Rates of Glucose Production and Utilization by the Foetus in Chronically Catheterized sheep

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1. Six well-nourished Scottish Blackface ewes were each prepared at 110–112 days of gestation with indwelling catheters in an umbilical artery and vein and at 2 days before experiment with catheters in each maternal jugular vein. Two-day experiments were conducted between days 124 and 134 of gestation when $[2\text{-}^3\text{H}, \text{U-}^{14}\text{C}]\text{glucose}$ was infused over 9 h into either the maternal or the foetal circulations, separate days being used for each infusion. 2. Plasma glucose specific radioactivities at plateau were used to determine, for a two-pool model representing the glucose system of the mother and foetus, the rates of glucose production, utilization and recycling by the mother and by the foetus and the rates of transfer of glucose between the mother and foetus. 3. The net rate of glucose utilization by the foetus, which we have called the 'foetal glucose requirement', was $7.6 \pm 0.6 \text{ mg/min per kg of foetus}$ ($n = 8$). Compared with the corresponding rate in the mother of $1.1 \pm 0.1 \text{ mg/min per kg of mother}$ ($n = 8$), this indicated a high rate of foetal metabolism. 4. The net rate of foetal uptake of glucose from the mother accounted for only $69 \pm 5\%$ of the foetal glucose requirement, and it has been argued that the remainder was supplied by foetal gluconeogenesis. 5. The rate of glucose recycling within the foetus was high, equal to $108 \pm 16\%$ of the net foetal glucose-utilization rate. The significance of this in terms of either Cori or futile cycling is discussed.

Glucose makes a large contribution to the energy requirements of the foetal sheep (see Battaglia & Meschia, 1978) and may also act as a precursor in several anabolic reactions, in particular the synthesis of foetal glycogen and fat. The rate of glucose utilization by the foetal sheep has been assessed in at least two different ways: (i) as the product of the umbilical blood flow rate and venous–arterial glucose concentration difference in chronically catheterized animals (see Battaglia & Meschia, 1978), and (ii) as the entry rate of glucose measured by administering radioactively labelled glucose as a 'single shot' (Warnes *et al.*, 1977a) or as a continuous infusion (Prior & Christenson, 1977) via indwelling catheters to foetal sheep. However, for these methods of measurement to be valid, the following assumptions must hold. First, maternal glucose must be the sole source of foetal glucose, otherwise input of glucose via foetal production *de novo* would cause method (i) to underestimate foetal glucose utilization. Secondly, the backflow of glucose from foetus to mother must be negligible, otherwise a proportion of foetal glucose would be lost by catabolism within maternal

and not foetal tissues, and method (ii) would overestimate foetal glucose utilization.

The object of the present work in sheep was to combine the results of infusions of radioactively labelled glucose into the maternal and foetal circulations in order to model the glucose system of the mother and foetus. The model provided estimates of the rate of glucose utilization by the sheep foetus and of the contribution of maternal glucose to the foetal glucose pool. We found that neither of the above assumptions held, and a new and more complete description of the kinetics of glucose metabolism in the pregnant ewe and its foetus is presented.

Materials and Methods

Animals and management

Six monotocous Scottish Blackface ewes weighing 48–63 kg were used. Each foetus had a catheter inserted into both an umbilical artery and vein (Mellor & Matheson, 1975) at days 110–112 of gestation. Catheters (Plextrocan 'E' type; Portex, Hythe, Kent, U.K.) were also inserted in each

maternal jugular vein 2 days before, and were removed at the end of, each experiment. The sheep were trained and housed as described by Hodgson & Mellor (1977) and received Ruminant Diet A, a complete diet of standardized composition (Wainman *et al.*, 1970). The diet was offered at a rate of 1.3 kg/day (three sheep) or 1.6 kg/day (three sheep), equivalent to 22.3 ± 1.2 g/kg per day and 30.0 ± 1.6 g/kg per day respectively. These feeding regimens were found to maintain individual ewe body weights constant throughout the period of study.

