Uptake of Monosaccharides by Guinea-Pig Cerebral-Cortex Slices

By P. JOANNY, J. CORRIOL AND H. HILLMAN

Laboratoire de Physiologie, University of Marseilles, France, and Department of Biological Sciences, University of Surrey Annex, 14 Falcon Road, London S.W.11

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By the use of 1 mM-iodoacetate to inhibit glycolysis in guinea-pig cerebral tissue slices, the kinetics of the uptake of monosaccharides on transfer of tissue from 0° to 37° were studied. D-Ribose, D-galactose, D-mannose, L-sorbose, and D-fructose showed diffusion kinetics, whereas 2-deoxy-D-glucose, D-glucose, D-arabinose and D-xylose showed saturation kinetics.

The transport of hexoses into muscle, erythrocytes, intestine and kidney has been extensively studied (Randle & Morgan, 1962; Le Fevre, 1961; Crane, 1960; Kleinzeller, Kolínšká & Beneš, 1967*a,b*), but comparatively little work has been done on transport of hexoses into cerebral tissue. Geiger, Magnes, Taylor & Veralli (1954) measured the uptake of glucose in the isolated and perfused cat brain, and found that the glucose content of the tissue diminished during the experiment, despite the maintenance of a high concentration of the hexose in the perfusion medium. Crone (1961) examined the movement of different substances across the blood-brain barrier, and suggested a relationship between their solubility in lipids and their rates of transport into the brain tissue; however, this relationship did not explain the transport of glucose at five times the rate of its isomer, fructose. Fishman (1963, 1964) examined the kinetics of glucose transport across the blood-brain barrier in the dog, and showed saturation and competitive inhibition. However, Gilbert (1965) seems to have been the first to study the kinetics of monosaccharide transport in mammalian cerebral slices *in vitro*, and Le Fevre & Peters (1966) examined the movement of monosaccharides from the blood to brain tissue *in vivo*. Both these studies suggested that D-glucose enters the brain by facilitated diffusion. Reports of the effects of alkali-metal ions and of inhibitors on the uptake of sugars *in vitro* have been published (Kleinzeller & Joanny, 1967; Joanny & Corriol, 1964).

In the present study iodoacetic acid was used to inhibit glycolysis, the high rate of which in the brain prevents the accumulation of significant concentrations of glucose. We show in this paper that iodoacetate does not affect the permeability of cerebral tissue to D-xylose, and thus the kinetics of

sugar uptake in cerebral slices can be studied in its presence, as has been done in renal slices (Kleinzeller *et al.* 1967*a,b*).

METHODS

Slices of adult guinea-pig cerebral cortex, 350 μ m. thick, were prepared and incubated in Krebs-Ringer bicarbonate saline, in an atmosphere of O₂ + CO₂ (95:5), as described by Joanny & Hillman (1963), in the rapid-transfer holders described by McIlwain & Rodnight (1962). The slices were incubated for 30 min. at 0° immediately after being cut, with Krebs-Ringer bicarbonate saline containing 1 mM-iodoacetate, 20 mM-sodium lactate as oxidizable substrate, and the monosaccharide being tested. The tissue was then transferred on the rapid-transfer holders to the same incubation medium at 37°, whereupon the concentration of sugar in the tissue immediately began to rise towards that in the medium. When anaerobic conditions were used, the tissue was incubated in an atmosphere of N₂ + CO₂ (95:5), with phosphorus in the centre well of the Warburg vessel.

Since there was no significant change in the dry wt./wet wt. ratio of the tissue in the presence of the different monosaccharides in this time (Table 1) the increase in monosaccharide content of tissue was interpreted as being due to its entry from the extracellular space into the intracellular space of the tissue. The tangent to the initial portion of the curve showing the increase in sugar content after incubation for 2 min. at 37° was used to construct Lineweaver-Burk plots.

The uptake of the monosaccharides was measured as the ratio of the concentration of sugar in the water of the incubated tissue to its concentration in the incubation medium. This was calculated by weighing the incubated tissue and then drying it for 12 hr. at 105° to constant weight. The difference between the initial and final weights was termed the tissue water.

After incubation, the slices were weighed on a torsion balance and deproteinized with 1 ml. of 5% ZnSO₄·6H₂O and 1 ml. of 0.3 M-Ba(OH)₂, in a Potter-Elvehjem homogenizer. After standing for 30 min. the suspension was centrifuged, and the supernatant was analysed.

