportional to osmotic flow, the ratio of the streaming potentials produced by the sugar to the streaming potential produced by an impermeable solute yields the sugar reflection coefficient. Sucrose (12 carbons) was chosen as the impermeant reference

solute since the streaming potentials for 15 fully hydroxylated compounds, ranging from 4-carbon (erythritol) to 18-carbon (raffinose) compounds, did not significantly differ. The advantage of this electrical method for determining reflection coefficient is that up to 12 measurements could be made in a single experiment. Thus, in one choroid plexus we could compare the σ's of closely related sugars, test the effects of inhibitors and cations, and investigate the kinetics of sugar transport.

Some insight into the kinetics of sugar transport across the choroid plexus can be obtained from reflection coefficients. Katchalsky and Kedem (1962) derived the following expression relating σ's to permeability coefficients,

$$\sigma = 1 - \frac{\omega \bar{V}_s}{L_p} - \frac{\omega f_{sm} d}{\phi_w} \tag{1}$$

where ω is the permeability coefficient ($\omega RT = P$), $\bar{V}_s$ is the partial molar volume of the solute, L_p is the hydraulic conductivity of the membrane, f_{sm} is the frictional coefficient between the solute and the membrane, d is the thickness of the membrane, and ϕ_w is the volume fraction of water in the membrane. In this equation, L_p, d and ϕ_w are parameters of membrane structure, $\bar{V}_s$ a parameter of the solute and f_{sm} a parameter of both the membrane and the solute. Thus, for each solute in a given membrane, Eq. (1) can be rewritten in the form

$$\omega = \frac{1-\sigma}{k} \quad \text{where} \quad k = \left(\frac{\bar{V}_s}{L_p} - \frac{f_{sm} d}{\phi_w}\right)^{*}. \tag{2}$$

Since the permeability coefficient ω is defined as the proportionality coefficient relating the rate of solute transport to the solute concentration (i.e., rate $= \omega \mathrm{RT}\, C$), the rate of transport can be described by the expression,

$$\text{rate} = \frac{1}{k}(1-\sigma)\,\mathrm{RT}C\,.$$

Although the value of k is unknown, the variation in the rate of transport with concentration for any given solute can be easily determined from σ measurements alone. Hence, some kinetic parameters of sugar transport

* This is the general expression for k where there is possible frictional interaction between the solute and water in the membrane; the frictional term is probably zero in the case of sugar transport across most biological membranes. It is expected that k is a constant independent of concentration over the sugar concentrations used in the present study.

can be determined from variations in σ with concentration; e.g., K_t, the concentration of the solute giving half the maximal rate of transport, can be determined from reciprocal plots of $\frac{1}{k}(1-\sigma)RTC$ against C.

Materials and Methods

The techniques used to measure reflection coefficients in the frog choroid plexus are described in the accompanying paper (Wright & Prather, 1970). The Ringer's solution normally contained 110 mmolal NaCl, 2.0 mmolal KCl, 1.0 mmolal $CaCl_2$ and was buffered to pH 8.2 with imidazole. In some experiments (mentioned specifically in the text), the NaCl and KCl of this solution were replaced by equimolar LiCl, KCl, RbCl or CsCl. The RbCl and CsCl were obtained from the Penn Rare Earth metal division of the Kawecki Chemical Co. (New York, N.Y.). Sugars were added to these saline solutions to give final concentrations varying from 25 to 100 mmolal. All sugars and related compounds were the purest grade commercially available, generally from CalBiochem (Los Angeles, Calif.), Sigma Chemical Co. (St. Louis, Mo.) or Mann Research Laboratories (Orangeburg, N.Y.). Sugars which were expected to undergo mutarotation were exposed to the choroid plexus within 90 sec of adding saline to the sugar.

The compound 1,5-difluoro-2,4-dinitrobenzene (FFDNB) was used to modify the permeability of the choroid plexus; 50 mg of this compound was dissolved in 0.5 ml of methanol, and this was added slowly, with vigorous stirring, to 80 ml of Ringer's solution in which 1.6 mmolal boric acid/NaOH buffer was used to maintain the pH at 8.2. Solutions of this inhibitor were made just prior to being tested because FFDNB undergoes slow hydrolysis in aqueous solution. The effect of anoxia on sugar transport was investigated by substituting nitrogen for the oxygen used to stir the ventricular and serosal solutions.

Sugar Fluxes

Sugar fluxes across the isolated choroid plexus were determined using ^{14}C labeled sugars. In these experiments, fluxes were measured from the ventricular to the serosal solutions by the addition of a tracer quantity of the radioactive isotope to the ventricular solution and by withdrawing samples of the serosal fluid. The radioactive samples were assayed by liquid scintillation counting techniques where each sample was counted to at least 1% counting efficiency. Evaporation of solution from the chambers amounted to about 1.5%/hr, and so this was ignored in the flux calculations. Fluxes were calculated in μmoles/cm^2 per hr.

In one series of experiments, we compared the fluxes of D-galactose and sucrose in each choroid plexus; in the second series, we compared D-arabinose and L-arabinose fluxes. A 50 mmolal sugar solution was placed on the ventricular side of the choroid plexus, a tracer quantity of the same labeled sugar was added to the ventricular solution, and samples were taken from the serosal solution every 20 min for a total of 120 min. The ventricular and serosal solutions were then changed repeatedly until the radioactive isotope in the saline bathing the choroid plexus approached background counting levels. Finally, a 50 mmolal solution of the second sugar was placed in the ventricular reservoir, and the fluxes were determined every 20 min over another 120-min period. This procedure was adopted to minimize the variation in fluxes from one choroid plexus to another. L-arabinose and sucrose ^{14}C isotopes were obtained from CalBiochem; the D-arabinose and D-galactose isotopes were obtained from New England Nuclear Corp.

(Boston, Mass.) and International Chemical and Nuclear Corporation (Irvine, Calif.), respectively.

All experiments were carried out at room temperature which varied between 22 and 24 °C. The variance is indicated by standard deviations.

