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The Transmission of Sugars across the Goat Placenta

By D. G. WALKER*

Department of Biochemistry, London Hospital Medical College, E. 1

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Fructose is known to occur in the foetal blood of a number of species, which include the sheep and the goat, and in the seminal plasma of most species. The presence of a considerable concentration of fructose in foetal blood and its virtual absence from the maternal blood indicate that the placenta must be impermeable to fructose in the direction foetus to mother. On the other hand, Huggett, Warren & Warren (1951) showed that, in the sheep, glucose rapidly passed between the bloods of ewe and foetus in both directions.

In the work to be described in this paper, goats have been used as experimental animals, and sufficient experiments similar to those of Huggett *et al.* (1951) on sheep have been performed to demonstrate that the placentae of these two species behave in an analogous manner. Further, a number of other sugars have been used in order to obtain some information as to the specificity of the permeability properties of the placenta.

A preliminary account of this work was given by Walker & Warren (1955).

* Present address: Department of Biochemistry, University of Shiraz, Iran.

METHODS

Animals. In most cases the date of mating of pregnant goats was known. For the remaining animals the stage of pregnancy was deduced from a graph of foetal age against foetal crown-rump length (R. J. Harrison, personal communication). The goats were kept in the animal house or in open-air pen for a period of from 2 days to several weeks. They were fed on hay and greenstuff and were allowed an unlimited supply of water.

Surgical procedure. This was based upon that used by Barklay, Haas, Huggett, King & Rowley (1949) and Huggett *et al.* (1951).

Anaesthesia was initially induced by injection of sodium pentobarbitone (Veterinary Nembutal, supplied by Abbott Laboratories Ltd., London) into a convenient vein in the forelimb of the goat. Between 8 and 18 ml. was required, depending upon the size of the goat. Thereafter, 1-2 ml. of the anaesthetic was given intravenously as indicated by variations in muscle tone. Administration of the anaesthetic during the course of an experiment did not appear to have any effect on the blood-sugar levels.

Cannulae were inserted into the jugular vein (for infusion of the supplementary doses of the anaesthetic and injection of the sugar solution) and carotid artery (for obtaining maternal-blood samples). Clotting in the cannulae was avoided by the liberal use of heparin. The goat was

placed in a 0.9% NaCl bath maintained at 37°. Laparotomy was performed and the foetuses were exposed by opening the uterus but were left attached to their mother by the umbilical cord. The foetus and the hind quarters of the mother were kept immersed in the 0.9% NaCl bath.

Infusion of the sugars. Sugar solutions were infused into the maternal circulation via the venous cannula at a rate of approx. 25 ml./min. Foetal infusions were made into the umbilical vein by means of a syringe and needle at the rate of about 2–3 ml./min.

Blood samples. The maternal-blood samples were obtained from the carotid artery. Foetal-blood samples were taken with a heparinized syringe and needle (size no. 15 was found most satisfactory), usually from one of the two veins of the umbilical cord (which carry the oxygenated blood). When the cord was brought out of the bath for blood sampling it was moistened with formalin-0.9% NaCl (1:9) to prevent vasoconstriction (Barcroft, 1946).

Cell/plasma volume ratios were determined in Wintrobe haematocrit tubes which were centrifuged at 3500 rev./min. for 30 min. Blood plasma was prepared from whole blood by centrifuging at 0° for 15 min. at 5000 rev./min.

Determination of sugars. The blood samples were deproteinized by the barium-zinc method of Somogyi (1945). Total reducing substances were determined on the protein-free filtrates by the method of Nelson (1944) and fructose by a modification of the Seliwanoff method due to Cole (as described by Bacon & Bell, 1948). Pentose was determined by the method of Mejbaum (1939).

In normal blood specimens, or in those which were known to contain glucose and fructose only, glucose was taken as the difference between the total reducing sugar and fructose values. Where a mixture of sugars was present in the blood, the usual colorimetric determinations were made and the information thus obtained was supplemented by evidence obtained by chromatographic methods.

