Transplacental Carbohydrate and Sugar Alcohol Concentrations and Their Uptakes in Ovine Pregnancy

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The concentrations of glucose, fructose, sorbitol, glycerol, and myo-inositol in sheep blood and tissues have been reported previously (1–5). However, the other polyols that are at low concentrations have not been investigated in pregnant sheep due to technical difficulties. By using HPLC and gas chromatography-mass spectrometry, seven polyols (myo-inositol, glycerol, erythritol, arabitol, sorbitol, ribitol, and mannitol) and three hexoses (mannose, glucose, and fructose) were identified and quantified in four blood vessels supplying and draining the placenta (maternal artery, uterine vein, fetal artery, and umbilical vein). Uterine and umbilical blood flows were measured, and uptakes of all the polyols and hexoses in both maternal and fetal circulations were calculated. There was a significant net placental release of sorbitol to both maternal and fetal circulations. Fructose was also taken up significantly by the uterine circulation. Maternal plasma mannose concentrations were higher than fetal concentrations, and there was a net umbilical uptake of mannose, characteristics that are similar to those of glucose. Myo-inositol and erythritol had relatively high concentrations in fetal plasma (697.8 ± 53 μ*M* and 463.8 ± 27 μ*M*, respectively). The ratios of fetal/maternal plasma arterial concentrations were very high for most polyols. The concentrations of myo-inositol, glycerol, and sorbitol were also high in sheep placental tissue (2489 ± 125 μ*M*/kg wet tissue, 2119 ± 193 μ*M*/kg wet tissue, and 3910 ± 369 μ*M*/kg wet tissue), an indication that these polyols could be made within the placenta.

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Key words: fetal polyols; fetal-maternal gradients; ovine placental polyols; myo-inositol; mannose; sorbitol; fructose

Fructose is the major form of carbohydrate in fetal blood for all Ungulate and Cetacea (1, 6). Previous studies have reported sorbitol and inositol concentrations in maternal and fetal blood for ovine pregnancies (5, 7). The accumulation of hexitols was first observed in perfused placental preparations by Huggett *et al.* (2). Battaglia and Meschia (5) reported the myo-inositol concentrations in fetal plasma and various fetal tissues for ovine pregnancies. However, it was difficult to determine the concentrations of those sugars and polyols present in ovine fetal blood at low concentrations because of technical limitations. In recent studies, some of these "trace" carbohydrates have been shown to be important physiologically in other species (8–13). Compounds such as myo-inositol and sorbitol are important structural components in tissues such as the nervous system and the lens of the eye. Thus, there is an appreciable accretion rate of these compounds in fetal life.

The present study utilizes the HPLC technique with selected carbohydrate columns to identify hexoses and their polyols in fetal and maternal circulations perfusing the placenta and in placental tissue. The concentrations were quantified at micromolar levels and were coupled with uterine and umbilical blood flow measurements so that uptakes could be calculated. All the polyols identified were further confirmed with gas chromatography (GC)/mass spectrometry (MS).

Materials and Methods

Experimental Design. Twenty-eight late gestation mixed-breed Columbia-Rambouillet ewes were used in the study. Surgery was performed under a combination of general pentobarbital (65 mg/ml) and spinal anesthesia (2 ml of 1% pontacaine) after a 48-hr fast with free access to water. Polyvinyl catheters were inserted into the following blood vessels: maternal femoral artery and vein, maternal uterine veins draining the pregnant uterine horn, fetal abdominal aorta, common umbilical vein, and fetal femoral vein. An amniotic catheter was also placed for the injection of antibiotics (500 mg of ampicillin) into the amniotic cavity. All catheters were tunneled subcutaneously through a maternal

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Table I. Carbohydrate Concentrations in Maternal Artery and Uterine Vein, Fetal Artery and Umbilical Vein, and Fetal-Maternal Arterial Ratio