Radioactive tracer infusions

D-[U- 14 C]Glucose and D-[2- 3 H]glucose were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Sterile NaCl solutions (9 g/litre) containing [U- 14 C, 2- 3 H]glucose and carrier glucose (50 mg/litre) were infused continuously with no priming dose at a rate of 4 ml/h by using a syringe pump (model SP15/A; Scientifica and Cook Electronics, London, W3 8AU, U.K.). Infusions lasted 9 h and were conducted on each of 2 consecutive days into either the maternal or the foetal circulation on day 1 via a jugular or umbilical catheter respectively (see Table 2 for route of foetal infusion) and into the uninfused circulation on day 2. This dual-infusion procedure lasted 33 h and constituted one experiment. It was assumed that over the period of an experiment any change in the kinetics of glucose metabolism would be small enough to be disregarded (see Hodgson & Mellor, 1977), and the results have been treated as though the mother and foetus were infused simultaneously. A total of eight experiments were conducted between days 124 and 134 of gestation by either (a) infusing each foetus in the sheep consuming 1.3 kg of diet/day with 4–6 μ Ci of each tracer/ml of infusate on day 1 and the mother with the same infusate on day 2 or (b) infusing each ewe consuming 1.6 kg of diet/day with 2.5–6.0 μ Ci of [U- 14 C]glucose and 5.0–8.5 μ Ci of [2- 3 H]glucose/ml of infusate on day 1 and the foetus with 5.0–8.5 μ Ci of each tracer/ml of infusate on day 2. Experiments were repeated after 5 days in each of two sheep using procedure (b).

A pilot study had shown that radioactivity persisted in maternal and foetal plasma glucose between days 1 and 2 of an experiment and in foetal plasma glucose between experiments, and that this residual radioactivity was relatively constant by the time a second infusion or repeat experiment was scheduled. The amounts, expressed as percentages of the preceding plateaux, were 1–4% ([14 C]glucose) and 0–1.5% ([3 H]glucose) in maternal glucose and 13–37% ([14 C]glucose) and 8–15% ([3 H]glucose) in foetal glucose immediately before infusion on day 2, and 3–10% ([14 C]glucose) and 0% ([3 H]glucose) in foetal glucose immediately before a repeat experi-

ment. The effect of this residual radioactivity on the glucose specific-radioactivity plateaux was allowed for by taking baseline samples before each infusion and subtracting the activity present from subsequent plateau values. For repeat experiments, procedure (b) was preferred to procedure (a), because it reduced the errors associated with the calculation of the contribution of maternal glucose to the foetal glucose pool.

Sampling and analyses

Blood samples (3 ml foetal, 10 ml maternal) from the uninfused foetal vessel and maternal jugular vein were withdrawn into heparinized syringes immediately before and at 6, 7, 8 and 9 h after the start of each infusion. The blood was centrifuged immediately and the plasma stored at -40°C until analysed.

Plasma glucose and fructose concentrations were determined by the methods of Trinder (1969) and Foreman *et al.* (1973) respectively. The standard penta-acetate method for measuring glucose specific radioactivities could not be used for foetal plasma samples as fructose also forms a penta-acetate derivative (see Jones, 1965), and, if labelled, would cause foetal glucose specific radioactivities to be overestimated. Therefore an alternative method was devised to determine the hexose specific radioactivities in both foetal and maternal plasma samples. Deproteinized plasma (Somogyi, 1945) was first deionized by passing it through a mixed ion-exchange resin [Amberlite IR-45 (OH) and IRC-50 (H) analytical-grade resins, 1.75:1, w/w] contained in glass columns (10 cm $\times$ 0.7 cm. Econo-Columns; Bio-Rad Laboratories, Bromley, Kent, U.K.). Samples were washed through with twice the column volume of deionized water, and the eluates were freeze-dried to remove $^3\text{H}_2\text{O}$. Radioactive contents were measured in an automatic liquid-scintillation spectrometer (NE8312; Nuclear Enterprises, Edinburgh, Scotland, U.K.) by adding a 1 ml sample reconstituted in water to 10 ml of NE250 scintillator (Nuclear Enterprises) and counting in low-potassium-glass scintillation vials (Searle Diagnostic, High Wycombe, Bucks., U.K.). Counting efficiencies were 28–31% and 40–44% for ^3H and ^{14}C respectively, with 24–26% of the ^{14}C radioactivity appearing in the ^3H channel.