Preparation of samples for chromatography. The barium-zinc blood filtrates were first desalted by treatment with the mixed ion-exchange resin Bio-Deminrolit (The Permutit Co. Ltd.). Approximately equal amounts of filtrate and resin (previously saturated with CO₂) were mixed and allowed to stand with periodic shaking for 30 min. Samples of 2 ml. of the salt-free filtrate were then dried in a desiccator, redissolved in 0.1 ml. of water and applied to the chromatograms. The filtrates from 0.2 ml. of the original whole blood were thus used in the preparation of each chromatogram.

Chromatography. Adequate resolutions of mixtures of simple hexoses such as glucose, fructose, galactose and mannose are difficult to obtain by one-dimensional chromatography, especially when one or other of them is in great excess. Two-dimensional chromatograms were therefore prepared as a routine from all the blood extracts where two or more hexoses were expected to be present. Phenol-water (80:20) and 2:4-lutidine-water (65:35) were used as the solvents, in that order. This system resolves the hexoses and pentoses in a manner almost identical with that of the system phenol-ammonia followed by *s*-collidine described by Partridge (1948). More compact spots were obtained when the phenol run was carried out in neutral and not alkaline conditions, possibly due to a diminished degree of oxidation of the sugars during the experiment. Whatman no. 4 filter papers were used throughout (size, 22 in. × 18 in.).

The positions of the spots on the developed chromatograms were revealed by a modification of the aniline hydrogen phthalate reagent described by Partridge (1949). The chromatograms were quickly dipped through a freshly prepared solution containing 2 ml. of aniline, 3.3 g. of phthalic acid, 5 ml. of water and 95 ml. of acetone. They were then heated at 105° for 5–10 min. Under these conditions hexoses give yellowish-brown spots and pentoses reddish-brown spots. When viewed in u.v. light it is found that the hexose spots fluoresce intensely whereas no fluorescence is obtained with the pentose spots. This fluorescence in u.v. light increases considerably the sensitivity of the method for hexoses. Fructose gives a weaker reaction with aniline phthalate than do the aldohexoses. Nevertheless, in the quantities present in all the foetal fluids examined it was always readily detectable.

Confirmation of the identity of the ketoses was also obtained with the phloroglucinol reagent (Horrocks & Manning, 1949) used to develop the spots. The chromatograms were dipped in a solution containing 0.6 g. of phloroglucinol, 10 ml. of water and 90 ml. of acetone, and then heated at 105° for 5 min. Fructose and sorbose give yellowish-brown spots which fluoresce brilliantly when viewed under u.v. light. Pentoses give greenish-blue spots which develop very much more slowly and increase in intensity on standing. The last-named do not fluoresce.

In experiments which were concerned only with the detection of the ketoses, fructose and sorbose, complete resolution of these substances could be achieved on one-dimensional chromatograms with phenol-water as the solvent.

No attempt at accurate quantitative evaluation after chromatographic separation was made. The relative amounts of the different sugars present in each case were arbitrarily graded as + + +, + +, + or trace according to the relative sizes and intensities of the spots obtained.

RESULTS

Normal blood-sugar values

The normal maternal blood-glucose levels (measured as total reducing sugar) in pregnant goats varied in the range 17–139 mg./100 ml., the mean value being 72 mg./100 ml. (Table 1). The total reducing sugar values for the foetal-goat bloods (including five foetuses not quoted in Table 1) for all foetal ages were in the range 44–296 mg./100 ml., mean value 154 mg./100 ml., and the corresponding figures for foetal fructose were: range 40–250 mg./100 ml., mean value 132 mg./100 ml. In general, the maternal blood-glucose level was lower than the foetal-blood total reducing sugar value, but higher than the foetal-blood glucose. The foetal glucose varied over the range 1–86 mg./100 ml. and was always less than the foetal fructose.

Maternal blood gave only a weak positive reaction to the Seliwanoff test and this may be accounted for as being due to the weak reaction given by glucose itself (Bacon & Bell, 1948).