	Maternal artery (μM)	Uterine vein (μM)	Fetal artery (μM)	Umbilical vein (μM)	Arterial ratio (fetal/maternal) (*P* value)
Inositol	21.1 ± 1.5	19.9 ± 1.6	697.8 ± 53.4	702.7 ± 53.1	35.8 ± 2.7 < 0.0001
Glycerol	44.4 ± 5.2	42.8 ± 4.6	62.5 ± 4.3	67.7 ± 4.7	1.9 ± 0.2 < 0.0001
Erythritol	9.6 ± 1.4	10.7 ± 1.2	463.8 ± 26.8	467.4 ± 27.3	64.9 ± 7.8 < 0.0001
Arabitol	10.4 ± 1.3	9.6 ± 1.3	56.6 ± 3.4	56.7 ± 3.1	10.8 ± 2.9 < 0.0001
Sorbitol	4.6 ± 0.6	6.4 ± 0.4	183.4 ± 15.2	187.8 ± 15.8	49.4 ± 4.9 < 0.0001
Ribitol	2.2 ± 0.2	1.6 ± 0.3	168.4 ± 12.7	171.3 ± 13.2	103 ± 9.7 < 0.0001
Mannitol	3.8 ± 0.8	2.0 ± 0.2	68.5 ± 5.8	68.9 ± 6.1	23.8 ± 3.0 < 0.0001
Mannose	72.5 ± 2.6	68.7 ± 1.8	37.9 ± 2.0	40.9 ± 2.1	0.5 ± 0.03 < 0.0001
Glucose	3898 ± 95	3699 ± 79	1309 ± 41	1511 ± 43	0.3 ± 0.01 < 0.0001
Fructose	33.5 ± 5.5	52.9 ± 7.6	7221 ± 539	7438 ± 576	290.4 ± 35 < 0.0001

Note. Values are mean ± SE. *P* values determined by comparing maternal arterial and venous concentrations versus fetal arterial and venous concentrations

skin incision and were kept within a plastic pouch secured to the ewe's flank. All animals were allowed at least 4 days for recovery and, in all cases, had returned to normal food intake before study. The animals had free access to alfalfa pellets, water, and a mineral block. The protocol was approved by the IACUC Committee.

One hour before the sampling, either ethanol (25.7 ml of absolute ethanol dl^{-1}) at a rate of 0.03 ml kg^{-1} min^{-1}, or 400 μCi of titrated water at a rate of 0.83 μCi/min (Amersham, Arlington Heights, IL) for a total volume of 3.6 ml over 2 hr was infused into the fetus to determine uterine and umbilical blood flows by the steady-state diffusion technique (14). Four sets of blood samples were drawn simultaneously at 30-min intervals into heparinized syringes from the maternal artery, uterine vein, fetal artery, and umbilical vein. Hemoglobin and O_2 saturation were measured spectrophotometrically (OSM-3 Radiometer, Copenhagen, Denmark). Plasma-tritiated water concentrations were determined by liquid scintillation counting using a Packard Tri-Carb 2300TR liquid scintillation counter. Plasma ethanol concentrations were measured spectrophotometrically using ethanol dehydrogenase. The blood samples for carbohydrate measurement were centrifuged at 4°C and were stored as plasma in a −70°C freezer until HPLC analysis. At the time of necropsy, the animals were anesthetized with an i.v. injection of Diazepam (0.11 mg/kg body weight)-ketamine (4.4 mg/kg body weight) and the uterus was removed. The animals were then injected with 12 ml of Sleepaway (Fort Dodge Animal Health, Fort Dodge, IA). At necropsy, catheter locations were confirmed, and fetal and placental weights were obtained. The cotyledons were separated from the placentomes manually, and were weighed, frozen in liquid nitrogen, and stored at −70°C. The percentage of water in the cotyledons was determined by drying to constant weight.

HPLC Analysis. The plasma was thawed quickly and deproteinization was achieved as follows: 0.1 ml of 0.3 N zinc sulfate containing 30 mg% xylitol as internal standard was added to 0.1 ml of plasma, the mixture was mixed well, and 0.1 ml of 0.3 N barium hydroxide was added. The mixture was centrifuged at 14,000*g* for 10 min and the supernatant was filtered through a 0.45-μm filter before loading on a refrigerated autosampler for HPLC analysis. For tissue analysis, cotyledons from six animals were used. The cotyledons were thawed, homogenized, and sonicated in distilled water at 4°C. After centrifuging, the tissue supernatant was deproteinized and analyzed as for plasma. On tissue samples, a separate analysis without the addition of xylitol as internal standard was performed to quantify the tissue concentration of xylitol in tissue.

