Assimilation of D and L I-C-14 Glucose into the Retina, Brain and Other Tissues

C. T. Dollery, M.B., F.R.C.P., P. Henkind, M.D., and M. L'E. Orme, M.B., M.R.C.P., London, England and New York

SUMMARY

Distribution of D and L 1-C-14-glucose radioactivity was measured in retina, brain and other tissues by a combustion technic at short intervals following an intravenous injection in rat, rabbit and guinea pigs. Five minutes after injection, the ratios of D/L radioactivity in the retina of the three species were 23.7, 11.6 and 8.3, respectively. Similar high ratios were found in the brain but not in voluntary muscle or liver. The explanation may lie in the special characteristics of capillaries in brain and retina, which have tight intracellular junctions. Substances that enter the brain and retina must therefore cross the endothelial cells and cannot diffuse between them as in other tissues. The results suggest the presence of a stereospecific carrier for glucose in the endothelial cells which controls entry of glucose into the retina, as it appears to in the brain. DIABETES *20*:519-21, August, 1971.

Diabetic retinopathy is now the leading cause of blindness in middle age. In the last two decades its clinical features, natural history and microvascular lesions have been extensively studied (Fine and Goldberg, 1969), but its pathogenesis remains obscure. For example, it is still uncertain whether the retinal angiopathy is a cause or a consequence of disordered metabolism in the retinal tissues (Dollery, 1969). The metabolism of the retina has been studied extensively in vitro (Graymore, 1970), but few observations have been made in the living eye because of technical problems. In the present paper we report observations of the assimilation of C-14 radioactivity from glucose into the retinas of three animal species having three different types of vascular supply to the retina.

From the Department of Clinical Pharmacology, Royal Postgraduate Medical School, London, England and the Department of Ophthalmology, Albert Einstein College of Medicine, Montefiore Hospital Medical Center, Bronx, New York.

METHODS

Either D or L-glucose-1-C-14, supplied by the Radiochemical Centre, Amersham, was administered by bolus intravenous injection into rats, guinea pigs and rabbits. In rats, 0.3 ml. of a solution containing 2.5 μCi of C-14 and 0.15 mg. of glucose was injected. Guinea pigs received 0.6 ml. and rabbits 0.9 ml. of the same solution. In the first experiment, pairs of rats were decapitated at one, three and five minutes after injection. The other animals were killed five minutes after injection. The whole retina and a sample of brain (cortex), skeletal muscle (psoas), liver and blood were removed within two minutes of the death of the animal. The tissues were weighed and dried and later burnt in oxygen using a plastic bag technic (Gupta, 1968). The CO_2 resulting from combustion was taken up in 15 ml. of phenylethylamine and 10 aliquots were counted with 10 ml. of butyl P.B.D. scintillator in toluene in a Packard Tricarb Liquid Scintillation Spectrometer. The methods used resemble those of Curtis-Prior, Trethewey, Stewart and Hanley (1969). These authors studied assimilation of labeled glucose into rat tissues five and forty minutes after an intravenous injection. At five minutes the proportion of activity in the brain and liver in a nonpolar form was less than 2 per cent of the total amount in those organs. Less than 0.1 per cent was expired as C-14-O_2 in the first five minutes. In the brain at this time 80 per cent of the radioactivity was soluble in isopropanol-water. These data suggest that at short periods after injection the majority of the activity injected remains as glucose.

In one experiment in rats, tritiated sorbitol was injected mixed with the labelled glucose as an extracellular fluid label. In subsequent experiments on two guinea pigs, 5 μl. of D or L-glucose, containing 0.042 μCi of C-14 and 2.5 μg glucose, was injected into the vitreous of one eye and 5 μl. of saline into the other eye. One

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/8/519/346432/20-8-519.pdf by guest on 03 December 2023

hour later the retina, vitreous and lens were removed and analyzed. Two rabbits injected with D or L-glucose were given an intravitreal injection of 0.1 ml. ouabain (containing 25 μg ouabain, with the objective of inhibiting sodium-potassium ATPase) or saline, one hour prior to the intravenous injection of glucose. Their retinas were removed and processed as above.