In the foetal blood, the glucose levels were very variable and showed no trend with foetal age, but

Table 1. *Normal maternal- and foetal-blood sugar levels*

Goat no.	Foetal age (days)	No. of foetuses	T.R.S., Total reducing sugar.			
			Maternal T.R.S. (mg./100 ml.)	Foetal		
				T.R.S. (mg./100 ml.)	Fructose (mg./100 ml.)	Glucose (mg./100 ml.)
6*	125	2	118	211	197	14
7	112	1	97	150	105	45
8	107	1	66	184	164	20
9	129	1	105	103	67	36
10*	86	2	57	157	140	17
11	103	2	93	171	135	36
				184	139	45
12*	95	4	92	126	105	21
13*	91	3	83	231	212	19
				248	221	27
15*	103	2	61	106	87	19
16	82	2	78	168	146	22
				198	168	30
17	84	2	59	123	101	22
				129	104	25
18	146	2	139	168	148	20
				208	176	32
19	78	1	88	236	188	48
20	104	2	56	125	124	1
				111	105	6
21	134	2	17	48	45	3
				46	45	1
22	103	2	52	112	107	5
				144	138	6
24	119	1	75	150	130	20
25*	102	3	82	212	209	3
				205	194	11
26	128	1	67	179	164	15
27	115	2	38	103	92	11
				113	97	16
30*	65	2	40	119	108	11
32	110	1	47	84	76	8
33	103	1	64	168	153	15
34*	111	2	49	112	91	21
35*	146	2	45	44	40	4
36	95	2	53	176	143	33
				171	156	15

* Blood samples were not taken from all the foetuses in these multiple pregnancies.

the total reducing sugar and fructose levels tended to decrease with foetal age (Table 2). Huggett *et al.* (1951) reported a similar type of result for foetal-sheep bloods but in this series of goats the differences between the two groups of foetuses is not significant.

Injection of sugars into the maternal and foetal circulation. Various mono- and di-saccharides have been infused into both the maternal and foetal circulations of goats with a view to obtaining information on two problems: (a) the permeability of

the goat placenta to the infused sugar and (b) the formation of fructose in the foetal circulation. In all cases the sugar was infused as an aqueous 20% (w/v) solution previously warmed to 37°. For convenience, the results of the two types of experiments will be presented together. All figures quoted refer to the sugar concentrations in whole blood.

Most of the experiments to be described have been repeated with similar results. The nature of the experiments and their expense have necessarily limited the number that could be performed.

Table 2. *Analysis of normal foetal-blood sugar levels for differences with foetal age*

Foetal age (days)	No. of foetuses	T.R.S., Total reducing sugar. T.R.S. (mg./100 ml.)		Fructose (mg./100 ml.)	
		Range	Mean $\pm$ s.d.	Range	Mean $\pm$ s.d.
60-105	22	106-248	165 $\pm$ 44	87-221	145 $\pm$ 40
106-146	19	44-296	143 $\pm$ 69	40-250	127 $\pm$ 59

Fig. 1. Pregnant goat no. 15. Foetal age 103 days; twins. Glucose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 36 and 44 min. ●—●, Maternal T.R.S.; ○—○, foetal glucose; △—△, foetal fructose; ●- - ●, T.R.S. in non-pregnant goat no. 28. Glucose (2 g./kg. body wt.) was injected intravenously between 15 and 25 min. In all the Figures T.R.S. indicates total reducing sugar.

Effects of injecting monosaccharides

Glucose into the maternal circulation. Glucose solution (1.5 g./kg. maternal body wt.) was injected into the maternal circulation at a constant rate after a few control specimens of blood had been withdrawn from both the goat and its foetus. Further blood samples were then taken at suitable intervals for a few hours. A typical result is shown in Fig. 1. It will be seen that, after the glucose injection, there was an immediate and rapid rise in the maternal blood total reducing sugar value. This was followed by a slower fall towards the initial normal values, this latter process extending over a period of from 3 to 4 hr. The maternal blood-glucose

Fig. 2. Pregnant goat no. 6. Foetal age 125 days; twins. Glucose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 31 and 49 min. One foetus was detached at 69 min., which remained alive. ●—●, Maternal T.R.S.; ○—○, attached foetal glucose; ○- - ○, detached foetal glucose; △—△, attached foetal fructose; △- - △, detached foetal fructose.