Results

Sugar Reflection Coefficients

In the course of this study of sugar transport across the frog choroid plexus, we measured the reflection coefficients of 41 sugars and related compounds. The results are presented in Table 1. Inspection of this table reveals that the choroid plexus is permeable ($\sigma < 1$) to monosaccharides ranging from trioses to hexoses, whereas the one heptose and the oligosaccharides are impermeant. A feature common to all but two of the permeant sugars is that they are aldose or ketose sugars which exist as pyranose or furanose rings in aqueous solution. (A pyranose ring is a six-membered ring containing five carbon atoms and one oxygen atom; a furanose is a five-membered ring containing four carbon atoms and one oxygen atom.) The two exceptions are dihydroxyacetone and glycerol, but it is probable that these compounds only permeate by virtue of their "lipid solubility". This view is supported by the position of glycerol in the σ/K_{ether} plot (Fig. 3 of Wright & Prather, 1970) and by comparison of the permeability of dihydroxyacetone and glycerol with the closely related 1,3-propanediol (Table 1 of Wright & Prather, 1970). D-erythrose, a four-carbon aldose, is the simplest furanose sugar (Deulofeu, 1932). The straight-chain polyhydric alcohols (D-mannitol, D-sorbitol, D-erythritol and D-ducitol) and the cyclic polyhydric alcohol (mesoinositol) are impermeant. The fact that inositol is impermeant implies that a ring oxygen is essential for sugar transport.

A detailed comparison of the sugar σ's in Table 1 yields considerable information about the nature of the transport process. First, sugar permeation through the choroid plexus is a stereospecific phenomena as judged by the σ's of optical isomers, e.g., D-glucose (0.74) vs. L-glucose (0.98), D-mannose (0.57) vs. L-mannose (1.02), D-xylose (0.36) vs. L-xylose (0.97), and L-arabinose (0.52) vs. D-arabinose (0.98). Only in the case of fucose is the choroid plexus permeable to both optical isomers. A second point is that the methyl glycosides are less permeant than their corresponding free sugars, e.g., α-methyl-D-glucose (1.00) vs. α-D-glucose (0.74), β-methyl-D-glucose (0.99) vs. β-D-glucose (0.77), β-methyl-D-galactoside (0.88) vs. D-galactose (0.48), and β-methyl-D-xyloside (0.90) vs. D-xylose (0.36). Either the free anomeric hydroxyl group, the carbon-one group, is

Table 1. *Sugar reflection coefficients (σ) in the choroid plexus*

Sugar[a]		σ[b]
Monosaccharides		
Trioses	Dihydroxyacetone	0.51 ± 0.04 (4)
	Glycerol	0.81 ± 0.06 (5)
Tetroses	D-Erythrose	0.36 ± 0.03 (3)
	D-Erythritol	1.01 ± 0.03 (4)
Pentoses	D-Ribose	0.80 ± 0.05 (8)
	2-Deoxy-D-ribose	0.65 ± 0.09 (5)
	D-Arabinose	0.98 ± 0.02 (4)
	L-Arabinose	0.52 ± 0.07 (12)
	D-Xylose	0.36 ± 0.05 (12)
	β-Methyl-D-xyloside	0.90 ± 0.09 (4)
	L-Xylose	0.97 ± 0.02 (5)
	D-Lyxose	0.41 ± 0.07 (7)
Hexoses	α-D-Glucose	0.74 ± 0.06 (8)
	β-D-Glucose	0.77 ± 0.03 (4)
	β-Methyl-D-glucoside	0.99 ± 0.01 (5)
	α-Methyl-D-glucoside	1.00 and 1.00 (2)
	2-Amino-2-deoxy-D-glucose (D-glucosamine)[c]	0.27 ± 0.10 (3)*
	N-Acetyl-D-glucosamine	0.99 ± 0.04 (4)
	2-Deoxy-D-glucose	0.70 ± 0.05 (7)
	3-0-Methyl-D-glucose	0.58 ± 0.04 (6)
	L-Glucose	0.98 ± 0.01 (3)
	D-Sorbitol	1.03 ± 0.02 (4)
	D-Mannose	0.57 ± 0.08 (10)
	L-Mannose	1.02 ± 0.03 (4)
	6-Deoxy-L-mannose (L-rhamnose)	0.99 ± 0.03 (4)
	D-Mannitol	1.04 ± 0.05 (3)
	D-Galactose	0.48 ± 0.06 (10)
	β-Methyl-D-galactoside	0.88 ± 0.03 (3)
	β-Methyl-D-thiogalactoside	0.96 ± 0.08 (4)
	2-Deoxy-D-galactose	0.40 ± 0.06 (5)
	6-0-Methyl-α-galactose	0.81 ± 0.07 (3)
	6-Deoxy-D-galactose (D-fucose)	0.43 ± 0.08 (5)
	6-Deoxy-L-galactose (L-fucose)	0.85 ± 0.04 (4)
	D-Dulcitol	1.00 ± 0.00 (5)
	D-Fructose	0.87 ± 0.10 (6)
	L-Sorbose	0.70 ± 0.06 (3)
	D-Tagatose	0.86 ± 0.13 (3)
	Meso-inositol	1.03 ± 0.03 (4)
Heptoses	D-Glucoheptose	1.03 ± 0.05 (4)
Oligosaccharides		
	Maltose	0.97 ± 0.05 (4)
	Raffinose	1.06 ± 0.11 (3)

[a] Arranged according to chemical classification.
[b] Average value ± standard deviation (number of estimates).
[c] D-glucosamine hydrochloride solution, pH 5.

required for sugar transport or the methyl group provides steric hindrance to the intermolecular interactions between the "carrier"[1] and the ring oxygen of the sugar. The third point to note is that there is no significant difference between the σ's of the α and β anomers of D-glucose. In addition, we noted no difference between the σ's obtained in freshly prepared sugar solutions and solutions which contained equilibrium mixtures of the anomers.