D-Glucose was measured by the specific method of Huggett & Nixon (1957) with glucose oxidase (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). L-Glucose, D-galactose, D-mannose, D-xylose, D-arabinose and D-ribose were measured by their reducing properties (Nelson, 1944), after correction for the 'endogenous' reducing agents by subtracting the reducing power of the tissue, determined in the absence of monosaccharides. Pentoses were measured by Tracey's (1950) method, with correction for endogenous pentoses; a few assays were also done after deproteinization and filtration through acid-washed charcoal to eliminate nucleotides (Tower, 1958); these gave similar results. L-Sorbose and D-fructose were measured by the colorimetric method of Roe (1934). 2-Deoxy-D-glucose was oxidized with periodate and measured colorimetrically (Waravdekar & Saslaw, 1957). D-Glucose, D-galactose, D-xylose, D-arabinose and D-ribose were obtained from Calbiochem, Los Angeles, Calif., U.S.A.; D-mannose, D-fructose and L-sorbose were from Sigma Chemical Co., St Louis, Mo., U.S.A. L-Glucose was from Koch-Light Laboratories Ltd., Colnbrook, Bucks.; this was shown by the glucose oxidase method to be free of D-glucose. D-[1-¹⁴C]Xylose was obtained from Calbiochem.

Since the aerobically incubated tissue contained endogenous pentoses (up to about 0.5 mM concentration; P. Joanny & H. Hillman, unpublished work), the uptake of xylose was examined by a radioactivity technique. Uptake of D-[1-¹⁴C]xylose (0.5 μ C/5 ml. of medium) in the presence of an excess of non-radioactive xylose (1–20 mM) was measured by counting 0.5 ml. of the supernatant for 7 min., after deproteinization with the ZnSO₄-Ba(OH)₂ mixture, with scintillation fluid NE 220 [Nuclear Enterprises (G.B.) Ltd., Edinburgh] in a Beckman 7066 scintillation counter. These counts were compared with the measured radioactivity of a similar volume of medium similarly treated; an external standard was used for the counting. Quenching by the tissue was measured by adding non-radioactive tissue and medium to the scintillator as a blank; the quenching was never more than 15%, and the radioactivity of the tissue was corrected with the blank reading.

RESULTS

On incubation of cerebral slices at 0° in the absence and presence of 1 mM-iodoacetic acid, the concentration of [1-¹⁴C]xylose in the tissue was 52% and 54% of that in the medium (mean values from six measurements each). The uptake of 1 mM-D-[1-¹⁴C]xylose shown in Fig. 1 was typical of the 1–20 mM concentration range tested, and this result showed that after 30 min. at 0°, only about 50% of the tissue water was accessible to xylose. When the cerebral slice was transferred to a medium of similar composition at 37°, the concentration of xylose in the tissue water increased considerably, to approximately that of the medium (Fig. 1, upper curve). Further, the rates of uptake at 37° of different concentrations of D-xylose in the absence and presence of 1 mM-iodoacetic acid were not significantly different (Table 2). Thus the inhibitor did not act by altering the permeability

of the tissue to D-xylose. Xylose is not metabolized by brain (Maddock, Hawkins & Holmes, 1939).

The concentration of D-glucose in the tissue water of slices incubated without iodoacetate in 10 mM-D-glucose for 30 min. at 37° was 5.9 ± 0.5 mM aerobically and 4.8 ± 0.6 mM anaerobically (each result is the mean $\pm$ S.D. of ten determinations). Aerobic incubation of slices at 37° with 1 mM-iodoacetate resulted in a D-glucose concentration in the tissue water of 8.9 ± 1.9 mM. Incubation

Table 1. *Dry wt./wet wt. ratios of slices before and after transfer from medium at 0° to medium at 37°*

The Krebs-Ringer solution contained 1 mM-iodoacetate and the appropriate substrate at 20 mM. Each value is the mean of the results for six slices.