The specific radioactivity of maternal glucose was measured as the ratio of radioactivity to glucose content in the reconstituted sample. The values obtained by this method were $98 \pm 1.4\%$ ($n = 30$ samples) of the specific radioactivities calculated after isolating maternal plasma glucose as the penta-acetate derivative (Jones, 1965). Before applying this calculation to foetal samples, a correction was necessary for radioactivity present as fructose. Fructose radioactivity in the reconstituted sample

was measured after extracting the glucose by (i) incubating the sample with glucose oxidase (type II; Sigma London Chemical Co., Poole, Dorset, U.K.) for 5 h at 37°C when glucose was quantitatively converted to gluconic acid, (ii) removing the gluconic acid by passing the incubated sample through Amberlite IR-45 (OH) resin in the columns described above and (iii) measuring the eluate radioactivity as before. The recovery of fructose during this process was $99 \pm 1\%$ and the glucose radioactivity was calculated by difference. Fructose specific radioactivities were 35–50% (^{14}C results) and 5–15% (^3H results) of the foetal plateau glucose specific-radioactivity values.

Fig. 1. A two-pool model of the glucose system of the mother and foetus in sheep

F and F' represent the flow rates calculated from [^{14}C]- and [^3H]-glucose results respectively. m indicates the maternal and f the foetal glucose pools respectively. The method of notation is taken from Shipley & Clark (1972).

Model parameter	Biochemical/physiological interpretation
F_{mo}	Maternal gluconeogenesis*
F'_{mo}	Maternal glucogenesis*
$F'_{mo} - F_{mo}$	Recycling of glucose within the maternal pool
F_{om}	The use of glucose for oxidation and tissue synthesis by all non-foetal tissues
$F_{fm} - F_{mf}$	Net uptake of maternal glucose by the foetus
F_{fo}	Foetal gluconeogenesis
F'_{fo}	Foetal glucogenesis
$F'_{fo} - F_{fo}$	Recycling of glucose within the foetal pool
F_{of}	The use of glucose for oxidation and tissue synthesis by the foetus (i.e. the 'foetal glucose requirement')

* May include small amounts of glucose absorbed from the gut

Model configuration, calculations and definitions used in the present analysis of the kinetics of glucose metabolism in the pregnant ewe and its foetus

The configuration of the model chosen to represent the glucose system of the pregnant ewe and its foetus and the biochemical/physiological interpretation of the model parameters are given in Fig. 1. The flow rates of the model were determined by substituting the plateau specific radioactivities of maternal and foetal plasma glucose and the glucose tracer infusion rates in formulae summarized by Shipley & Clark (1972) [equations 3 and 4 (p. 150), equations 15 and 16 (p. 104)]. By applying the formulae to results from both [^{14}C]- and [^3H]-glucose infusions, model flow rates either exclusive ([^{14}C]glucose results) or inclusive ([^3H]glucose results) of glucose recycling were calculated (cf. Judson & Leng, 1972). The rates of glucose recycling within the maternal and foetal pools were then determined as the difference between the rates of flow into each pool calculated from the two sets of results (see the legend to Fig. 1).

Presentation of results

When required for the purposes of comparison, glucose flow rates have been expressed on a body-weight basis. To convert the rates in the mother, the live weight of the pregnant ewe was used; to convert the rates in the foetus, the foetal weight, which probably comprised over 85% of the combined foetal-placental weight (Barcroft, 1946; Mellor *et al.*, 1977; D. J. Mellor, unpublished work), was used. Foetal weight at the time of experiment was calculated from the lamb birth weight by using published growth curves (Agricultural Research Council, 1980).

Results

The mean concentrations of foetal plasma glucose and fructose and of maternal glucose during each experiment are shown in Table 1. Within- and between-day variations in concentration were small, and it was assumed that steady-state conditions with respect to these metabolites were achieved. Maternal plasma glucose concentrations were significantly higher ($P < 0.001$) at the higher level of feeding, but foetal plasma hexose concentrations were similar at both levels of intake.

Fig. 2 is an example of ^{14}C specific radioactivities in maternal and foetal plasma glucose during infusions to mother and foetus. Similar results were obtained for ^3H specific radioactivities of plasma glucose. Plateau values were reached after about 6 h in the mother and after about 7 h in the foetus, and the mean $\pm$ s.e.m. values are presented in Table 2. A greater variability was observed in the analyses of foetal compared with maternal samples as a result of

Table 1. *Concentrations of glucose and fructose in foetal plasma and of glucose in maternal plasma*
Results are means $\pm$ S.E.M.