Further analysis shows that the presence of a hydroxyl group on carbon two is not necessary to facilitate the movement of sugars across the epithelium. The 2-deoxy sugars [2-deoxy-D-ribose (0.65), 2-deoxy-D-glucose (0.70) and 2-deoxy-D-galactose (0.40)] and 2-amino-2-deoxy-D-glucose (D-glucosamine, 0.27) are all quite permeant. However, the reflection coefficient for 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) was found to be 0.99. The $-NHCOCH_3$ group on carbon two of N-actyl-D-glucosamine may reduce sugar permeation because of steric factors. A comparison of D-glucose (0.74) and 3-0-methyl-D-glucose (0.58) also shows that the presence of a free hydroxyl group on carbon three is not essential. As yet we have been unable to obtain carbon-four derivatives.

The effect of various groups on carbon five can best be illustrated by the comparison of sugars in one homomorphous series, e.g., L-arabinose (0.52), D-fucose (0.43), D-galactose (0.48) and 6-0-methyl-D-galactose (0.81). In a homomorphous series, the configuration of the pyranose rings are identical, and the members of the series differ only in the group present on carbon five, e.g., $-H$, $-CH_3$, $-CH_2OH$, $-CH_2OCH_3$, etc. It is apparent that the choroid plexus is permeable to all members of the galactose series. Similar results were obtained for the mannose series [D-lyxose (0.41) and D-mannose (0.57)] and the glucose series [D-xylose (0.36) and D-glucose (0.74)], except that the heptose (D-glucoheptose) was impermeant.

In summary, the results of Table 1 show that aldose or ketose sugars permeate across the frog choroid plexus by a stereospecific process. There is no absolute requirement for a free hydroxyl group on any carbon atom except perhaps the anomeric hydroxyl group on carbon one. The presence of large groups on sugars, e.g., $-NHCOCH_3$ and $-CHOHCH_2OH$, on carbon atoms two and five may inhibit transport owing to steric hindrance between the carrier and the sugar molecule.

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1 The term "carrier" is used in this paper to denote that component of the membrane which specifically increases the sugar partition coefficients between the membrane and the external solutions. The term is meant to have no further implication as to the mechanism of sugar permeation.

The reflection coefficients in Table 1 were obtained by the addition of sugars to the ventricular solution, a procedure which measures the permeation of sugar from the ventricular to the serosal solutions. To determine if sugars were also transported from the serosal to ventricular solutions, we measured the reflection coefficients when sugars were added to the serosal solution. In four experiments, we measured the σ's for a total of three sugars (L-arabinose, D-xylose and D-glucose) from both the ventricular and serosal sides of the plexus. The results are shown in Table 2, where it can be seen that there was close agreement between the σ's obtained from both sides of the preparations. Therefore, the rates of sugar permeation are similar from either side of the choroid plexus. In the remainder of our experiments, σ's were determined only from the ventricular solution. More σ's can be determined per experiment from the ventricular side than from the serosal side since the half time for the buildup of the ventricular streaming potential is much shorter than the half time for the serosal streaming potential (Wright & Prather, 1970).

Sugar Fluxes Across the Choroid Plexus

To confirm the validity of the reflection coefficient measurements summarized in Table 1, we determined the fluxes of four sugars across the choroid plexus. Sugar fluxes were measured from the ventricular to serosal solutions when the ventricular sugar concentration was 50 mmolal. After the addition of the radioactive sugar isotope to the ventricular solution, the sugar flux reached a steady state within 20 to 30 min. In each preparation two sugar fluxes were measured in two consecutive 120-min periods; the order of the sugars was reversed from one experiment to the next. Two series of experiments were performed: one in which we compared the fluxes of sucrose and D-galactose, and one in which we compared the fluxes of D-arabinose and L-arabinose.

It can be seen from Table 3 that the D-galactose flux is three times greater than the sucrose flux, and the L-arabinose flux is 1.4 times greater than the D-arabinose flux. The flux of sucrose across the choroid plexus gives a sucrose permeability coefficient of 2×10^{-6} cm/sec. As the sucrose σ is indistinguishable from 1.0, this flux implies that the choroid plexus L_p is less than 2×10^{-6} cm/sec per atm (i.e., L_p is less than $1/100\ \omega \bar{V}_s$), which is in the range of the L_p obtained in the rabbit choroid plexus by Welch (1967). It also can be seen in Table 3 that the flux of D-arabinose is twice that of sucrose even though the σ's for the two compounds are close to 1.0. This apparent discrepancy is accounted for by the difference in the partial molar volumes of the two solutes. It is also possible, however, that a proportion

Table 2. *Sugar reflection coefficients (σ) determined from the ventricular and serosal solutions. σ's were determined first by addition of sugars to the ventricular solution and then by addition to the serosal solution*

Experiment no.	Sugar	Ventricular solution σ	Serosal solution σ
1	L-Arabinose	0.54	0.46
	D-Xylose	0.33	0.21
2[a]	L-Arabinose	0.75	0.74
	D-Xylose	0.73	0.70
3	D-Xylose	0.36	0.36
4[a]	L-Arabinose	0.85	0.67
	D-Glucose	0.78	0.66

[a] 5 mmolal glucose was present in all solutions, resulting in partial inhibition of sugar transport.

Table 3. *Sugar fluxes across the choroid plexus*

Experiment series	Sugar	Flux[a] (μmoles/cm^2/hr)	P[b]
1	D-Arabinose	0.72 ± 0.28 (20)	
			>0.025
	L-Arabinose	1.00 ± 0.43 (30)	
2	D-Galactose	1.10 ± 0.41 (15)	
			>0.001
	Sucrose	0.36 ± 0.13 (20)	

[a] In all experiments, the sugar concentration in the ventricular solution was 50 mmolal; the serosal solution was sugar-free. The fluxes given are from the ventricular to the serosal sides of the epithelium $\pm$ standard deviation (number of estimates).

[b] P value represents the significance of the difference between the two fluxes in each series of experiments.

of these solute fluxes is related to damage at the edge of the tissue when the plexus is clamped between the two lucite chambers (*compare* the edge effects reported for the frog skin by Dobson & Kidder, 1968). Nevertheless, it can be concluded that the fluxes and reflection coefficients are consistent for these four sugars (i.e., the choroid plexus is more permeable to D-galactose than sucrose and the permeation process is stereospecific).