curve was, in fact, very similar to the control glucose-tolerance curve for a non-pregnant goat. There was a smaller peak in the foetal-glucose concentration but this occurred after the maternal blood sugar had begun to fall. This result suggested that the goat placenta was freely permeable to glucose in the direction mother to foetus. The foetal-glucose level fell in a similar manner to the maternal glucose. As soon as the foetal blood glucose rose above its initial value, a rise in the foetal blood fructose was observed. The ascending fructose curve invariably crossed the descending glucose curve. This experiment has been performed 11 times and in all cases the rise in foetal fructose was at the rate of 20-40 mg./100 ml./hr. This rate of rise appeared to be independent of the foetal age (range 90-146 days).

Fig. 2 shows the results of a similar experiment in which one of twin foetuses was detached immediately after the glucose infusion into the mother. The foetal-blood fructose increased only in the foetus which remained attached to the parent via the placenta. In the detached foetus the blood fructose began to fall immediately after the separation from the mother.

Glucose into the foetal circulation. Glucose (1 g./kg. foetal body wt.) was injected via the umbilical vein into the foetal circulation. The foetal glucose immediately rose and then fructose formation commenced as the glucose began to return slowly to its initial value. Fructose formation stopped when the foetal-blood glucose approached its initial value. A small but transient rise in the maternal total reducing sugar occurred immediately after the glucose infusion. This type of result was also obtained in another experiment in which twin foetuses were infused with glucose at different times (Fig. 3). In each of the foetuses, the foetal-blood fructose level began to rise immediately after the glucose injection. The shape of the maternal total reducing sugar curve again suggested that some glucose passed over into the maternal circulation, although this would not be expected to give a marked rise because of the large dilution such glucose would undergo owing to the greater blood volume.

Fig. 3. Pregnant goat no. 27. Foetal age 115 days; twins. Glucose (1 g./kg. foetal body wt.) was injected into the umbilical vein of foetus A between 34 and 43 min. and of foetus B between 148 and 155 min. ●—●, Maternal T.B.S.; ○—○, foetus A glucose; ○—○—○, foetus B glucose; △—△, foetus A fructose; ▲—▲—▲, foetus B fructose.

Fructose into the maternal circulation. The fact that fructose was present in the foetal blood but not in the maternal blood suggested that the goat placenta was impermeable to fructose, at least in the direction foetus to mother. Fig. 4 shows the result of injecting 1.5 g. of fructose/kg. maternal body wt. into the maternal blood. The maternal-blood fructose concentration fell rapidly from the high value thus induced and returned almost to zero in the course of 2 hr. This represents a greater tolerance of the maternal goat for fructose than for glucose. The rise in the maternal glucose suggests that some of the fructose was rapidly converted into glucose, presumably by the maternal liver. In the foetal blood, however, the initial rise in fructose was negligible, and only a very small increase in the fructose level occurred. The rise in the maternal glucose was reflected in a small increase in the foetal glucose. The apparent impermeability of the placenta to fructose in the direction mother to foetus was also demonstrated in another similar experiment in which, after the injection of fructose into the maternal circulation resulting in the attainment of a maternal fructose level of over 500 mg./100 ml., the fructose values in the twin foetuses increased from 222 to 242 mg./100 ml. and from 228 to 249 mg./100 ml. respectively over a time period of 40 min. This represents a similar rate to those observed after an injection of glucose into the maternal circulation, and might be explained by some slow active process of fructose transfer across the placenta. There was no rapid transfer of

Fig. 4. Pregnant goat no. 4. Foetal age 130 days; singleton. Fructose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 26 and 34 min. ●, Maternal glucose; △, maternal fructose; ○, foetal glucose; ▲, foetal fructose.

fructose across the placenta as there is of glucose under comparable conditions.

Fructose into the foetal circulation. The foetal-fructose level in a singleton foetus was increased to 450 mg./100 ml. by injection of fructose into the umbilical vein. Fig. 5 shows that the maternal total reducing sugar value did not increase at all. No fructose could be detected in the maternal blood either by the Seliwanoff test (i.e. over and above the normal very small positive reaction) or by chromatographic methods. In another experiment (pregnant goat no. 8, foetal age 107 days) the foetal fructose was increased to 557 mg./100 ml. and a similar result was obtained.

