Foetal and maternal rates of glucose production and utilization in chronically catheterized ditocous ewes

J. Christopher HODGSON, D. J. MELLOR and Alexander C. FIELD
Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH, Scotland, U.K.

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1. Five well-nourished Scottish Blackface ewes carrying twin lambs were used. An umbilical artery and vein of each foetus in two sheep and one foetus in three sheep were catheterized at 109–112 days of gestation and both maternal jugular veins were catheterized 2 days before experiment. Two-day experiments were conducted between days 124 and 141 of gestation when $[2\text{-}^3\text{H}, \text{U-}^{14}\text{C}]$ glucose was infused intravenously over 9 h into either the mother or one foetus, separate days being used for each infusion. 2. Plasma glucose specific radioactivities of the mother and infused foetus at plateau were used to determine the glucose flux rates within and between mother and foetus. The corresponding values for the uninfused foetus were calculated by using the relative weights of each twin foetus and were used to correct the maternal flux rates for the presence of the second foetus. 3. The net rate of glucose utilization by the foetus was 11.2 ± 1.3 mg/min per kg of foetus (mean $\pm$ S.E.M.; $n = 11$). The corresponding rate in the mother was 1.1 ± 0.1 mg/min per kg of live weight ($n = 11$). 4. The net rate of foetal uptake of glucose from the mother accounted for only $56 \pm 4\%$ of the glucose used by the foetus. The remainder was provided by an input of 4.9 ± 0.7 mg/min per kg of foetus to the foetal glucose pool via foetal gluconeogenesis. 5. The rate of glucose futile cycling in the foetus was high, equal to $95 \pm 19\%$ of the net foetal glucose-utilization rate. 6. The rate of glucose utilization (mg/min per kg of foetus) decreased progressively over the observational period of 124–141 days of gestation. There was no increase in the rates of net foetal glucose uptake or of foetal gluconeogenesis to counter this decline.

In fed monotocous ewes during the last 30 days of pregnancy, mean umbilical uptakes of glucose of 11–18 mg/min have been estimated to account for only 41–52% of foetal O_2 consumption (James *et al.*, 1972; Boyd *et al.*, 1973). However, in studies of similar ewes and their foetuses infused with radioactively labelled glucose, Hodgson *et al.* (1980) showed that a mean ($\pm$ S.E.M.) umbilical uptake of 14.1 ± 1.4 mg/min ($n = 8$) provided only 69% of the glucose used by the foetus, and attributed the source of the remainder to foetal gluconeogenesis. Thus a larger proportion of foetal O_2 consumption was required for the complete oxidation of foetal glucose than could be calculated from umbilical glucose uptake values alone, which reinforced the concept of glucose as the major energy substrate for ovine foetal metabolism (see also Hodgson *et al.*, 1979).

The present experiments were designed to determine, for well-nourished ditocous sheep, the rates of maternal and foetal glucose production and

utilization and of net foetal uptake of glucose from the mother, and to compare these parameters with those calculated for monotocous ewes. The results on a body-weight basis for gestational ages of 124–134 days showed that, compared with monotocous ewes, in ditocous ewes the rates of maternal glucose production and utilization and of net foetal uptake were similar, the rates of foetal glucose production and utilization were significantly higher and foetal gluconeogenesis supplied a greater proportion of the glucose used by the foetus. Results from repeat observations in the same animals showed that between gestational ages of 124 and 141 days the rate of maternal glucose production increased but that the foetal rate of glucose utilization, expressed per kg of foetal weight, decreased. These findings are used to suggest metabolic reasons for the known decrease in growth rate of similar foetuses from about 120 days of gestation to term (Mellor & Murray, 1981).

Materials and methods

Animals and management

Five ditocous Scottish Blackface ewes weighing 54–75 kg were housed, trained, maintained and catheterized as described previously (Hodgson & Mellor, 1977; Hodgson *et al.*, 1980). An umbilical artery and vein of each foetus in two sheep and of one foetus in three sheep were catheterized. Each animal received 1.4–1.7 kg of Ruminant Diet A per day, equivalent to 24.7 ± 0.9 g/kg live wt. per day. In some animals the dietary intake was adjusted between experiments in an attempt to maintain constant plasma glucose concentrations within and between sheep throughout the work.

Radioactive tracer infusions

Eleven experiments were conducted between days 124 and 141 of gestation using the glucose tracers and infusion procedures described previously (Hodgson *et al.*, 1980). All infusions (to the mother via a jugular vein and to the foetus via an umbilical vein) were at a rate of 4 ml/h. In sheep H308 and H310 one foetus (foetus A) was infused on day 1 of an experiment with $6.0 \mu\text{Ci}$ of each tracer/ml of infusate and its mother with the same infusate on day 2. In the other three sheep the mother was infused on day 1 with 3.0 – $8.5 \mu\text{Ci}$ of $[\text{U-}^{14}\text{C}]$ glucose and 7.5 – $8.5 \mu\text{Ci}$ of $[\text{2-}^3\text{H}]$ glucose/ml of infusate and the corresponding foetus A on day 2 with 6.0 – $6.5 \mu\text{Ci}$ of $[\text{U-}^{14}\text{C}]$ glucose and 6.5 – $8.0 \mu\text{Ci}$ of $[\text{2-}^3\text{H}]$ glucose/ml of infusate. Experiments were repeated at 5-day intervals twice in sheep H308 and F816 and once in sheep F847 and F879. The infused foetuses were identified after birth by injecting ^{125}I -labelled albumin through the corresponding umbilical catheters 2–3 days before birth.