A Dionex HPLC analyzer equipped with a CarboPac MA1 anion-exchange column was used for the separation of the hexoses and polyols (Dionex, Sunnyvale, CA). The analysis was run isocratically with 500 mM sodium hydroxide for 25 min, followed by a step change to 400 mM sodium hydroxide for 20 min at ambient temperature. The flow rate was 0.4 ml/hr. The sodium hydroxide solution was prepared with degassed, deionized water. All the peaks were quantified using a pulse amperometric detector with a gold working electrode (14). The Dionex PeakNet software was used for instrument operation and data analysis. The concentrations of each sugar were calculated by using the integrated area under the peak. The internal xylitol standard was used to correct for instrument variances with the equation: Concentration (in μM) = $(A_u/A_s) \cdot K \cdot D \cdot (X_s/X_u)$.

GC-MS. Plasma samples and placental tissues previously homogenized and sonicated were deproteinized with absolute ethanol. The ethanol mixture was centrifuged, the supernatant was evaporated, and the residue was redissolved in water. The solution was passed on a DEAE Sephadex A-25 anion exchange resin, eluted with water, and the eluate was evaporated to dryness (16). The dried samples were then derivatized and analyzed by gas liquid chromatography and MS as described for the authentic carbohydrates below.

Authentic carbohydrates were converted to their alditol acetates and trimethysilyl (TMS) derivatives. Alditol acetates were prepared by reducing the carbohydrates with sodium borohydride, followed by acetylation (17, 18). TMS

derivatives were obtained by dissolving the dry sugar residue in warm pyridine (for approximately 10 min), followed by derivatizing with hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) at room temperature for 2 hr or more (pyridine:HMDS:TMCS, 5:2:1, v/v) (19). Precipitate was removed by centrifugation, the supernatant was evaporated, and the residue was redissolved in a mixture of bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% TMCS and acetonitrile (BSTFA/TMCS, acetonitrile, 1:5, v/v).

Gas Liquid Chromatography/MS. The derivatized samples were analyzed by GC-MS and the sugars were identified on GC by comparing their relative indices (methylene units) to those of the authentic carbohydrates as well as by their mass spectra.

The GC was performed on a capillary gas chromatograph (Carlo Erba) fitted with a DB1 column (30 m by 0.25 mm o.d.) as described earlier (17). The oven was held for 4 min at an isothermal temperature of 160°C (or lower, i.e., 110°C for the low-molecular-weight carbohydrates) and was then programmed to 250°C at 1°C/min.

Mass spectra were obtained on a Hewlett Packard (Palo Alto, CA) 5790 mass spectrometer (MSD) with the same temperature settings as for the GC. Ethanol concentrations were determined using Sigma (St. Louis, MO) diagnostic kit.

Calculations and Statistics. Uterine (Q_m) and umbilical (Q_f) blood flows were calculated by the application of the steady-state transplacental diffusion method with either ethanol or tritiated water as a flow-limited marker (14). The plasma flows were calculated from the blood flow: plasma flow = blood flow × (1 − Ht), where Ht is the hematocrit; the umbilical and uterine plasma uptakes of carbohydrates were calculated by the application of the Fick principle: umbilical plasma uptake = $Q_f\ (\Upsilon - \alpha)_{plasma}$, where Υ and α refer to umbilical venous and umbilical arterial plasma concentrations; and uterine plasma uptake = $Q_m\ (A - V)_{plasma}$, where A and V refer to maternal artery and uterine vein plasma concentrations. All the values were expressed as $\mu M \cdot min^{-1} \cdot kg_{fetus}^{-1}$. All data are expressed as mean and standard error (SE). The maternal and fetal concentration differences were tested by analysis of variance (ANOVA) with two fixed effects (two types of vessels by two circulations) and one random effect (28 animals). The P values for the maternal-fetal concentration differences are presented in Table I. The uterine and umbilical uptakes were analyzed for significance from 0 by using paired student τ test after testing for a normal distribution. Two-tailed values were considered significance at $P < 0.05$.