Food intake (kg/day)	Sheep	Foetal age (days)	Day of expt.	Hexose concentrations (mm)		
				Foetal		Maternal glucose
				Glucose	Fructose	
1.3	H260	124-125	1	1.37 $\pm$ 0.06	4.03 $\pm$ 0.04	3.20 $\pm$ 0.04
			2	1.24 $\pm$ 0.05	3.91 $\pm$ 0.06	3.14 $\pm$ 0.03
1.3	H297	126-127	1	1.00 $\pm$ 0.05	5.15 $\pm$ 0.18	3.38 $\pm$ 0.04
			2	1.05 $\pm$ 0.05	5.42 $\pm$ 0.04	3.46 $\pm$ 0.03
1.3	H256	133-134	1	1.31 $\pm$ 0.05	3.92 $\pm$ 0.01	3.50 $\pm$ 0.10
			2	1.29 $\pm$ 0.05	4.16 $\pm$ 0.02	3.37 $\pm$ 0.03
1.6	F817	124-125	1	1.21 $\pm$ 0.02	4.36 $\pm$ 0.05	3.73 $\pm$ 0.11
			2	1.25 $\pm$ 0.02	4.23 $\pm$ 0.09	3.76 $\pm$ 0.14
1.6	F834	124-125	1	1.45 $\pm$ 0.01	6.32 $\pm$ 0.06	4.14 $\pm$ 0.18
			2	1.50*	6.47*	4.38 $\pm$ 0.19
1.6	F883	124-125	1	1.33*	5.85*	4.17 $\pm$ 0.10
			2	1.24*	5.99*	4.21 $\pm$ 0.09
1.6	F817	131-132	1	1.24 $\pm$ 0.03	4.46 $\pm$ 0.14	3.80 $\pm$ 0.11
			2	1.17 $\pm$ 0.04	4.33 $\pm$ 0.06	3.87 $\pm$ 0.04
1.6	F834	131-132	1	1.79 $\pm$ 0.05	6.01 $\pm$ 0.12	3.97 $\pm$ 0.11
			2	1.65 $\pm$ 0.06	6.06 $\pm$ 0.11	3.91 $\pm$ 0.11

* These values are from analysis of the 8 and 9 h samples only.

Table 2. *Mean specific radioactivities $\pm$ S.E.M. (corrected for residual activity and to an infusion rate of 1 μ Ci/min) of foetal and maternal plasma glucose and the proportion of foetal glucose derived from the mother*

Abbreviations: SR is used to denote specific radioactivity, the superscript to denote the circulation infused and the subscript to denote the circulation sampled; UV, umbilical vein; UA, umbilical artery.

Food intake (kg/day)	Sheep	Foetal age (days)	Label	Plateau specific radioactivities (μ Ci/g of glucose carbon)				Route of foetal infusion	Proportion of foetal glucose derived from mother
				SR _m ^m	SR _f ^m	SR _f ^f	SR _m ^f		
1.3	H260	124-125	U- ¹⁴ C	43.1 $\pm$ 0.2	30.7 $\pm$ 4.1	82.6 $\pm$ 4.8	12.3 $\pm$ 0.1	UV	0.71
			2- ³ H	29.9 $\pm$ 2.1	15.2*	34.0 $\pm$ 3.3	8.2 $\pm$ 0.3	UV	0.51
1.3	H297	126-127	U- ¹⁴ C	28.6 $\pm$ 1.9	20.8 $\pm$ 3.0	106.4*	13.0 $\pm$ 0.1	UA	0.72
			2- ³ H	23.1 $\pm$ 1.6	11.4 $\pm$ 1.3	51.2*	8.7 $\pm$ 0.7	UA	0.49
1.3	H256	133-134	U- ¹⁴ C	41.4 $\pm$ 2.1	32.9 $\pm$ 4.0	79.9 $\pm$ 8.5	8.2 $\pm$ 0.1	UV	0.80
			2- ³ H	30.3 $\pm$ 1.5	12.6 $\pm$ 2.9	26.1 $\pm$ 1.3	5.9 $\pm$ 0.2	UV	0.42
1.6	F817	124-125	U- ¹⁴ C	25.0 $\pm$ 0.9	23.1 $\pm$ 0.3	107.3*	10.2 $\pm$ 0.1	UV	0.92
			2- ³ H	22.4 $\pm$ 0.9	10.7 $\pm$ 0.9	99.1*	9.9 $\pm$ 0.2	UV	0.48
1.6	F834	124-125	U- ¹⁴ C	33.1 $\pm$ 1.2	29.4 $\pm$ 0.8	74.1*	17.2 $\pm$ 0.8	UA	0.89
			2- ³ H	32.7 $\pm$ 1.8	19.7 $\pm$ 4.2	60.1*	15.5 $\pm$ 0.8	UA	0.60
1.6	F883	124-125	U- ¹⁴ C	32.4 $\pm$ 1.5	27.6*	134.1*	5.8 $\pm$ 0.2	UA	0.85
			2- ³ H	23.0 $\pm$ 2.1	11.2*	97.8*	3.5 $\pm$ 0.3	UA	0.49
1.6	F817	131-132	U- ¹⁴ C	37.1 $\pm$ 1.0	30.6*	88.4*	10.3 $\pm$ 0.3	UV	0.82
			2- ³ H	35.6 $\pm$ 1.8	16.1*	62.3*	11.0 $\pm$ 0.5	UV	0.45
1.6	F834	131-132	U- ¹⁴ C	34.7 $\pm$ 0.5	25.8*	64.4*	17.1 $\pm$ 0.2	UA	0.74
			2- ³ H	34.9 $\pm$ 0.5	16.8*	42.9*	12.4 $\pm$ 0.8	UA	0.48