RESULTS

The main feature of the results was the high ratio of D to L-glucose activity found in the brain and retina at one, three and five minutes after intravenous injection (figure 1). For comparative purposes the ratios of activity in brain and retina following D and L-glucose injection are shown for the three species studied (table 1). All three species have a high ratio of D to L in both retina and brain. The D/L ratio in retina varied from 23.7 in the rat to 8.3 in the guinea pig.

In the rat experiments using tritiated sorbitol, the ratio of C-14-H_3 in the intravenous dose has been taken as unity. Five minutes after injection, the ratio of C-14-H_3 in plasma was 1.47 for D-glucose and 1.28 for L-glucose. Corresponding ratios for the retina were 2.91 for D-glucose and 0.65 for L-glucose. We have no explanation for the low ratio in the retina for L-glucose, and further studies will be needed to clarify this point. Technical problems with tritium-counting cannot be excluded as an explanation.

In the rabbits in which one eye was pretreated with 25 μg intravitreal ouabain, the ratio of D/L in the retina was 7.5 compared with 11.6 in the saline-controlled eyes. In the guinea pigs given intravitreal-labelled glucose there was a high ratio of D to L-glucose in the lens (20.9) but not in the retina (2.8). In this experiment the lens appeared free of vitreous, but it was impossible to free the vitreous completely from the retina. Thus, some of the activity in the "retinal" samples in this experiment may have been still in the vitreous. It is also probable that labelled glucose which had entered the retina from the vitreous may have been metabolized and removed in the one hour between intravitreal injection and cessation of circulation.

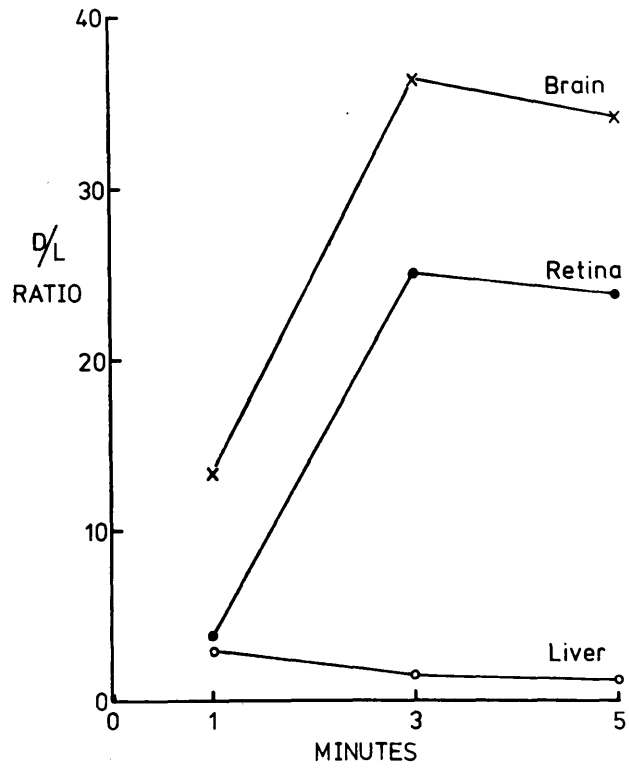

FIG. 1. Ratio D/L radioactivity in retina, brain and liver after intravenous injection of I-C-14-glucose.

TABLE 1

Results at five minutes after injection

Organ	D-Glucose/C-14 (dpm/100 mg. wet wt.)	L-Glucose/C-14 (dpm/100 mg. wet wt.)	Species	D/L ratio
Retina	4,384	185	Rat	23.7
Retina	2,312	195	Rabbit	11.6
Retina	5,863	487	Guinea Pig	8.3
Brain	6,519	191	Rat	34.1
Brain	3,569	106	Guinea Pig	33.7
Blood	4,717	4,796	Rat	0.98
Blood	1,886	2,280	Rabbit	0.81
Blood	3,530	2,839	Guinea Pig	1.24
Muscle	908	813	Rat	1.1
Muscle	312	675	Guinea Pig	0.5
Liver	4,991	3,608	Rat	1.4
Liver	6,344	2,922	Guinea Pig	2.2