Table II. Gestational Age, Fetal, and Placental Weights, Umbilical, and Uterine Blood Flows, Umbilical, and Uterine O_2 Uptakes ($n = 28$)

Gestational ages (days)	130 ± 1
Fetal weights (g)	2841 ± 113
Placental weights (g)	569 ± 30
Blood flows (ml · min^{-1} · kg_{fetus}^{-1})	
Umbilical	224 ± 12
Uterine	558 ± 36
O_2 uptakes (μmol · min^{-1} · kg_{fetus}^{-1})	
Umbilical	363 ± 21
Uterine	701 ± 34

ERYTHRITOL

395 = M − 15 (CH3)

307 = M − 103 (CH2-OTMS)

293 = M − 15 (CH3) − 103 (CH2-OTMS) + H+

277 = M − 15 (CH3) − 117 (CH2-OTMS) + H+

|

CH2

ARABITOL AND RIBITOL

422 = M − 90 (HOTMS)

319 = M − 90 (HOTMS) − 103 (CH2-OTMS)

307 = M − 205 (CH2-OTMS)

|

CHO-TMS

293 = M − 219 (CH2-OTMS)

|

CHOTMS

|

CH2

277 = M − 235 (CH2-OTMS)

|

CHOTMS

|

CH2O

Figure 1. Presents the diagnostic fragments for the three sugar alcohols, erythritol, arabitol, and ribitol. The numbers 395, 307, etc. refer to the mass-to-charge ratio (m/e, in atomic mass units [a.m.u.] of the fragments from the molecular ion [M+]) Weight "M" is in a.m.u. HOTMS, trimethylsilanol (an alcohol); OTMS, the anion of trimethylsilanol; CH2OTMS and CHOTMS, trimethylsilyl ethers.

Results

Twenty-eight animals were studied; all had normal values for fetal and placental weights, uterine and umbilical blood flows, and uterine and umbilical oxygen consumptions (Table II).

Erythritol, arabitol, and ribitol were identified by interpretation of the diagnostic fragments in the mass spectra and by comparison of these fragments with those present in the authentic carbohydrates (Fig. 1).

The hexoses and polyols found in the fetal plasma are myo-inositol, glycerol, erythritol, arabitol, sorbitol, ribitol, mannitol, mannose, glucose, and fructose. The HPLC chromatograms in Figure 2 represent all the hexose and polyol peaks detected in maternal and fetal plasmas. Fructose, myo-inositol, glycerol, and sorbitol had been reported previously in pregnant sheep (1, 2, 5). This is the first report, however, of erythritol, arabitol, ribitol, and mannitol concentrations in both maternal and fetal plasmas (Table I). Maternal plasma concentrations of glucose and mannose were higher than fetal plasmas. By contrast, all polyols showed significantly higher concentrations in umbilical plasma than uterine plasma. As reported previously, fructose had very high concentrations in fetal plasma, 7221 ± 539 μM, which is 290 times higher than that of the maternal plasma. Fetal fructose concentrations are ~5-fold that of fetal glucose concentrations. The fetal artery/maternal artery concentration ratios for all compounds are presented in Table I.

The uterine and umbilical uptakes are presented in Figure 3, A and B. There was a net release of sorbitol from the placenta into both the maternal and fetal circulations. Mannose and glucose had a similar pattern in that both sugars had higher concentrations in maternal blood (Table II), a significant uterine uptake into the placenta, as well as a significant umbilical uptake into the fetal circulation (Fig. 3). There was a significant net release of fructose into the uterine circulation.

Most polyols, especially sorbitol, had relatively higher concentrations in placental tissue than that of the fetal plasma (Fig. 4). Fructose had a higher concentration in fetal plasma than placental tissues. Xylitol was detected in pla-