Mannose into the maternal circulation. Mannose, being a sugar very similar to glucose, might be expected to pass freely across the placenta. Further, it might act as a precursor of foetal fructose. The results of this and most of the remaining experiments demanded use of chromatographic methods in addition to the quantitative colour tests. This particular experiment will therefore be described in rather more detail to illustrate the type of reasoning used in deducing the results of the succeeding experiments.

Fig. 6 shows the results of the quantitative determinations. The upper graph shows the varia-

Fig. 5. Pregnant goat no. 34. Foetal age 111 days; twins. Fructose (1 g./kg. foetal body wt.) was injected into the umbilical vein between 21 and 29 min. ●, Maternal T.R.S.; ○, foetal T.R.S. (the foetal-glucose values remained below 20 mg./100 ml. throughout the experiment).

tion of the maternal total reducing sugar with time. This will include mannose in addition to the glucose after the mannose injection (which was made over the period 32–42 min.). The lower graph shows the foetal fructose and foetal 'glucose plus mannose' values. The latter were obtained by subtracting the foetal fructose from the foetal total reducing sugar values. This information does not, by itself, decide whether or not the rise in the foetal 'glucose plus mannose' is due to glucose alone, or to a rise in both sugars. Fig. 7 illustrates the chromatograms obtained from blood samples. The semi-quantitative data thus provided are given in the legend to Fig. 6. The samples (called A, B, C and D in the legend) correspond to the same blood samples as those similarly marked on the graphs. Hence it is seen that a large intense mannose spot occurred in sample A, which was taken immediately after the injection of mannose into the maternal blood. The foetal-blood sample (B) taken before the mannose injection shows the presence of fructose and a trace

Fig. 6. Pregnant goat no. 17. Foetal age, 84 days; twins. Mannose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 34 and 42 min. ●, Maternal T.R.S. (i.e. glucose and mannose); ○, foetal glucose and mannose; △, foetal fructose. The table below shows the chromatography results (two-way chromatograms) corresponding to the blood samples marked on graphs.

	Glucose	Fructose	Mannose
A	++	—	Very high
B	Trace	+	—
C	+	+	+++
D	+	++	++

of glucose (the latter corresponding to 24 mg./100 ml.). After the mannose injection into the maternal blood, mannose was clearly demonstrated to be present in the foetal blood (sample C). At a later stage, sample D, the intensity of the mannose spot had decreased, whereas that of the fructose spot had increased. Exactly the same results were obtained for the blood samples taken from the second of the twin foetuses.

The results of this experiment (and also of a duplicate on pregnant goat no. 9, singleton foetus, foetal age 129 days) indicated a rapid transfer of mannose from mother to foetus, and a small but significant rise in the foetal fructose. These experiments do not, however, indicate whether this fructose formation took place directly from mannose, or whether the mannose was first converted into glucose which was in turn transformed into fructose.

Mannose into the foetal circulation. Two injections of mannose were given to one of twin foetuses (Fig. 8). The foetal 'glucose plus mannose' values were again derived by subtracting fructose from the total reducing sugar values. The peaks on the foetal side (such as sample A) were reflected by peaks (represented by samples B and C) in the

maternal total reducing sugar curve. The chromatograms showed that the latter peaks were due, at least in part, to the passage of mannose from the foetus to the mother. There was again a small but significant rise in the foetal-fructose value. In another similar experiment (pregnant goat no. 32, foetal age 110 days) the rise in the foetal fructose was from 76 to 92 mg./100 ml.

Galactose into the maternal circulation. When galactose was injected into the mother, the usual type of maternal total reducing sugar curve was obtained (Fig. 9). The foetal 'glucose plus galactose' values, obtained by subtracting the foetal fructose from the foetal total reducing sugar values, showed a peak a few minutes after the maternal peak, and the chromatograms showed that this rise was due, at least in part, to the passage of galactose across the placenta into the foetal blood. No fructose production from galactose occurred. In a duplicate experiment (pregnant goat no. 31, foetal age 61 days) galactose transfer was again demonstrated but the foetal-fructose values did not increase.