* These values are from analysis of 8 and 9 h samples only.

the small amounts of sample available for, and the greater complexity of, the foetal analyses. The fraction of the total flow into the foetal pool of glucose derived directly from the maternal pool

(calculated as the ratio of the mean plateau specific radioactivity of foetal plasma glucose to that of maternal plasma glucose during a maternal infusion) was 0.81 ± 0.03 and 0.49 ± 0.02 when calculated

Fig. 2. Specific radioactivities of maternal (O) and foetal (●) plasma glucose during a constant infusion of [U-¹⁴C]glucose into the mother (a) or foetus (b). The mother was infused at a rate of 0.42 μCi/min and the foetus, via an umbilical vein, at a rate of 0.49 μCi/min. Specific radioactivities have been corrected for residual radioactivity.

from [¹⁴C]glucose and [³H]glucose results respectively (Table 2). As a measure of the sensitivity of this calculation to the variation observed in the plateau values of glucose specific radioactivity, the ratio was also calculated from the results of the 9 h samples alone, which should have been most representative of the achieved plateau. Means of 0.78 ± 0.05 ([¹⁴C]glucose results) and 0.56 ± 0.05 ([³H]glucose results) were obtained which, although associated as expected with a higher degree of error, were not significantly different ($P > 0.05$) from the original mean values.

Glucose fluxes in the maternal-foetal glucose model

The rates of gluconeogenesis, glucose recycling, net utilization and net transfer, as defined in Fig. 1, within and between mother and foetus, are given in Table 3.

The mean foetal glucose requirement (F_{of}) was 20.6 ± 1.7 mg/min, equivalent to 7.6 ± 0.6 mg/min per kg of foetus. Although the mean total flux of glucose from mother to foetus (F_{fm}) was 26.2 ± 2.6 mg/min, more than enough to meet the foetal requirement, there was a substantial backflow

Table 3. Allocation of model flow rates to the various processes of metabolism within and transfer between mother and foetus

Food intake (kg/day)	Foetal age (days)	Weight of ewe plus foetus (kg)	Estimated weight of foetus (kg)	Mother				Foetus			
				Input		Output		Input		Output	
				Gluconeogenesis (F_{mo})	Recycling ($F'_{mo} - F_{mo}$)	Net utilization (F_{om})	Net foetal uptake ($F_{fm} - F_{mf}$)	Gluconeogenesis (F_{fo})	Recycling ($F'_{fo} - F_{fo}$)	Requirement (F_{of})	
1.3	H260	63.0	2.42	55.3	16.9	40.8	14.5	9.7	31.9	24.2	
1.3	H297	60.0	2.99	83.9	14.1	76.9	7.0	7.1	20.0	14.1	
1.3	H256	53.0	2.92	59.1	11.5	38.7	20.4	7.0	54.7	27.3	
1.6	F817	54.5	2.33	99.4	5.9	86.0	13.4	2.0	12.2	15.3	
1.6	F834	47.4	2.50	72.9	-5.6	57.3	15.6	4.9	14.6	20.5	
1.6	F883	63.0	2.50	76.5	30.2	63.5	13.0	3.3	10.3	16.2	
1.6	F817	57.5	2.95	65.9	-3.0	48.8	17.1	5.6	18.4	22.7	
1.6	F834	47.5	3.17	66.1	-6.9	54.0	12.1	12.3	22.8	24.5	