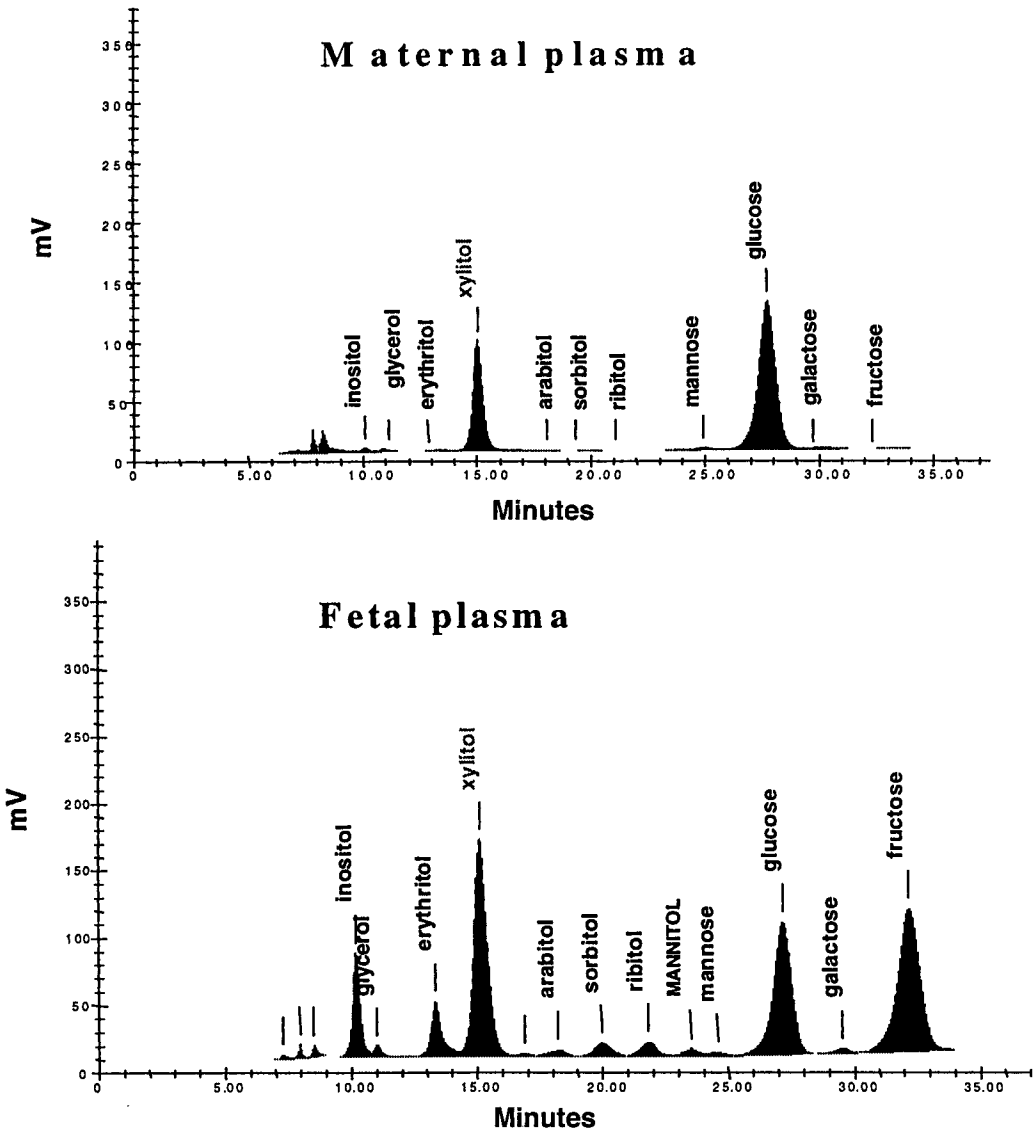

Figure 2. The HPLC chromatograms depicting the peaks for the various sugars and polyols are presented for maternal and fetal plasmas. The much higher peaks for the polyols in fetal blood are clearly evident in a comparison of the two figures.