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/8/519/346432/20-8-519.pdf by guest on 03 December 2023

TABLE 2

Distribution one hour after intravitreal injection (dpm/100 mg. wet tissue) (guinea pig)

Tissue	D-Glucose	L-Glucose	Ratio D/L
Lens	6,332	302	20.9/1
Vitreous	11,798	6,738	1.7/1
Retina	8,947	3,242	2.8/1

DISCUSSION

The three species studied possess differing types of retinal vascularization. The rat has a retina whose vascularization is similar to that of man and other primates in that its inner half is fully vascularized (holangiotic). The rabbit retina has only a few vessels on its inner surface (merangiotic). The guinea pig retina lacks vessels (anangiotic) and is nourished solely by the choroid.

The pigment epithelial cells which separate the choriocapillaris from the photoreceptors, and the endothelial cells lining the retinal vessels have tight intercellular junctions which have been shown to act as a barrier to diffusion of larger molecules (Ashton, 1965; Shiose, 1970).[5,6] The capillaries of the central nervous system are the only other capillaries that have tight junctions of this kind. The similarity of results in the three species suggests that the barrier to diffusion of glucose in the vascular endothelium and the pigment epithelium is probably similar.

The retina is an actively metabolizing tissue with a high consumption of glucose (Graymore, 1970). Glucose is relatively insoluble in lipid and its diffusion into a number of tissues is facilitated by a stereospecific carrier. In the red blood cell L-glucose is able to enter by diffusion, but at only about one ninth the rate of 3 o-methyl D-glucose (Regen and Morgan, 1964).[7] The liver also possesses a stereospecific carrier to facilitate glucose diffusion (Williams, Exton, Park and Regen, 1968). Crone (1970), using indicator dilution technics, has demonstrated with D and L-glucose that there is virtually no extraction of the latter during one transit through the brain, but that a substantial fraction of D-glucose is lost from the blood. He concluded that the barrier to diffusion of glucose was in or very close to the vascular endothelium of the brain capillaries. Technical problems rule out the application of the same technic to the retina, but the method used in the present study permits separate analysis of the entry of the two stereoisomers over short periods after injection. The high ratios of D to L activity in both brain and retina favor the hypothesis that a similar situation occurs in both tissues. It is interesting to note that the same ratio may also be true of lens when glucose is injected into the vitreous.

Further work will be needed to examine the implications of our preliminary findings.

ACKNOWLEDGMENT

This work was supported by the Medical Research Council, The Wellcome Trust, England, and Grant Number EY 00469 of the National Eye Institute, United States.

REFERENCES

[1] Goldberg, M., and Fine, S.: *In* Treatment of Diabetic Retinopathy. Symposium held at Warrington, Virginia, Sept.-Oct., 1968. U.S. Public Health Service, 1968.

[2] Dollery, C. T.: *In* Scientific Basis of Medicine Annual Reviews. University of London Athlone Press, 1969, p. 44.

[3] Graymore, C. N.: *In* Biochemistry of the Eye. C. N. Graymore, (Ed.). London, Academic Press, 1970, p. 645.

[4] Gupta, G.: Microchem. J. *13*:4, 1968.

[5] Curtis-Prior, P. B., Trethewey, J., Stewart, G. A., and Hanley, T.: Diabetologia *5*:384, 1969.

[6] Ashton, N.: Trans. Ophthal. Soc. U.K. *85*:199, 1965.

[7] Shiose, Y.: Jap. J. Ophthal. *14*:73, 1970.

[8] Regen, D. M., and Morgan, H. E.: Biochim. Biophys. Acta *79*:151, 1964.

[9] Williams, T. F., Exton, J. H., Park, C. R., and Regen, D. M.: Amer. J. Physiol. *215*:1200, 1968.

[10] Crone, C., and Thompson, A. M.: *In* Capillary Permeability. Second Alfred Benzon Symposium. C. Crone, and N. A. Lassen, (Eds.). Academic Press, 1970.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/8/519/346432/20-8-519.pdf by guest on 03 December 2023