Sorbose into the maternal circulation. Sorbose was injected into the maternal circulation and its fate followed by total reducing sugar and ketose (Seliwanoff) values. Both sorbose and fructose will

Fig. 7. Drawings of sections of two-way chromatograms [on Whatman no. 4 filter paper; solvents: phenol-water (right to left) and 2:4-lutidine-water (bottom to top): spots were detected with aniline hydrogen phthalate]. A, B, C and D correspond to the respective points on the graphs in Fig. 6.

Fig. 8. Pregnant goat no. 21. Foetal age 134 days; twins, only one foetus used. Mannose (1 g./kg. foetal body wt.) was injected into the umbilical vein between 41 and 45 min. and again between 116 and 120 min. ●, Maternal T.R.S. (glucose and mannose); ○, foetal glucose and mannose; △, foetal fructose. Chromatograms (two-way) gave the following results.

	Glucose	Fructose	Mannose
A	+	+	Very high
B	+	-	+
C	+	-	+

contribute to the latter (sorbosose has about half the chromogenicity of fructose in the Seliwanoff reaction). The maternal total reducing sugar and ketose curves (Fig. 10) fell from their high values after the injection in a similar way. In the foetal blood there was a definite rise in the ketose values after the injection. Chromatography showed in two experiments that the rise in foetal ketose was caused by the transfer of sorbose from mother to foetus. The quantitative results for both experiments (pregnant goats nos. 11 and 30) indicate that the amount of sorbose which passed across was less and its passage slower, than for glucose, mannose and galactose.

Xylose into the maternal circulation. In the two experiments performed with this sugar, determinations were made on all blood specimens for total reducing sugar, ketose and pentose. No attempt

was made to determine the individual sugars because fructose contributes to the pentose colour. The results (Fig. 11) show that, after an injection of xylose into the mother, there was an immediate rise in foetal total reducing sugar which could be accounted for by the rapid passage of xylose from mother to foetus. Similar results were obtained in a duplicate experiment (pregnant goat no. 12, foetal age 95 days).

Effects of injecting disaccharides

In experiments in which disaccharides were injected into the maternal circulation no disaccharides appeared in the foetal blood, showing that the goat placenta is impermeable to disaccharides.

Maltose. Although the maternal total reducing sugar value rose to over 600 mg./100 ml. after the injection of maltose (1.5 g./kg. body wt.) there was virtually no difference in the total reducing sugar or

Fig. 9. Pregnant goat no. 10. Foetal age 86 days; twins, only one foetus used. Galactose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 71 and 79 min. ●, Maternal T.R.S. (glucose and galactose); ○, foetal glucose and galactose; △, foetal fructose. Chromatograms (two-way) gave the following results.

	Glucose	Fructose	Galactose
A	++	-	Very high
B	+	-	+++
C	+	+	-
D	+	+	++

Fig. 10. Pregnant goat no. 11. Foetal age 103 days; twins. Sorbose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 61 and 70 min. ●—●, Maternal T.R.S. (glucose and sorbose); ●—●, maternal ketose (sorbitol); ○—○, foetus A ketose; ○—○, foetus B ketose. Chromatograms (two-way) gave the following results.

	Glucose	Fructose	Sorbose
A	+	-	Very high
B	++	++	-
C	+++	+++	Trace

fructose levels in the foetal blood. Chromatograms failed to detect any maltose in the foetal blood, and thus it was clear that the goat placenta was impermeable to maltose.

Lactose. Two experiments were performed in which lactose was injected into the maternal circulation (1.5 g./kg. body wt.). No changes occurred in the sugar concentrations in the foetal blood, and no lactose could be detected in the foetal blood.