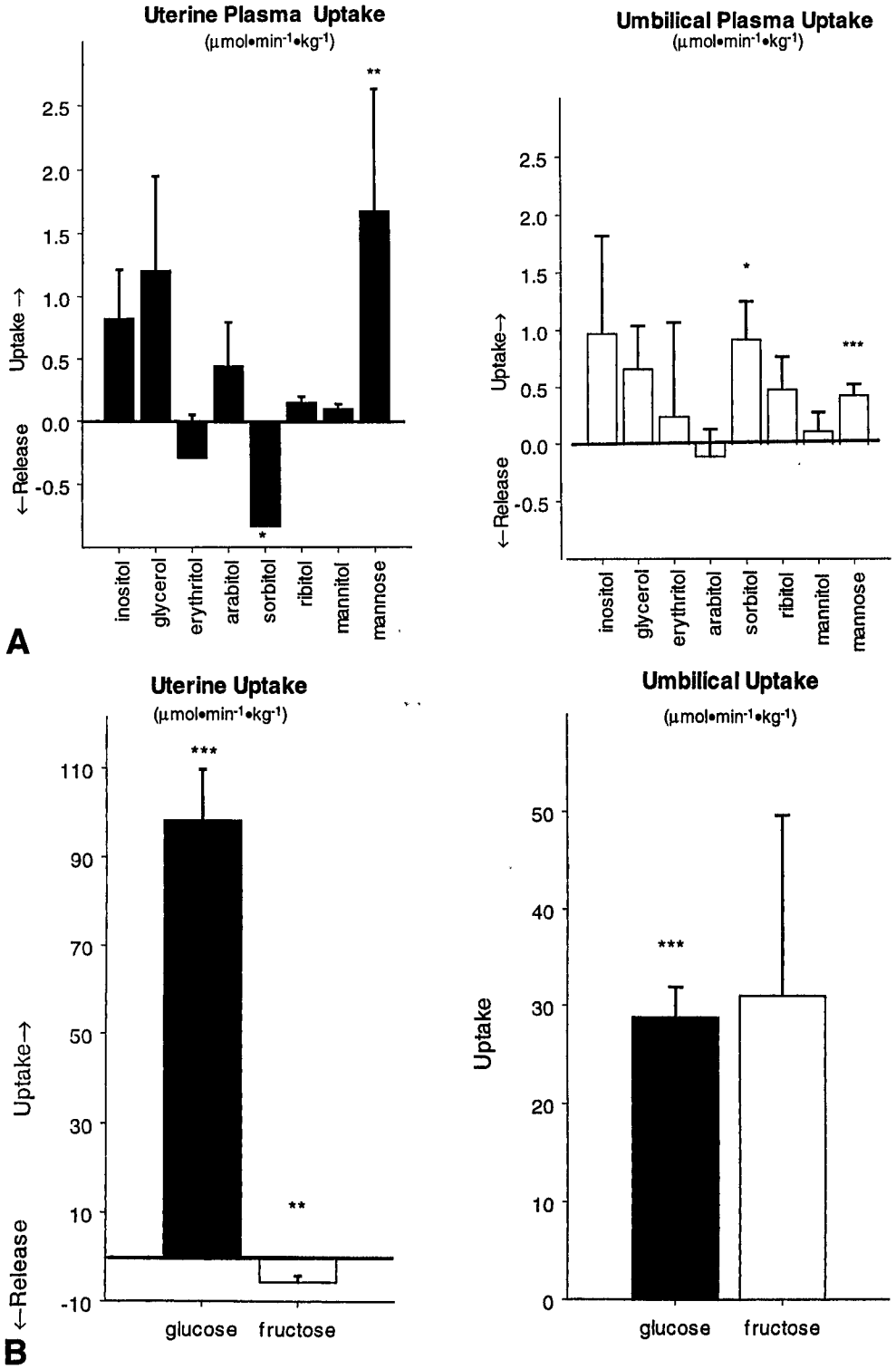

Figure 3. (a) The uterine and umbilical plasma uptakes for all of the polyols and mannose. For each of the uptakes calculated, significance of the difference from zero is shown by the asterisk; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Glucose and fructose are not shown in a because of their much higher uptakes. (b) The uterine and umbilical uptakes of glucose and fructose with the same significance for the asterisks. Note the large variance to the umbilical uptake measurement of fructose that is a function of the extremely high fructose concentration in fetal blood.

cental tissue in small amounts, but was undetectable in maternal and fetal plasma. The concentrations in tissue were expressed as micromoles per kilogram of wet weight (Table III).