of glucose from foetus to mother (F_{mf}) of 12.0 ± 2.5 mg/min. This indicated that the efficiency of uptake by the foetus of maternal glucose was only 54%. Consequently the net uptake of maternal glucose by the foetus (14.1 ± 1.4 mg/min) supplied only $69 \pm 5\%$ of the foetal glucose requirement. The discrepancy was met by the input of glucose to the foetal pool via F_{fo} , which we have attributed to foetal gluconeogenesis (see the Discussion section). The calculated values for F_{fo} were significantly different from zero ($P < 0.001$) and the mean value, 2.4 ± 0.4 mg/min per kg of foetus, was equivalent to $31 \pm 5\%$ of the foetal glucose requirement. The results from repeat experiments in the same sheep indicated that the foetal glucose requirement increased with gestational age, and that the proportional contribution of foetal gluconeogenesis to the glucose requirement also increased.

The mean rate of glucose production by the mother (F_{mo}) was 72.4 ± 5.1 mg/min, of which $21 \pm 3\%$ was accounted for by the net foetal uptake of glucose. The mean rate of glucose utilization by the mother (F_{om}) was 58.3 ± 5.9 mg/min or 1.1 ± 0.1 mg/min per kg of ewe plus foetus, which was only 0.14 of the foetal rate (F_{of} , 7.6 ± 0.6 mg/min per kg of foetus).

There was an inverse relationship between the foetal glucose requirement (Y mg of glucose/min) and the rate of maternal glucose utilization (X mg of glucose/min), such that $Y = 36.3 - 0.27X$ ($r = 0.92$; $P < 0.001$). This complied with the suggestion made by others (Lindsay, 1971; Setchell *et al.*, 1972) that part of the increased requirement for glucose during pregnancy might be offset by a reduction in the utilization of glucose by maternal tissues.

The mean $\pm$ S.E.M. rate of glucose recycling, when expressed as a fraction of the glucose-utilization rate, was 0.14 ± 0.08 and 1.08 ± 0.16 for mother and

foetus respectively. The variability in the calculation of this rate (see Table 3) was largely due to the lower accuracy of the determination of [3 H]glucose compared with [14 C]glucose specific radioactivities.

In order that the work of others who used different methods of calculating the kinetic variables might be compared with ours, the rates of total entry, irreversible loss and recycling of glucose, as defined by Leng (1970), were calculated. The results for these parameters are presented in Table 4.

Discussion

The infusions of [U- 14 C]- and [2- 3 H]-glucose into the maternal circulation of the sheep used in this study produced lower plateau specific radioactivities in the plasma glucose of the foetus compared with the mother. This showed that only a proportion of the foetal glucose was derived from the mother, the calculation of which (0.81 from [14 C]glucose results and 0.49 from [3 H]glucose results) was independent of any particular model formulation. The lower foetal specific radioactivities must result from the input of unlabelled glucose from one or both of two sources, foetal gluconeogenesis and foetal glycogen turnover. The likely contributions made by these two sources are now discussed.

Foetal gluconeogenesis

The possibility of glucose being synthesized by the sheep foetus has been discussed before (e.g. Boyd *et al.*, 1973), and it has been reaffirmed that the necessary enzymes are present in foetal liver (Warnes *et al.*, 1977b). The major gluconeogenic precursors are likely to be amino acids and lactate, which can enter foetal plasma from the mother and from turnover of foetal tissue. About half of the amino acid nitrogen transferred from mother to

Table 4. Maternal and foetal rates of total entry, irreversible loss and recycling of glucose