Sucrose. Sucrose was estimated as the difference in the total reducing sugar values before and after hydrolysis by dilute mineral acid. Such values agreed well with fructose values (assuming that sucrose reacts as 50% fructose in the Seliwanoff reaction). When sucrose (1.5 g./kg. maternal body wt.) was injected into the maternal circulation (pregnant goat no. 25, foetal age 125 days), the total reducing sugar value after hydrolysis was 620 mg./100 ml. and this decreased over a period of 3 hr. to 197 mg./100 ml. In the foetal blood the total reducing sugar value after hydrolysis rose very slowly from 206 to 245 mg./100 ml. and the fructose value from 212 to 240 mg./100 ml. How-

ever, these small increases must have been due to glucose or fructose or both, for no sucrose could be detected chromatographically in samples of foetal blood taken at any stage of the experiment. In a duplicate experiment (pregnant goat no. 19, foetal age 78 days) the maternal total reducing sugar after hydrolysis rose to over 700 mg./100 ml., and then fell to 233 mg./100 ml. in 2 hr. The rise in the foetal blood total reducing sugar after hydrolysis was 32 mg./100 ml. and that of the foetal fructose was 17 mg./100 ml. Four foetal-blood specimens were found to contain no sucrose by chromatographic methods. The total reducing sugar values before and after hydrolysis suggested that some sucrose was broken down, forming glucose and fructose. In the foetal blood, the total reducing sugar after hydrolysis was approx. 10–15 mg./100 ml. higher than the total reducing sugar before hydrolysis throughout the experiment. The amount of sucrose equivalent to this difference would have been detectable chromatographically. It was concluded that the goat placenta was impermeable to sucrose.

Fig. 11. Pregnant goat no. 26. Foetal age 128 days; singleton. Xylose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 34 and 42 min. △, Maternal pentose; ○, foetal T.R.S.; ▲, foetal pentose; □, foetal fructose. Chromatograms (two-way) gave the following results.

	Glucose	Fructose	Xylose
A	+	+	++
B	+	+	++

Table 3. *Cell-plasma distribution of sugars*

Pregnant goat no. 35. Foetal age 146 days; twins. Glucose (1.5 g./kg. maternal body wt.) was injected intravenously into the maternal circulation between 59 and 68 min. T.R.S., Total reducing sugar.

Sample	Time (min.)	Plasma			Blood cells		
		T.R.S. (mg./ 100 ml.)	Fructose (mg./ 100 ml.)	Glucose (mg./ 100 ml.)	T.R.S. (mg./ 100 ml.)	Fructose (mg./ 100 ml.)	Glucose (mg./ 100 ml.)
Maternal 1	0	48	—	—	33	—	—
Maternal 2	37	59	—	—	39	—	—
Maternal 3	73	402	—	—	105	—	—
Maternal 4	123	287	—	—	69	—	—
Maternal 5	177	212	—	—	59	—	—
Maternal 6	229	148	—	—	50	—	—
Foetal 1	2	45	41	4	56	38	18
Foetal 2	42	51	43	8	49	29	20
Foetal 3	78	135	56	79	120	47	73
Foetal 4	126	159	82	77	129	65	64
Foetal 5	180	159	107	52	123	81	42
Foetal 6	232	145	115	30	121	100	21

Cell-plasma distribution of sugars

In studying the relative concentrations of the sugars on either side of the placenta the concentrations in foetal and maternal blood plasma will be those actually defining the gradient across the barrier. Experiments were performed in which similar techniques to that described above for the injection of glucose into the maternal circulation were used, except that glucose and fructose assays were performed to determine both the cell and plasma concentrations. The concentrations of the sugars within the cells were calculated from a knowledge of the concentrations in whole blood and plasma and the haematocrit value. Table 3 shows the results of an experiment in which glucose was injected into the maternal circulation. It can be seen that the relations between the sugar concentrations in the maternal- and foetal-blood plasmas were very similar to the results obtained for whole blood. Similar results were also obtained in a duplicate experiment (pregnant goat no. 37, foetal age 106 days).

DISCUSSION

The occurrence of fructose in the foetal blood of sheep and goats raises the questions (a) from what is the fructose formed, and (b) how and where is the fructose formed from its precursor? The answers were partly provided by Huggett *et al.* (1951) in their experiments on sheep, in which they showed that foetal-blood glucose is the immediate precursor of foetal-blood fructose, and that the site of formation is the placenta. Experiments now reported on the goat confirm the previous findings on the sheep and indicate that the placentae of these two species behave in a similar manner when studied in this way.