Discussion

CarboPac MA1 columns separates sugar alcohols as their anions by high-performance anion-exchange chromatography (HPAE). The detection of the complex carbohy-

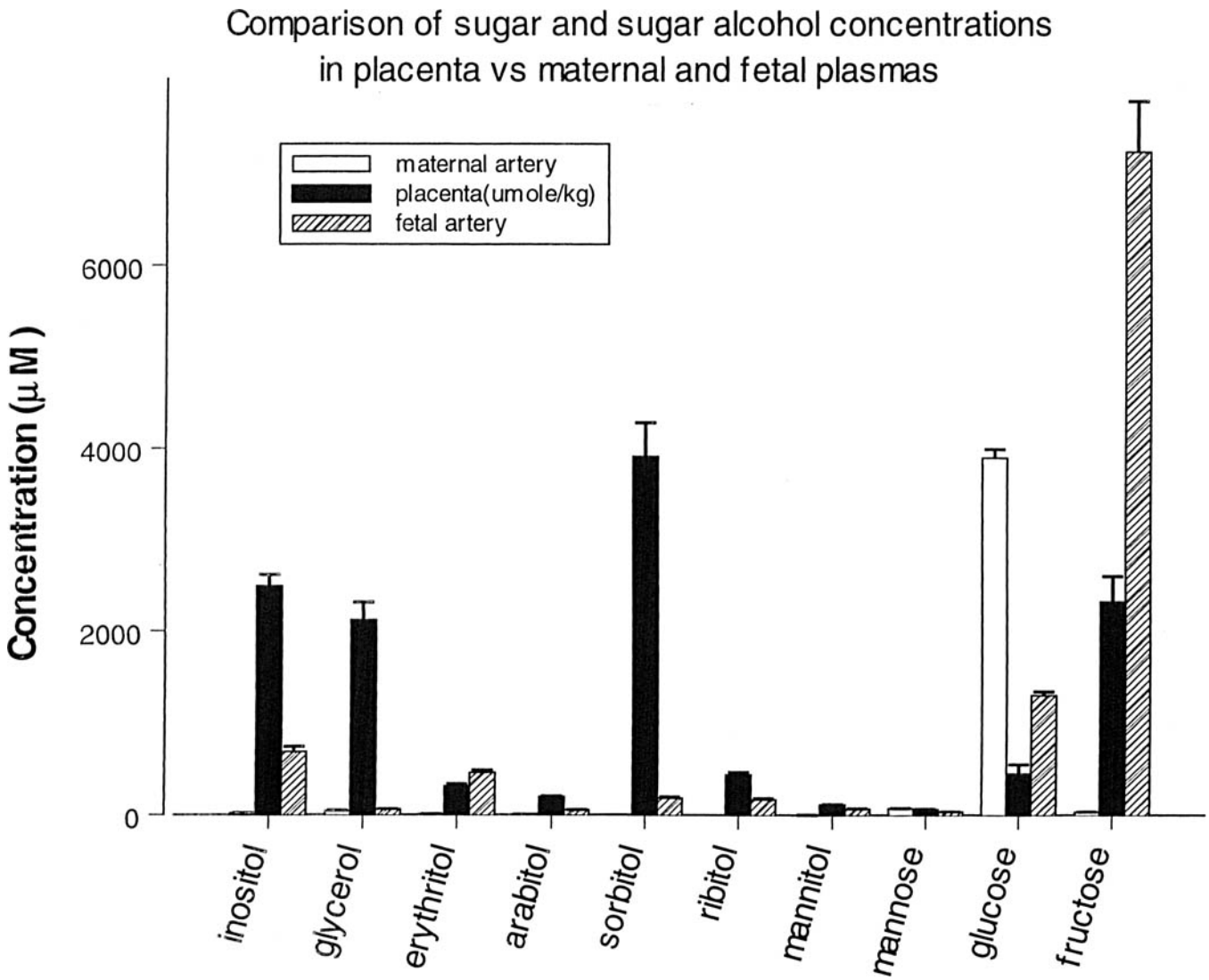

Figure 4. Presents the concentrations of the polyols and carbohydrates in the ovine cotyledon at the time of delivery. Concentrations are expressed in micromoles per kilogram of tissue water.

drate compounds after separation by HPAE has been optimized with the use of a pulsed amperometric detector (15). This technique offers the following advantages: unique selectivity compared with metal-loaded resins, higher sensitivity than refractive index detection, no sample derivatization is required, and separation is at ambient temperature.