Effects of FFDNB and Anoxia

The effects of FFDNB and anoxia on sugar reflection coefficients are shown in Table 4. In the first three experiments, FFDNB produced an

Table 4. *Effect of FFDNB and nitrogen on sugar transport*[a]

Experiment no.	Sugar	Control σ	FFDNB σ	Nitrogen σ
1	L-Arabinose	0.40	0.83	—
	D-Glucose	0.76	0.83	—
2	L-Arabinose	0.56	0.98	—
	D-Glucose	0.79	0.93	—
3	L-Arabinose	0.51	0.71	—
	Urea	0.51	0.37	—
	Diethylene glycol	0.28	0.21	—
4[b]	D-Xylose	0.40	—	0.38
	L-Arabinose	0.46	—	0.54

[a] The choroid plexus was exposed to 2.8 mM FFDNB for an average of 12 min. The σ's were determined in normal solution approximately 1 hr after removal of FFDNB.

[b] In Exp. 4, 100 % oxygen was replaced with 100 % nitrogen for 135 min. Results similar to these were obtained in two other experiments.

increase in L-arabinose and D-glucose σ's, whereas there was a slight decrease in the σ's for urea and diethylene glycol. Control experiments with methanol and sodium fluoride showed that the effect on sugars was due to FFDNB and not to the methanol or the fluoride released from hydrolysis of the compound. These results indicate that, although FFDNB produces a general increase in the permeability of the plexus to nonelectrolytes, the reagent specifically inhibits the transport of sugars. The related compound FDNB produced similar effects on sugar transport across the red cell membrane (Bowyer & Widdas, 1958), and it is probable that both reagents combine with sugar carriers in an irreversible fashion. In the fourth experiment, the tissue was made anoxic by replacing the oxygen with nitrogen. The lack of oxygen had no significant effect on the D-xylose and L-arabinose σ's.

The Effect of Cation Composition

Nonelectrolyte transport across a number of biological membranes is sensitive to the external sodium concentration; e.g., sugar transport across the small intestine requires the presence of sodium in the mucosal solution (*see* review by Crane, 1965). The influence of the alkali metal cations on galactose σ's in the choroid plexus is shown in Table 5. In these six experiments, the D-galactose σ was independent of the cation in the Ringer's solution. This is taken as evidence that sugar transport across the *in vitro* choroid plexus is insensitive to the external cation composition.

Table 5. *Effect of cation composition on* D-*galactose* σ*'s in the choroid plexus*

Experiment no.	D-Galactose σ				
	NaCl	LiCl	KCl	RbCl	CsCl
1	0.39	0.55	—	—	—
2	0.51	0.46	—	—	—
3	0.50	—	0.56	—	—
4	0.49	—	0.48	0.48	—
5	0.54	—	—	0.66	0.42
6	0.43	—	—	—	0.57

[a] D-Galactose σ's were determined in normal NaCl solution before and after NaCl was replaced with one of four other alkali cations. The control σ's are the average of the values obtained before and after each replacement.

The Kinetics of Sugar Permeation

The mechanism of sugar transport across the choroid plexus can be clarified by kinetic analysis. If the mechanism is simple diffusion, both permeability coefficients and reflection coefficients would be expected to be independent of concentration. The variation in reflection coefficients as a function of concentration is shown for four solutes in Fig. 1. The urea, diethylene glycol and L-xylose σ's were virtually independent of concentration over the range 25 to 100 mmolal, but the D-glucose σ increased from 0.33 at 25 mmolal to 0.77 at 100 mmolal. On the basis of Fig. 1, it can be concluded that urea, diethylene glycol and L-xylose permeate the choroid plexus by simple diffusion. The results obtained with D-glucose indicate that the permeability coefficient of this compound decreases with increasing concentration, a saturation phenomenon suggestive of a process other than simple diffusion.

As outlined in the Introduction, the variation in σ with concentration can be used to estimate the kinetic parameters of sugar transport. The rate of transport, $(1-\sigma)CRT/k$, can be plotted against concentration to yield both the maximum rate of transport (V_{max}) and the concentration of sugar which gives half the maximum rate of transport (K_t). A convenient method used to extract these parameters is to plot the reciprocal of the rate of transport as the ordinate against the reciprocal of the concentration as the abscissa (Lineweaver & Burk, 1934). Such a plot is illustrated in Fig. 2 for D-glucose and D-mannose. The intercept of the extrapolated regression lines on the abscissa is the negative reciprocal of the K_t and the intercept on the ordinate is the reciprocal of the V_{max}. The K_t values for D-glucose and D-mannose in these two experiments were 17 and 67 mmolal, respectively.

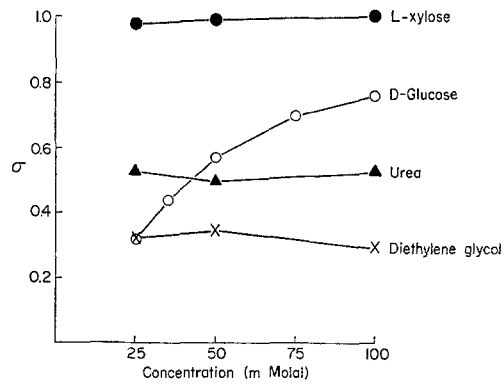


Fig. 1. Nonelectrolyte σ's plotted as a function of concentration. The L-xylose, D-glucose, urea and diethylene glycol σ's were measured at concentrations varying from 25 to 100 mmolal. At each concentration, the nonelectrolyte σ's were obtained by measuring the streaming potentials produced by the compound and by sucrose