Once the fructose is formed it might be expected to pass into the maternal blood unless the placenta acted as a barrier to its transmission. Experiments described in this paper indicate that the impermeability of the placenta to fructose is virtually complete in both directions. The foetal-fructose level has been raised to about 1000 mg./100 ml. of whole blood, but no fructose could be detected in the maternal blood (that is, no increase in the small initial values given by the blood samples taken during the control period was found). In the other direction, fructose injected into the maternal circulation gave rise only to a slow increase in the foetal-blood fructose, the rate of increase being similar to that when glucose is infused into the maternal circulation.

Mannose and galactose, like glucose, passed rapidly across the placenta in both directions. The ketohexose sorbose was able to traverse the placenta but at a much slower rate than the aldohexoses. Thus it would appear that sorbose behaves in a manner somewhere between that of the aldohexoses and fructose. The only pentose studied, xylose, passed rapidly across the placenta whereas the organ appeared to be completely impermeable to the disaccharides examined. These results indicate that fructose may hold a unique position in being the only monosaccharide to which the placenta of the goat is virtually completely impermeable.

As far as the production of foetal fructose is concerned, it would appear that, in addition to glucose, mannose might also be a precursor. Galactose, however, did not appear to give rise to fructose.

Whereas the fructose, which is always present in the body fluids of the foetal goat, presumably arises by a steady formation from foetal glucose in

the placenta, it appears that one important factor in maintaining its concentration continuously at such high levels must be the specific impermeability of the placenta to this sugar which prevents its passage into the maternal circulation. Evidence has been reported (Karvonen & Rih, 1954; Davies, 1955) that a similar relative placental impermeability to fructose may occur also in the rat, rabbit and guinea pig, and similar observations have been made independently in this Laboratory. None of these species normally contains appreciable concentrations of fructose in the foetal blood. This suggests that the phenomenon of a specific impermeability of the placenta to fructose may be a quite general one. If this is so, it is perhaps related only somewhat fortuitously to the phenomenon of placental fructose formation, which is peculiar to the ungulates (Goodwin, 1956), in that, by helping to maintain high concentrations of fructose once it is formed, it accentuates the effect.

The remarkable disparity in placental permeability between fructose and other naturally occurring monosaccharides raises many fascinating problems about the nature of the transport mechanisms involved. The work reported in this paper offers more information on the specificity of the placental barrier but does not attempt to explain the mechanism of transfer or barrier effect. At the moment, virtually nothing is known about the details of such processes although the problems have been discussed (Widdas, 1952; Chinard, 1954). The only other monosaccharide which at all resembles fructose in this respect is the ketohexose sorbose. It may be that there is some peculiarity in the detailed nature of the transport processes which favours aldose rather than ketose groupings.

SUMMARY

1. The goat placenta has been shown to act in a similar manner to that of the sheep in that it is the

site of formation of foetal-blood fructose from foetal-blood glucose.

2. The goat placenta is freely permeable to glucose, mannose, galactose and xylose but is impermeable to fructose, sucrose, maltose and lactose. Sorbose can traverse the placenta but only at a slow rate.

3. The role of the remarkable relative impermeability of the placenta to fructose is discussed with reference to the occurrence of fructose in goat foetal blood and the mechanism of sugar transmission.

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The Preparation of Soluble Vasopressinase from Human Placenta

By K. C. HOOPER

Medical Research Council Clinical Endocrinology Research Unit, University of Edinburgh

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Hooper & Jessup (1959) indicated that oxytocin and vasopressin were destroyed by separate enzyme systems present in human placenta. Previously, Hawker (1956) showed that homogenate of placenta destroyed both hormones. Some properties of the enzymes were revealed by means of inhibitors, and it appeared likely that both oxytocinase and vasopressinase were peptidases, the former, in addition,

possessing esterase activity (Hooper, 1959). A study of the distribution of the enzymes in the placenta (Hooper & Jessup, 1959) showed that oxytocinase was present in the supernatant obtained by centrifuging the homogenate at 113 000 g and thereby removing particulate matter. Vasopressinase, on the other hand, was found to be predominantly in the mitochondrial and microsomal