Substrate	$\frac{\text{Dry wt.}}{\text{Wet wt.}} \times 100$	
	After 30 min. at 0°	After 2 min. at 37°
D-Glucose	12.5	11.5
2-Deoxy-D-glucose	12.0	11.9
L-Glucose	11.3	11.5
D-Galactose	11.4	12.7
D-Mannose	12.9	13.0
L-Sorbose	12.4	12.0
D-Fructose	12.9	12.9
D-Ribose	12.3	11.8
D-Xylose	12.5	12.7
D-Arabinose	11.0	12.2

Fig. 1. Uptake of 1 mM-D-[¹⁴C]xylose (0.1 μ C/ml. of medium) by guinea-pig cerebral-cortex slices *in vitro*, incubated in Krebs-Ringer bicarbonate saline containing lactate. For details see the Methods section. The ordinate is the ratio of the radioactivity in tissue water to the radioactivity in the same volume of medium, expressed as a percentage. Each point is the mean of the values for 12 cerebral slices, with the S.D. represented as a vertical bar.

Fig. 2. Lineweaver-Burk plots of the tangents to the initial slopes of curves of uptake of monosaccharides by cerebral slices after transfer from Krebs-Ringer bicarbonate saline containing 20 mM-sodium lactate and 1 mM-iodoacetate at 0° to the same medium at 37°. Details of the incubation are given in the Methods section. Each point is the mean of the values from 12–15 slices. (a) ○, D-Fructose; ●, L-sorbose; (b) ○, D-galactose; ●, D-mannose; (c) ○, D-ribose; ▲, D-xylose; ●, D-arabinose; (d) ○, L-glucose; ○, 2-deoxy-D-glucose; ●, D-glucose.

Table 2. Effect of 1 mM-iodoacetate on the rate of uptake at 37° of different concentrations of D-xylose

For experimental details, see the Methods section. Each value is the mean of results for six slices. The velocity constant $V_{\max}$, (m-moles/l. of tissue water/min.) was determined graphically.

Concn. of D-xylose (mM)	$V_{\max}$, (m-moles/l./min.)	
	Without iodoacetate	With 1 mM- iodoacetate
10	118	120
25	230	240
40	390	370
50	450	460

in the presence of 10 mM-2-deoxy-D-glucose resulted in a concentration of this sugar in the tissue water of 9.2 ± 1.3 mM (mean $\pm$ S.D. of ten determinations). Thus the inhibitor of glycolysis would appear to have preserved the normally metabolized D-glucose,

which had entered the tissue water, so that it could be measured there.

Extrapolations of the Lineweaver-Burk plots for the uptake of L-sorbose and D-fructose, which are both ketoses and isomers of D-glucose, both made intercepts very close to the origin (Fig. 2a); they had high half-saturation concentrations (apparent K_m values were 6.5×10^{-1} M and 6.6×10^{-1} M respectively) and high maximum velocities (apparent $V_{\max}$ values were 63.0 and 66.5 m-moles/l./min. respectively). At infinite concentrations of sugar, the rate of transport would be infinite, and the rate of uptake of sugar was proportional to the concentration difference, as would be expected from Fick's Law governing simple diffusion.

D-Galactose, D-mannose and D-ribose showed similar characteristics (Figs. 2b and 2c). However, extrapolation of the graphs for D-arabinose and D-xylose made an intercept on the $1/s$ axis of -5 , which shows some reaction of the sugar with the membrane.

Comparison of the results obtained with L-glucose, 2-deoxy-D-glucose and D-glucose (Fig. 2d) showed the L-glucose curve passing close to the origin with apparent K_m $8.0 \times 10^{-1} M$ and V_{max} , 100 m-moles/l./min., and the values for D-glucose were K_m $1.25 \times 10^{-1} M$ and V_{max} , 20 m-moles/l./min., and for 2-deoxy-D-glucose K_m $0.57 \times 10^{-1} M$, and V_{max} , 8.4 m-moles/l./min. Thus the naturally occurring D-glucose showed less affinity than a 'foreign' inhibitor, 2-deoxy-D-glucose (Fig. 2d). The tissue showed stereospecificity in its uptake of the D-glucose rather than the L-glucose.

