

FRUCTOSE AND GLUCOSE UTILIZATION BY LAMB AND SHEEP BRAIN*

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Fructose can be utilized by the mammalian brain *in vitro* as judged by oxygen uptake and by lactate production, although with some preparations lactate production was slower from fructose than from glucose (Loebel 1925; Dickens and Greville 1933; Edson and Leloir 1936; Geiger 1940; Klein 1944; Meyerhof and Wilson 1948). An increase in oxygen uptake by brain slices *in vitro*, during application of electrical pulses to the tissue, occurs when glucose is present in the medium; this also occurs when fructose is present in the medium, but higher concentrations of fructose than glucose are needed for the same effect (McIlwain 1953).

On the other hand, it has generally been accepted that fructose is not utilized by the brain *in vivo*, as it does not restore normal function or a normal electroencephalogram in the hypoglycaemic, hepatectomized dog (Mann and Magath 1922; Maddocks, Hawkins, and Holmes 1939). However, Corvilain *et al.* (1958) and Tagnon and Corvilain (1959) have recently suggested that fructose administered with insulin prevents clinical symptoms of hypoglycaemia, without increasing blood glucose concentration.

In view of these discrepancies and as fructose is normally present in foetal and new-born lamb blood, the uptake of fructose by lamb and sheep brain was of some interest, although it has been observed that fructose does not relieve hypoglycaemic symptoms in the lamb (Alexander, personal communication).

In vivo Methods

Fifteen normal Merino or Corriedale lambs were lightly anaesthetized with pentobarbitone sodium ("Sagatal", May & Baker) given by intravenous injection within 24 hr of birth. The skull was trephined and a blood sample (10 ml) removed from the superior sagittal sinus; immediately 10 ml arterial blood was collected by opening the carotid artery. Heparin (10 units/ml) was added to each sample, portion of which was centrifuged immediately (3000 *g* for 20 min) to obtain plasma, while sodium fluoride (10 mg/ml) was added to the remainder for preservation as whole blood. All samples were stored at -20°C until analysed.

In vitro Methods

Pieces of cerebral cortex were removed from two non-pregnant ewes under pentobarbitone sodium ("Nembutal", Abbott Laboratories) anaesthesia and from lambs (foetal ages in Table 1) delivered by caesarian section from ewes under "Nembutal" anaesthesia.

The tissue was chopped into prisms approximately 0.3 by 0.3 mm and 100–500 mg added to 2 ml buffered saline (Tris-saline, Setchell 1959) containing 4 $\mu\text{moles/ml}$ glucose or fructose or 4 $\mu\text{moles/ml}$ of each. The air in the containers was replaced with

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oxygen. Half of the samples were shaken in a water-bath at 39°C for 3 hr while the remainder were stood in iced water in a refrigerator. At the end of this time, the contents of both lots of samples were centrifuged (3000 *g* for 5 min) and 1 ml of the supernatant mixed with 5 ml water, 2 ml 0.3*N* NaOH, and 2 ml 5% ZnSO₄·7H₂O (w/v) to precipitate proteins. After centrifuging again, the supernatants were stored at -20°C until analysed.

Chemical Methods

Glucose was determined by the method of Huggett and Nixon (1957) on whole plasma and protein-free filtrates. Fructose was determined by heating for 10 min with thiourea-resorcinol in glacial acetic acid and ferric chloride in concentrated HCl at 80°C in the dark, a slight modification of the method of Roe, Epstein, and Goldstein (1949). Lactate was determined by the method of Ryan (1958).

TABLE 1
UPTAKE OF FRUCTOSE AND GLUCOSE FROM BUFFERED SALINE IN VITRO BY
CHOPPED LAMB AND SHEEP BRAIN TISSUE

Each value is the mean of two or three samples. Agreement was within 10%

	Foetal Age (days)	Fructose Uptake (μmoles/g fresh wt./hr)		Glucose Uptake (μmoles/g fresh wt./hr)	
		Fructose Alone	Fructose + Glucose	Fructose + Glucose	Glucose Alone
Lamb 1	128	1.68	1.47	5.9	7.6
Lamb 2	146	2.94	1.00	9.4	11.7
Lamb 3	146	1.33			
Lamb 4	146	1.41			
Sheep 1		2.10	1.45	>7	> 7
Sheep 2		2.25	1.86	8.7	15.5

In vivo Results

In 15 pairs of plasma samples, the mean arteriovenous difference for fructose was -0.04 ± 0.34 mg/100 ml which is not significantly different from zero. In eight cases the difference was slightly positive (0.2–2.2 mg/100 ml) and in seven it was slightly negative (0.3–3.0 mg/100 ml). The plasma fructose concentrations ranged from 7 to 51 (mean 19.2) mg/100 ml. In the same samples, the mean arteriovenous differences for glucose was 7.7 ± 1.5 mg/100 ml ($P < 0.001$). All differences were positive (range 1.8–22.4 mg/100 ml). The plasma glucose concentrations ranged from 27 to 136 (mean 78) mg/100 ml. The regression of arteriovenous differences on arterial concentration was not significant for either fructose or glucose nor was there a significant regression of arteriovenous difference for fructose or glucose on arterial fructose to glucose ratio, the mean of which was 0.30.

In vitro Results

Both foetal lamb and adult sheep brain utilized fructose. However, the rate of utilization was only about 20% of that for glucose (Table 1). The utilization of fructose

was depressed in the presence of equimolar concentrations of glucose and vice versa. The uptake of glucose for adult sheep was slightly lower than that reported earlier for sheep brain slices (Setchell 1959) but the slices were incubated for only 90 min and damage to the tissue would be less than for chopped tissue used in the present study. The production of lactate could account for about 50% of both the fructose and glucose disappearing, as has been shown earlier for glucose (Setchell 1959).

Discussion

The results show that fructose is not taken up in significant amounts by the brain of the new-born lamb *in vivo* whereas glucose is. The arteriovenous difference for glucose is of the same order as that seen in the adult sheep (McClymont and Setchell 1956) although a quantitative comparison is not possible without the respective values for blood flow.

Although no fructose was phosphorylated in the presence of an equivalent concentration of glucose by a purified sheep brain hexokinase (Slein, Cori, and Cori 1949), the rate of fructose utilization *in vitro* by chopped sheep brain in the present studies is about 20% of that for glucose when both are present in similar concentrations. With the considerable arteriovenous differences for glucose, one might expect that some differences for fructose would be seen *in vivo* especially in the samples where fructose concentration was similar to that of glucose. This was not the case, and the lamb would therefore seem to be similar to the cat in which some fructose is utilized *in vitro* but none *in vivo*, possibly due to differences in rate of passage of the two sugars through the "blood brain barrier" (Klein, Hurwitz, and Olsen 1946; Park *et al.* 1956).

This finding does not disagree with the suggestion of Shelley and Dawes (1962) that most of the fructose present at birth in the lambs is excreted, not metabolized. However, in view of the small number of organs so far studied, it is felt that a more thorough examination of the fructose metabolism of the tissues of the foetal and new-born lamb is indicated before their suggestion is accepted.

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