		Glucose flux rates (mg/min per kg)						
		Maternal			Foetal			
Food intake (kg/day)	Sheep	Foetal age (days)	Total entry	Irreversible loss	Recycling	Total entry	Irreversible loss	Recycling
1.3	H260	124-125	1.3	0.9	0.4	30.3	12.5	17.8
1.3	H297	126-127	1.8	1.5	0.3	16.3	7.9	8.4
1.3	H256	133-134	1.6	1.2	0.4	32.8	10.7	22.1
1.6	F817	124-125	2.1	1.8	0.3	10.8	10.0	0.8
1.6	F834	124-125	1.6	1.6	0	16.7	13.5	3.2
1.6	F883	124-125	1.7	1.2	0.5	10.2	7.5	2.7
1.6	F817	131-132	1.2	1.2	0	13.6	9.6	4.0
1.6	F834	131-132	1.5	1.5	0	18.4	12.2	6.2

foetus is retained in foetal tissues (Lemons *et al.*, 1976). The extent to which the excess acids, which are in dynamic equilibrium with foetal protein, are used as precursors for foetal gluconeogenesis has received some attention. Prior & Christenson (1977) found that carbon was transferred from alanine into glucose in foetal lambs, in sufficient amounts to account for about 0.02 of the foetal glucose-entry rate. This contribution is small, accounting for 0.07 of the alanine turnover rate (Prior & Christenson, 1977), but constitutes 0.49 of the net uptake of alanine estimated for foetal sheep from the data of Lemons *et al.* (1976). Other foetal amino acids may be used to a similar extent, and this is supported by our unpublished observations on the incorporation of carbon into foetal glucose from a mixture of 16 labelled amino acids. Foetal amino acids may therefore provide a major part of the carbon required for the rates of foetal gluconeogenesis reported here.

A net uptake of lactate from the placenta by the foetal lamb has been demonstrated (Burd *et al.*, 1975; Char & Creasy, 1976), and lactate will also be formed during foetal metabolism (e.g., of glucose, Warnes *et al.*, 1977a). In adult sheep, lactate can be used for glucose synthesis (see Bergman, 1973), but this may not be the case in foetal sheep, since Warnes *et al.* (1977a) found no evidence of a transfer of carbon from lactate to glucose when [U- ^{14}C]lactate was given as a 'single shot' to foetal sheep. An important corollary to their observation that lactate does not serve as precursor for foetal glucose synthesis is that the turnover of extra-hepatic glycogen reserves, which releases lactate and not glucose into plasma, cannot contribute to our estimation of foetal gluconeogenesis.

Foetal glycogen turnover

From the above argument it is apparent that only glucose released during the turnover of foetal liver glycogen need be considered here. Foetal liver glycogen reserves in sheep are small (5–8 g; Shelley, 1960), and, for their turnover to contribute significantly to our estimate of foetal gluconeogenesis (9.4 g of glucose/day), a half-life of 6–12 h would be necessary. In fact, a short half-life for liver glycogen is unlikely, because (i) the activities of the enzymes involved are low in foetal lambs (Walker, 1968) and (ii) the level of labelling of foetal liver glycogen after a 5 h infusion of [U- ^{14}C]glucose is very low (J. M. Bassett, personal communication relating to Setchell *et al.*, 1972), even in a situation where the fraction of foetal glucose derived from the mother (0.73 ± 0.05 , $n=4$; Setchell *et al.*, 1972) is less than that reported here. It has been suggested (Lindsay, 1970) that, if glycogen were not uniformly labelled during a [^{14}C]glucose infusion, then glucose of a high specific

radioactivity might be lost from the periphery of glycogen during its isolation from tissues and therefore that the specific radioactivity of total glycogen could be underestimated. However, the suggestion still awaits experimental verification and, from the work of Setchell *et al.* (1972) in which recoveries of glycogen were high (cf. Shelley, 1960), it appears that the turnover of foetal liver glycogen is low. Accordingly, we conclude that foetal gluconeogenesis constitutes the major part of the non-maternal glucose input to the foetal glucose pool.