DISCUSSION

These experiments suggest that entry of monosaccharides into guinea-pig cerebral slices has saturation kinetics for 2-deoxy-D-glucose, D-glucose, D-arabinose and D-xylose, in order of diminishing affinity. However, D-ribose, D-galactose, D-mannose, L-sorbose and D-fructose appear to enter by simple diffusion, or to have a very low affinity for the tissue. There is stereospecificity for the transport of glucose, but not its isomers, which is a characteristic of facilitated diffusion (Wilbrandt & Rosenberg, 1961). Incubation of rat brain slices with glucose largely inhibits the liberation of $^{14}CO_2$ from [^{14}C]fructose (Quastel, 1961), and competition between D-glucose and D-xylose has been shown in preliminary experiments (P. Joanny, J. Corriol & H. Hillman, unpublished work). A high temperature coefficient for the uptake of D-glucose has previously been shown (Joanny, Corriol & Papy, 1966), which is further evidence against entry of this sugar by simple diffusion. Gilbert (1965) suggested that 1 mM-D-xylose was accumulated aerobically by cerebral-cortex slices against a concentration gradient, which is contrary to findings in muscle and isolated heart (Randle & Morgan, 1962; Morgan, Post & Park, 1961).

The present results agree with the findings *in vivo* by Fishman (1963) suggesting a greater affinity of the brain for deoxy-D-glucose than for D-glucose, and also suggesting that D-fructose enters by simple diffusion. Le Fevre & Peters (1966) proposed that there is transport of D-glucose into mouse brain cells *in vivo*, and evidence for transport of D-mannose was obtained in rats as well. Further, competition was shown between D-glucose and D-mannose, but not between D-galactose and D-xylose. This corresponds to the present finding of very small affinity for D-galactose (Fig. 2b). Le Fevre & Peters (1966) remarked that the failure of glucose injected into the cerebrospinal fluid to relieve hypoglycaemic stress in the cerebral cortex or in the spinal cord (Fishman, 1964) suggested that 'Fishman's well-documented blood CSF sugar transport apparatus would not appear . . . to be properly located to

assist the sugars appreciably in reaching the brain cells from the blood'. The present experiments indicate that the high rate of cerebral glycolysis might have been responsible for the failure to detect the expected concentrations of glucose in the brain.

Bachelard (1967) suggested that only low concentrations of glucose are found in the absence of iodoacetate in guinea-pig cerebral tissues. Further, very little of the sugars could be detected in the hearts of normal or diabetic rats (Morgan *et al.* 1961). The present results, showing the presence of D-glucose in physiological concentrations in the tissue space when an inhibitor of glycolysis was present, indicate that the transport across the cell membrane rather than the intracellular metabolism was the rate-limiting step in the utilization of glucose in cerebral tissue.

In skeletal muscle (Randle & Morgan, 1962), in erythrocytes (Le Fevre & Marshall, 1958), in gut (Crane, 1960) and in other tissues in which it has been tested, D-glucose penetrates by non-accumulative facilitated diffusion. However, 2-deoxy-D-glucose, for which the affinity is greatest in the present experiments, is not actively transported by gut or erythrocytes, which transport D-galactose (see Crane, 1960; Le Fevre & Marshall, 1958). In contrast, 2-deoxy sugars are accumulated against a concentration gradient by rabbit kidney slices (Kleinzeller *et al.* 1967b). Crane, Miller & Bihler (1961) suggested that sugars actively transported into gut must have a pyran ring, a methyl group or substituted methyl group at C-5 of the ring and a hydroxyl group at C-2. This rule clearly does not apply to cerebral slices. The affinities of the various tissues for sugars other than glucose are very different, and there are probably also species differences (Le Fevre & Peters, 1966); thus it is highly unlikely that the same carrier is used in all mammals for substances other than D-glucose, or that the particular affinities of different tissues in different mammals have their origin in particular chemical conformations of the same 'carrier' molecule. It is also notable that the affinity of cerebral tissue for monosaccharides other than 2-deoxy-D-glucose is in general no more than 5% of that found in other tissues by the authors quoted in this paragraph.

The present experiments demonstrate that D-glucose is not taken up by cerebral slices by simple diffusion, but shows saturation kinetics and stereospecificity. The fact that the rate of uptake is more rapid with low concentrations but less rapid with very high concentrations than occurs with simple diffusion is evidence suggestive of facilitated diffusion (Wilbrandt & Rosenberg, 1961). To distinguish unequivocally between membrane adsorption and facilitated diffusion, the

phenomenon of counter-transport must be demonstrated in the latter case; experiments to show this are complicated by the probable masking of the effect of the high rate of such transport, and by the relatively large extracellular space.

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