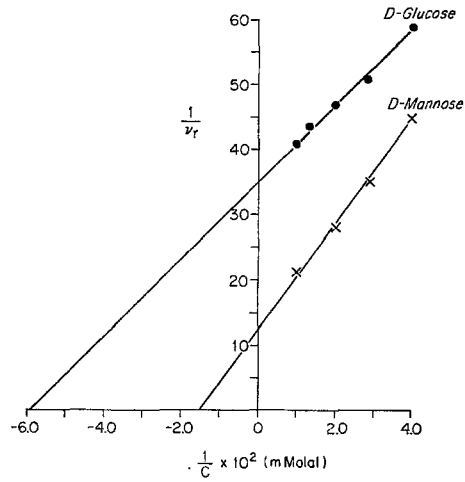


Fig. 2. Double reciprocal plots of transport rate against concentration for D-glucose and D-mannose. The reciprocal rate of transport v_r [where $v_r = 1/k\,(1-\sigma)\,RTC$] is plotted as the ordinate, and the reciprocal of concentration is plotted as the abscissa. The D-glucose experiment is that shown in Fig. 1. The useful range of concentration for the osmotic method in this tissue is from 25 to 100 mmolal; below 25 mmolal the method is not sufficiently accurate, and above 100 mmolal a nonlinear relation exists between the streaming potential and the sucrose concentration (Wright & Prather, 1970)

Similar plots were constructed for a total of 15 permeant sugars; the K_t and V_{max} rates are summarized in Table 6. The standard deviations of these parameters represent the variation from one choroid plexus to another. In all experiments, sugar permeation across the choroid plexus formally followed Michaelis-Menten enzyme kinetics; i.e., in double reciprocal plots,

Table 6. *Kinetic parameters of sugar transport across the choroid plexus (obtained from reciprocal plots such as those in Fig. 2)*

Sugar	K_t[a] (mmolal)	$\frac{V_{max}\text{ (sugar)}}{V_{max}\text{ (glucose)}}$
D-Glucose	20±16(5)	1.0
3-0-Methyl-α-D-glucose	50±7(3)	1.8
2-Deoxy-D-glucose	55±29(5)	1.8
D-Mannose	95±33(5)	3.0
2-Deoxy-D-galactose	115±17(2)	1.9
L-Sorbose	145±103(3)	2.3
D-Ribose	165±60(3)	2.0
D-Galactose	180±49(2)	4.5
D-Xylose	240±64(6)	6.5
D-Fucose	290±43(3)	8.3
L-Arabinose	290±65(4)	5.6
D-Lyxose	370±90(3)	8.3
6-0-Methyl-α-D-galactose	>500(2)	8.3
2-Deoxy-D-ribose	>500(2)	7.2
Methyl-β-D-xyloside	>500(1)[b]	—

[a] Mean ± standard deviation (number of estimates).

[b] This K_t is included for comparison, although the plot is almost indistinguishable from simple diffusion.

the data was best fitted by straight lines. Since K_t is analogous with the Michaelis constant K_m, the K_t may be interpreted, at least to begin with, as an index of the dissociation constant of the sugar carrier complex. The precise significance of the K_t depends on the detailed nature of the transport process; the significance of K_t or K_m, and V_{max} is particularly complicated in multistep non-equilibrium, or steady state, kinetics (*see*, e.g., Dixon & Webb, 1966, pp. 92–100). In all cases, however, K_t is a function of the carrier-sugar dissociation constant.

The sugars in Table 6 are arranged in order of increasing K_t, the sugar with the lowest K_t, and presumably the highest affinity, coming first. Analysis of the results give us some insight into the factors which govern the formation of the sugar-carrier complex. The high K_t of β-methyl-D-xyloside (>500) vs. D-xylose (240±64) indicates either that the free anomeric hydroxyl group is essential for the binding process or that the methyl group blocks binding by steric hindrance. In contrast, the presence of hydroxyl groups at carbons two and three appears to be unimportant as judged by the K_t of sugar derivatives, e.g., D-glucose (20±16) vs. 2-deoxy-D-glucose (55±29), D-galactose (180±49) vs. 2-deoxy-D-galactose (115±17), and D-glucose (20±16) vs. 3-0-methyl-D-glucose (50±7). However, the pentoses-ribose (165±60) and 2-deoxy-D-ribose (>500) – present an exception to this pattern.

The hexoses exhibit lower K_t's than their homomorphous pentoses, e.g., D-galactose (180 ± 49) vs. L-arabinose (290 ± 65), D-mannose (95 ± 33) vs. D-lyxose (370 ± 90), and D-glucose (20 ± 16) vs. D-xylose (240 ± 64). Further information concerning the effect of substituents on carbon fixe can be obtained from a comparison of the homomorphous series: L-arabinose (–H), D-fucose ($-CH_3$), D-galactose ($-CH_2OH$) and 6-0-methyl-D-galactose ($-CH_2OCH_3$). There was no difference between D-fucose (290 ± 44) and L-arabinose (290 ± 65), whereas the affinity for 6-0-methyl-D-galactose (> 500) was substantially less than the affinity for all other members of the series. These results suggest that the hydroxyl group on carbon six promotes the binding of the sugar to the carrier and that a $-CH_2OCH_3$ group on carbon five blocks binding by steric hindrance.

Overall, the analysis of the K_t values suggests, that sugars combine with a component of the membrane to a degree which is controlled by the stereochemical configuration of the sugar molecule and by the presence of free hydroxyl groups on carbons one and six, but which is relatively unaffected by the presence or absence of hydroxyl groups on carbon atoms two and three. In addition, the low K_t of L-sorbose (145 ± 103) shows that both aldoses and ketoses can combine with carriers to a high degree.

The V_{max} in Table 6 represents the maximum rate of sugar transport through the choroid plexus. In this table, V_{max} is quoted as the ratio of the V_{max}(sugar)/V_{max}(glucose) since the absolute rates of sugar transfer are not known; i.e., the value of the constant k in the term $1/k(1-\sigma)RTC$ is not yet accessible. It is seen in Table 6 that the relative V_{max} is not the same for all sugars but increases as the K_t of the sugar increases. One interpretation of this phenomenon could be that each sugar, or group of sugars, permeates via different carriers, but this is unlikely as seen from the experiments on mutual inhibition shown in Fig. 3 and Table 7. A more probable explanation is that we are dealing with steady state kinetics where the V_{max} depends on the dissociation constant and other rate constants (*see* Dixon & Webb, 1964, pp. 92–100, for a more detailed discussion).