Foetal glucose recycling

Lower specific radioactivities in [^3H]glucose compared with those in [^{14}C]glucose in plasma after the intravenous administration of [2- ^3H , U- ^{14}C]glucose have been attributed to the recycling of glucose through lactate and glycogen, when the ^3H label, but not the ^{14}C label, is lost from the glucose molecule (Katz & Dunn, 1967). However, as indicated above, the recycling of glucose through lactate and glycogen in the foetal lamb can largely be discounted. Instead, the low [^3H]glucose plateau specific radioactivities observed in foetal plasma glucose in the present work may reflect the presence of an active 'futile' cycle (see Katz & Rognstad, 1978) in the foetal sheep, which would remove ^3H but not ^{14}C from foetal glucose during the repeated interconversions of glucose, glucose 6-phosphate and fructose 6-phosphate. Thus the rates given in Tables 3 and 4 for foetal glucose recycling probably refer to the operation of such a cycle. The amounts of glucose involved are large (23.1 ± 5.1 mg/min) and the cycle would consume substantial amounts of energy, which may contribute significantly to the very low efficiency of energy utilization (12–14%; Sykes & Field, 1972) in foetal sheep.

Distribution of maternal glucose between maternal and foetal tissues

The mean net rate of transfer of glucose between the maternal and foetal pools ($F_{\text{fm}} - F_{\text{mf}}$) of 14.1 ± 1.4 mg/min may be compared with other published data of glucose fluxes in monotocous sheep obtained by alternative techniques. The net umbilical uptake of glucose, calculated from umbilical blood flow rates and venous–arterial glucose concentration differences, was 11.0 ± 1.2 mg/min (James *et al.*, 1972) and 18.0 ± 1.4 mg/min (Boyd *et al.*, 1973), whereas the net uterine uptake of glucose, calculated from uterine blood flow rates and arterial–venous glucose concentration differences, was 59.0 ± 12.0 mg/min (Setchell *et al.*, 1972), 49.6 mg/min (Prior & Christenson, 1978) and 72.5 mg/min (Christenson & Prior, 1978). We conclude from this comparison that our calculation of the net transfer of glucose between mother and foetus represents the net umbilical uptake of glu-

cose, and that glucose metabolism by the uterine and placental tissues (approx. 30–55 mg of glucose/min when calculated from the above values for the net uterine uptake corrected for the net umbilical uptake) is largely included in our estimate of the rate of maternal glucose utilization (F_{om} , 38.7–86.0 mg/min; Table 3).

Rates of total entry and irreversible loss of glucose in mother and foetus

As far as we are aware there are no similar reports in the literature with which to compare our model of maternal–foetal glucose kinetics in sheep. However, the calculations of the rates of total entry and irreversible loss of glucose (Table 4) do allow some comparisons between published work and certain aspects of the present work.

The mean values for maternal rates of total entry and irreversible loss of glucose (1.6 ± 0.1 and 1.4 ± 0.1 mg/min per kg respectively) are lower than those published by Steel & Leng (1968, 1973), who obtained mean values in pregnant ewes at similar gestational ages (136 days approximately) and food intakes (22.4 g/kg per day) of 2.8 ± 0.2 and 2.2 ± 0.2 mg/min per kg ($n = 4$) respectively. However, maternal glucose-entry rates are related to the level of feeding (see Leng, 1970; Steel & Leng, 1973), and it is important to note that Steel & Leng (1968, 1973) effectively doubled the rate of intake of their animals by giving each day's entire ration over the 12 h allocated to each experiment.

The mean value for the foetal rate of irreversible loss was 10.5 ± 0.8 mg/min per kg of foetus, which compares with a mean of 7.5 ± 0.3 mg/min per kg of foetus obtained by Warnes *et al.* (1977a) using the 'single-shot' technique. Mixing problems prevented Warnes *et al.* (1977a) from calculating the foetal glucose total entry rate and would also have produced an underestimation in their calculation of irreversible loss. Prior & Christenson (1977) reported 'total plasma glucose turnover' in foetal lambs of 8.3 ± 2.6 mg/min per kg of foetus. However, as the label used by these workers more closely mimics the behaviour of [U- 14 C]- than [2- 3 H]-glucose (see Judson & Leng, 1972), the values are more closely representative of the rate of irreversible loss of foetal glucose.

The measurement confined to the foetal glucose irreversible loss (see, e.g., Prior & Christenson, 1977) can seriously overestimate the foetal glucose requirement, because it includes foetal glucose that is transferred to the mother to be metabolized by maternal tissues. In the present work we found that 0.14 ± 0.03 ([14 C]glucose results) of maternal glucose was derived from the foetal pool and that consequently the parameter foetal glucose irreversible loss overestimated the foetal glucose requirement by 35–40%.

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