These advantages permit the accurate measurement of many polyols and hexoses in blood and placental tissue at the micromolar level. In the pregnant sheep, glucose, fructose, sorbitol, myo-inositol, and glycerol had been reported previously in fetal blood (1–5). However, this is the first demonstration that there is a net release of sorbitol into both circulations, which establishes the fact that there is placental sorbitol production *in vivo*. The sorbitol pathway for production from glucose using aldose reductase has been shown to be present in human umbilical cord tissue (9). The present study presents fetal/maternal concentration ratios of the polyols in plasma. These ratios are surprising and much

Table III. Carbohydrate Concentrations in Placental Tissue

	Placental tissue μmol/kg water[a]	Placental tissue μmol/kg wet tissue
Inositol	2895.4 ± 138.7	2353.8 ± 98.8
Glycerol	2495.3 ± 228.8	2037.8 ± 189.5
Erythritol	369.4 ± 29.8	300.9 ± 25.5
Arabitol	230.4 ± 138.7	195.4 ± 8.2
Xylitol	50.7 ± 11.0	36.0 ± 9.2
Sorbitol	4623.9 ± 479.7	3758.7 ± 368.4
Ribitol	523.2 ± 32.3	425.1 ± 25.9
Mannitol	133.2 ± 8.4	108.1 ± 6.5
Mannose	76.6 ± 6.5	62.6 ± 5.7
Glucose	536.1 ± 118.2	441.4 ± 100.5
Fructose	2907.9 ± 375.4	2224.676 ± 282.8

Note. Values are mean ± SE.
[a] Wet tissue contains 83.5% water.

higher than those found for any amino acids. The high fetal and placental concentrations of the polyols, particularly erythritol (~65-fold higher in fetal plasma) and ribitol (~100-fold higher), have not been reported previously. This finding suggests production of polyols within the conceptus (fetus and placenta) and a very low permeability from the placenta into the maternal circulation. Enzymatic studies of the aldose reductases for these polyols and tracer studies will be required to confirm this hypothesis and to establish the primary site of synthesis (fetus versus placenta). The presence of so many polyols in the fetal circulation may reflect a difference in redox state of fetal versus maternal tissues. This relationship to redox state and the high polyol concentrations in fetal tissues was pointed out by Toh *et al.* (11). The reduced redox state of fetal tissues was pointed out by several early studies of lactate/pyruvate ratios in fetal tissues (11). However, the redox state, although accounting for increased polyol production, does not explain the maintenance of such large maternal-fetal concentration gradients.

The fact that there is no significant umbilical uptake of fructose is due to two factors. First, the very high fetal fructose concentrations could mask a significant fetal uptake because of a low coefficient of extraction (CE). For example, a 2% CE across the umbilical circulation would not be detectable by these techniques, yet it would represent a venoarterial concentration difference of 150 μM, a very significant value nutritionally. Second, ovine placental tissue has been shown to have a high aldose reductase activity, but a relatively low sorbitol dehydrogenase activity (2). The data in the present report are consistent with the enzymatic studies given the current findings of a relatively high placental sorbitol concentration and low fructose concentration compared with fetal plasma.

The finding that despite a low maternal concentration of mannose (4.6 μM), there is a significant uptake of mannose into the placenta from the maternal circulation and a significant uptake into the fetal circulation suggests there may be a mannose transporter in the trophoblast, as had been described for other tissues (20). The studies of mannose affinity for glucose transporters had described a very low affinity compared with glucose (21). Since glucose concentration in the maternal circulation is much higher than mannose, the *in vivo* data suggest the presence of a specific mannose transporter. The umbilical uptake of mannose may be important for the fetus despite the fact that theoretically mannose requirements could be met by mannose synthesis from glucose. Recent studies with human fibroblasts have shown that an external source of mannose is used preferentially for *N*-glycosylation rather than mannose produced from glucose (21). This may also be true for fetal tissues, heightening the importance of an umbilical uptake of mannose. The placental supply of mannose may meet the fetal requirements.

It is surprising that the placenta is able to maintain a fetal/maternal concentration ratio of close to 60-fold for erythritol given the fact that erythritol is a relatively small neutral molecule. It suggests that the ovine placenta is relatively impermeable to erythritol.

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