The effect of D-glucose on the permeability of a second sugar is shown in Table 7. In five experiments, the σ's of D-xylose, L-arabinose and D-mannose were determined in the absence or presence of glucose in both the ventricular and serosal solutions. In all five experiments, there was a significant increase in the sugar σ in the presence of glucose. In the sixth experiment, D-xylose σ's were determined in the absence and presence of 5 mmolal mannose, and it can be seen that mannose, like glucose, produces a significant increase in the D-xylose σ. Therefore, the presence of a second permeant sugar decreases the rate of permeation of other permeant sugars. We

Table 7. *The effect of a second sugar on reflection coefficients* (σ)

Experiment no.	Sugar	Concn. (mmolal)	Control σ[a]	σ in presence of second sugar[b]
1	D-Xylose	100	0.34	0.58
	L-Arabinose	100	0.57	0.77
2	D-Xylose	100	0.28	0.48
	L-Arabinose	100	0.40	0.62
3	D-Xylose	100	0.34	0.48
	D-Xylose	25	0.20	0.36
4	D-Mannose	100	0.48	0.55
	D-Mannose	50	0.27	0.38
5	D-Mannose	25	0.23	0.32
	D-Mannose	50	0.29	0.41
6	D-Xylose	50	0.30	0.43

[a] Values were obtained before and after the second sugar was added to both the ventricular and serosal solutions, and represent the average value.

[b] The second sugar in Exps. 1 – 5 was 5 mmolal D-glucose, and 5 mmolal D-mannose in Exp. 6.

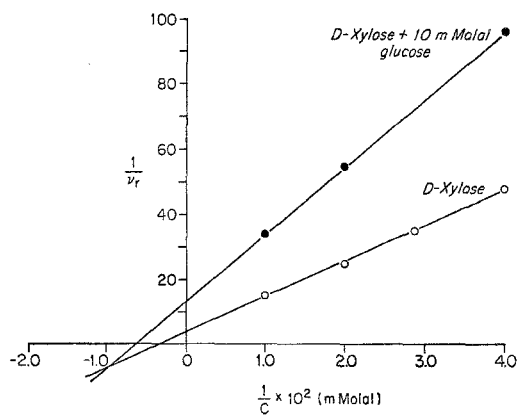


Fig. 3. Inhibition of D-xylose transport by D-glucose. The reciprocal of the relative rate of transport (v_r) as the ordinate is plotted against the reciprocal of concentration for D-xylose both in the presence and absence of 10 mmolal glucose. All data in this graph were obtained in one choroid plexus

investigated these effects further by measuring the kinetic parameters of sugar permeation in the presence and absence of D-glucose. Such an experiment is illustrated in Fig. 3. Glucose produced a decrease in both the xylose K_t and V_{max}; the K_t decreased from 290 to 155 mmolal, and the V_{max} decreased by a factor of 3.5. The glucose inhibition constant, estimated by the graphical method of Hunter and Downs (1945), was found to be

$14 \pm 7(3)$ mmolal, which agrees well with the glucose K_t given in Table 6. Similar results were obtained with other pairs of sugars, e.g., D-mannose and D-glucose, and 2-deoxy-D-galactose and D-glucose. This inhibition of sugar transport by a second sugar is a "mixed-type" inhibition (Dixon & Webb, 1964, pp. 324–325) as the second sugar acts on both the V_{max} and K_t. The changes in V_{max} and K_t produced by a second sugar are difficult to interpret mechanistically as it appears that sugar transport across the choroid plexus is described by multistep non-equilibrium kinetics.

Discussion

This study strongly suggests that sugars are transported across the frog choroid plexus by facilitated diffusion. Danielli (1954) defined facilitated diffusion as a process which results in a rate of permeation which is greater than that predicted for simple diffusion but which does not lead to transport against a concentration gradient. The characteristics of this process in the choroid plexus include: (1) sugar movement is down the concentration gradient and is not linked directly to the metabolism of the cell (Tables 2 & 4); (2) the rate of permeation is greater than predicted from the lipid: water partition coefficients (Wright & Prather, 1970, Fig. 3); (3) the process is stereospecific (Table 1); (4) permeation shows saturation kinetics (Figs. 1 & 2); (5) there is competition between substrates of analogous structure (Table 7 & Fig. 3); and (6) permeation is specifically reduced by inhibitors (FFDNB, Table 4). In addition, sugar transport across this epithelium is insensitive to the external monovalent cation composition (Table 5). These features are also common to sugar transport across the human red cell membrane (*see* Stein, 1967, for an extensive review).

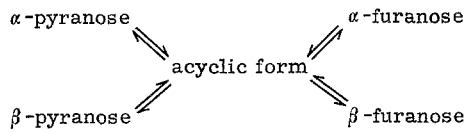
These results, which were obtained for an *in vitro* preparation of the frog choroid plexus, are very similar to those obtained in mammals for the distribution of sugars between blood and CSF. Bradbury and Davson (1964) perfused the cerebral ventricles of the rabbit and measured transport from blood to the perfusion fluid. They found evidence for carrier-mediated transport of D-glucose, D-xylose and probably D-fructose in and out of the CSF. Fishman (1964) measured sugar distribution between blood and CSF in dogs. He also found evidence of carrier-mediated D-glucose and 2-deoxy-D-glucose transport. In neither of these studies was there any indication of active sugar transport.

Csáky and Rigor (1964) have obtained evidence for the uphill accumulation of D-glucose and D-galactose by the isolated choroid plexuses of the dog. This accumulation was inhibited by anoxia, dinitrophenol, phlorrhizin

and ouabain, by lowering the external sodium concentration and by increasing the external sugar concentration. The process was found to saturate at approximately 1 mM. This accumulation is therefore quite distinct from sugar transport across the choroid plexus.

The Relation Between Sugar Conformation and Transport

Sugars in solution exist as a complex equilibrium mixture of at least one open-chain form and four cyclic isomers, i.e.,



The majority of pentoses and hexoses in aqueous solutions occur almost exclusively as the α and β anomers of the pyranose ring with only a small quantity ($<1\%$) in the furanose or acyclic forms (Pigman & Isbell, 1968). The pyranoses are six-membered rings in which an oxygen atom bridges carbons one and five, and most, like the cyclohexanes, can occur in two forms of Sache strainless rings, i.e., the chair and boat forms. However, unlike the cyclohexanes, the sugars may occur in two different chair configurations and six different boat configurations owing to the presence of one hetero atom in the ring (oxygen) and the substituent groups.

Reeves (1949, 1951), in a study of the formation and nature of sugar cuprammonium complexes, has demonstrated that the aldopyranoses prefer the chair to the boat conformations. In the chair conformations, substituent groups are attached to carbon atoms of the ring either parallel to the axis of symmetry of the ring (axial bonds) or at a $\pm 20°$ angle with the horizontal plane of the ring (equatorial bonds). The most stable conformation is the chair conformation with fewer bulky axial groups; bulky equatorial groups (–OH and CH_2OH) have the least interaction with adjacent non-bonded groups. Consequently, pyranose rings twist in order to be in the chair conformation with the lowest free-energy content. The relative stability of the two chair conformations (denoted C1 and 1C by Reeves[2]) has been determined by: (1) analysis of the sugar cuprammonium complexes (Reeves, 1949, 1951; Kelly, 1957); (2) calculations of atomic overlap of non-bonded atoms (Barker & Shaw, 1959); and (3) calculation of interaction energies (Angyal in Eliel, Allinger, Angyal & Morrison, 1965). As a result, it has

2 The original nomenclature introduced by Reeves (1949, 1951) is retained in the present paper in order to distinguish between the conformations of the two enantiomorphs.

been predicted that almost all of the D-pyranoses have the C1 conformation and the L-pyranoses the 1C. In a substantial number of cases, these predictions have been confirmed by X-ray crystallography (Capon & Overend, 1960), measurements of optical rotation (Whiffen, 1956; Brewster, 1959), infrared absorption spectra (Tipson & Isbell, 1960), and nuclear magnetic resonance spectra (Lemieux, 1961, cited by Eliel *et al.*, 1965).

The two chair conformations of α-D-glucose are shown in Fig. 4. In the C1 conformation, the hydroxyl groups at carbons two, three and four and the hydroxymethyl group at carbon five are in the equatorial plane, but the anomeric hydroxyl group is in the axial position. In the alternative 1C conformation, bulky substituents on carbons two through five are axial and the anomeric hydroxyl group is equatorial; i.e., the orientation of each group is reversed when one chair conformation is twisted into the other. Angyal's calculations of interaction energies show that it is the C1 conformation which is most stable for α-D-glucose. In contrast, it is the 1C conformation which has the lowest free-energy content for the optical isomer L-glucose.

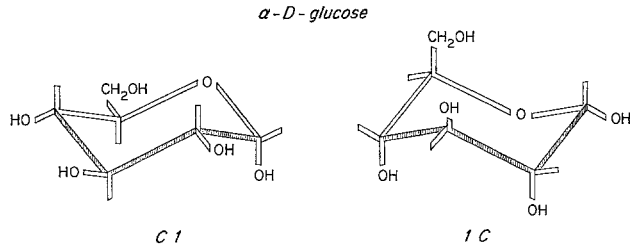


Fig. 4. The two chair conformations of α-D-glucose

Le Fevre was the first to recognize the importance of sugar conformation in sugar transport across biological membranes (Le Fevre & Marshall, 1958; Le Fevre, 1961). The present study provides another example of the striking correlation between conformation and transport. For example, all the permeant hexoses and pentoses in Table 1 are in the C1 conformation (exceptions are L-sorbose and D-fructose). A more detailed correlation between sugar transport across the choroid plexus and the conformation of the sugar is summarized in Table 8. All the sugars in Table 8 are both in the C1 chair conformation and are transported to some extent across the epithelium. Even though there is a considerable deviation in the value of K_t from one preparation to another, it can be seen that there is a fairly good correlation between the number of equatorial hydroxyl groups and the K_t; the sugar with the lower K_t has the greater number of equatorial hydroxyl groups.

Table 8. *The relation between aldose conformation and K_t's*

Sugar[a]	Conformation	% of α anomer in equilibrium solution	No. of equatorial –OH groups on molecule	K_t
D-Glucose	C1 (i, ii, iii, iv)[b]	36 % (viii, iii)	1, 2, 3, 4, 6	20
3-0-Methyl-D-glucose	C1 (vii)	—	(1), 2, 4, 6	50
2-Deoxy-D-glucose	C1 (vii)	—	(1), 3, 4, 6	55
D-Mannose	C1 (i, ii, iii)	69 % (viii, iii)	3, 4, 6	95
2-Deoxy-D-galactose	C1 (vii)	—	(1), 3, 6	115
D-Ribose[c]	C1 (i, ii, iii)	16 % (viii, iii)	1, 2, 4	165
D-Galactose[d]	C1 (i, ii, iii)	30 % (viii, iii, ix)	1, 2, 3, 6	180
D-Xylose	C1 (i, ii, iii)	35 % (viii, iii)	1, 2, 3, 4	240
D-Fucose	C1 (vii)	—	(1), 2, 3	290
L-Arabinose[e]	C1 (i, ii, iii, v) C1 = 1C (i, ii)	74 % (viii)	1, 2, 3	290
D-Lyxose	C1 (iii)	76 % (viii, iii)	3, 4	370
6-0-Methyl-α-D-galactose	C1 (vii)	—	(1), 2, 3	>500
2-Deoxy-D-ribose	C1 (vi, vii)	—	(1), 4	>500
β-Methyl-D-xylose	C1 (i, ii)	—	2, 3, 4	>500

[a] Aldose sugars and derivatives are listed according to K_t values.

[b] Small roman numerals refer to: (i) Reeves, 1949, 1951; (ii) Kelly, 1957; (iii) Eliel *et al.*, 1965, Chapt. 6; (iv) McDonald & Beevers, 1952; (v) Hordvik, 1961; (vi) Lemieux, 1961, cited by Eliel *et al.*, 1965, showed that 2-methyl-2-deoxy-ribose has the C1 conformation; (vii) predicted conformation; (viii) Pigman & Isbell, 1968; (ix) Sweeley, Bentley, Makita & Wells, 1963.

[c] In aqueous solution, 8.5 % of ribose is in the straight-chain form (Pigman & Isbell, 1968) and 24 % is in the furanose ring (Angyal & Pickles, 1967).

[d] About 12 % of galactose in aqueous solution is in the furanose form (Eliel *et al.*, 1965, Chapt. 6).

[e] 3 % of D-arabinose is the furanose in aqueous solution (Angyal & Pickles, 1967). The ring configuration of α-L-arabinose is equivalent to that of β-D-galactose.

The position of D-ribose in Table 8 may be anomalous owing to the fact that 8.5% is in the acyclic form and 24% is in the furanose ring when the sugar is dissolved in aqueous solutions (Pigman, 1957; Angyal & Pickles, 1967). This would result in an underestimate of the D-ribose K_t using the osmotic procedure.

A $-CH_2OH$ group is attached to the anomeric carbon atom in the ketose sugars. Three ketoses were used in the present study: L-sorbose, D-fructose and D-tagatose. L-sorbose [σ $0.70 \pm 0.06(3)$ and K_t $145 \pm 103(3)$] occurs as the α-pyranose in aqueous solution (Pigman & Isbell, 1968; Karabinos, 1952). As already indicated, this sugar has the 1C conformation although it permeates through the choroid plexus quite readily. Construction of Corey-Pauling Koltun molecular models, however, shows that the keto-

pyranose 1C conformation is formally equivalent to the aldopyranose C1 conformation; i.e., Reeves' terminology for the two chair forms has to be reversed for the ketopyranoses. The value of the experimentally determined L-sorbose K_t is close to that predicted for an aldopyranose sugar (C1 chair) containing four equatorial hydroxyl groups.

The reflection coefficients for D-tagatose and D-fructose were found to be $0.86 \pm 0.13(3)$ and $0.87 \pm 0.10(6)$, respectively. In solution, D-tagatose is in the C1 conformation, whereas D-fructopyranose is in the 1C conformation (Eliel *et al.*, 1965). About 15% of D-fructose is present as the co-planar furanose ring (Verstraeten, 1967). Therefore, we expect the sequence of ketopyranose K_t's to be: L-sorbose < D-fructose < D-tagatose. The only sugar used which is known to exist mainly as the furanose in solution is D-erythrose; only the furanose ring is structurally feasible with the aldotetroses. This sugar is quite permeable (σ 0.36) compared with mesoerythritol (σ 1.01), and this suggests that at least some of the non-planar furanose compounds are able to permeate through the choroid plexus.

In summary, these results imply that for the majority of sugars which are transported across the choroid plexus the carrier is able to distinguish between the three-dimensional conformations of the two chair forms. In addition, the binding of the sugar to the carrier is promoted by the presence of three or more equatorial hydroxyl groups. This is consistent with the observations that equatorial groups are more reactive than axial groups, e.g., in the formation and hydrolysis of glycosides, and the oxidation of aldoses by halogens (Eliel *et al.*, 1965).

The Nature of the Transport Process

Since some sugars permeate through the epithelial cells of the choroid plexus much more rapidly than expected from their lipid:water partition coefficients (Wright & Prather, 1970), it appears that there are specific intermolecular forces which increase the effective sugar partition coefficients. Permeation of sugars through "pores" is highly improbable, since we have shown in the accompanying paper (Wright & Prather, 1970) that only compounds with less than four carbon atoms can permeate to any significant extent via a polar pathway. The results presented in the present paper enable us to draw some conclusions about the nature of the transport process. The first conclusion is that sugars interact with a component of the membrane which is present in limited amounts. This is suggested by the observed saturation kinetics of the process. By analogy with other sugar transport systems (Stein, 1967), these carriers are mobile rather than fixed

sites. The kinetics of permeation through the choroid plexus further suggests that the mobility of the carriers may not be rate limiting compared with the association/dissociation of the sugar carrier complex; i.e., the system exhibits non-equilibrium kinetics.

The specificity of the transport system (Tables 1, 6 & 8) provides more information about sugar:carrier interactions. The sugars which are transported are pyranose (or furanose) rings in a particular chair conformation without large substituent groups. Thus the carrier can distinguish between the three-dimensional conformation of the isomers, and the approach to the carrier can be blocked by large groups on the sugar causing steric hindrance. The correlation between the K_t and the number of equatorial hydroxyl groups, and the observation that inositol is impermeable, further suggest that both the ring oxygen and the equatorial groups are involved in the interactions. There is also some evidence that the hydroxyl groups on carbons one and six are preferred sites.

The specificity of the process can be explained by the presence of a site on the carrier composed of a three-dimensional constellation of polar groups. By analogy with the enzyme-active sites (Koshland, 1959), sugar could combine with the site in either a "lock-key" or an "induced-fit" relationship. In either case, combination of the sugar with the carrier would increase the partition of the sugar into the membrane interior from the external solution. The carrier, which can be referred to as an amphiphile (i.e., a water-insoluble lipid molecule containing localized polar groups), would be expected to form the sugar:carrier complex at the lipid:water interface, to diffuse across the membrane as the result of thermal agitation, and to dissociate at the trans-face of the membrane. In the field of ion transport, such amphiphiles are known and well characterized, e.g., the macrocyclic antibiotics and cyclic polyethers (Eisenman, 1968).

In conclusion, the evidence presented in this paper suggests that sugars are transported across the choroid plexus by facilitated diffusion. This transport system appears to be fairly primitive in character as it exhibits a broad specificity and is insensitive to the external sodium concentration. The significance of a facilitated sugar transport system in this tissue is related to the fact that the choroidal epithelium constitutes a barrier to the distribution of solutes between the blood and cerebrospinal fluid. We have shown (Wright & Prather, 1970) that the properties of this barrier are very similar to those shown by most other cell membranes. Therefore, it is not surprising that the choroid plexus has a special mechanism for sugar transport in order to accommodate the heavy nutritional demands of the brain.

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