Sampling and analyses

Foetal and maternal blood samples were taken immediately before and at 6 (mother only), 7, 8 and 9 h after the start of each infusion. When both foetuses in a ewe were catheterized, both were sampled during experiments but only foetus A was infused. Details of the sampling and analytical procedures have been published previously (Hodgson *et al.*, 1980).

Mathematical analysis

The form of modelling used in the present paper makes no attempt to define sub-models representing glucose metabolism within the mother or foetuses and is dependent on the assumption that all tracer and tracee glucose pass before utilization through the sampled pools, i.e. the maternal and foetal blood. The model used for the analysis of glucose kinetics in ditocous sheep should include pools for the mother and both foetuses. However, the complete

Fig. 1. A three-pool model of the maternal-foetal glucose system in ditocous ewes

F , R and F' , R' represent the flow rates calculated from $[\text{14C}]$ - and $[\text{3H}]$ -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 mother
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 foetus A†
F_{fo}	Gluconeogenesis within foetus A†
F'_{fo}	Glucogenesis within foetus A†
$F'_{fo} - F_{fo}$	Recycling of glucose within foetus A†
F_{of}	The use of glucose for oxidation and tissue synthesis by foetus A†

* May include small amounts of glucose absorbed from the gut.

† Similar considerations apply to foetus B, substituting R and R' for F and F' respectively.

solution to a three-pool model (Fig. 1) requires infusions to, and specific radioactivity measurements in, the mother and each foetus separately. Using the present experimental technique this was considered impractical for two main reasons. (i) The duration of experiments would need to be increased from 33 to 57 h and this would make the assumption necessary for the simultaneous treatment of all results (see Hodgson *et al.*, 1980) less tenable. (ii) The residual radioactivity in plasma glucose would be increased and would decrease the accuracy of measurement of plateau glucose specific radioactivities. In the present paper a compromise solution is offered in which the fluxes of the three-pool model are calculated from the available results in two stages. (1) The flow rates of a two-pool model

representing the mother and foetus A were calculated as described previously (Hodgson *et al.*, 1980). This analysis correctly represents the glucose kinetics of foetus A but overestimates the rates of maternal glucose production and utilization (see Hodgson & Mellor, 1977). (2) The flow rates between the maternal pool and a third pool representing foetus B, and the rates of glucose production and utilization within foetus B, were calculated as the product of the equivalent rates for foetus A and the foetus B/foetus A weight ratio. The observation that the four [^{14}C]glucose SR_f^m (see the legend to Table 2 for definition of this term) values obtained for foetus B were similar to those obtained for each corresponding foetus A (Table 2) supported the assumption that twin foetuses would exhibit similar kinetics of glucose metabolism. The true rates of maternal glucose production (F_{mo}^*) and utilization (F_{om}^*) were then obtained by subtracting from the rates calculated in stage (1) the contribution from, and the utilization of maternal glucose by, foetus B respectively.

Hence:

$$F_{mo} = F_{mo}^* - R_{fo} [R_{mf}/(R_{mf} + R_{of})]$$

and

$$F_{om} = F_{om}^* - R_{fm} [R_{of}/(R_{mf} + R_{of})]$$

where F_{mo}^* and F_{om}^* are the values obtained for the maternal rates of production and utilization respectively from the two-pool model. The model flow rates for foetus A calculated in stage (1), the estimated rates for foetus B and the true rates for the mother are the results reported in the present paper.

Results

As before (Hodgson *et al.*, 1980) the within- and between-day variation in plasma glucose and fructose concentrations in mother and foetuses during

experiments was small and it was assumed that steady-state conditions with respect to these metabolites were maintained. However, there were some within-animal differences in concentrations between experiments as indicated in Table 1. The

Fig. 2. Specific radioactivities of maternal (O) and foetal (●) plasma glucose during a constant infusion of [^{14}C]glucose into the mother (a) or foetus (b). The mother was infused at a rate of $0.62 \mu\text{Ci}/\text{min}$ and the foetus at a rate of $0.38 \mu\text{Ci}/\text{min}$. Specific radioactivities have been corrected for residual radioactivity.

Table 1. Plasma hexose concentrations in foetus A and mother during experiments

Different superscripts denote significantly different means within animals ($P < 0.01$). The absence of superscripts indicates no significant difference between means. Results are means $\pm$ S.E.M.

Sheep	Experiment	Plasma concn. (mm)		
		Foetal glucose	Foetal fructose	Maternal glucose
H308	1	1.26 ± 0.02^a	4.19 ± 0.11^a	3.69 ± 0.01^a
H308	2	1.01 ± 0.02^b	3.81 ± 0.04^b	3.11 ± 0.12^b
H308	3	0.78 ± 0.02^c	3.20 ± 0.08^c	3.07 ± 0.03^b
H310	1	1.20 ± 0.02	3.30 ± 0.07	3.60 ± 0.06
F816	1	1.00 ± 0.03	5.88 ± 0.21^a	3.03 ± 0.04^a
F816	2	1.05 ± 0.02	4.24 ± 0.07^b	2.95 ± 0.07^{ab}
F816	3	1.02 ± 0.02	4.35 ± 0.06^b	2.83 ± 0.04^b
F847	1	0.89 ± 0.05	5.72 ± 0.04^a	2.71 ± 0.04^a
F847	2	0.91 ± 0.02	4.41 ± 0.09^b	2.94 ± 0.02^b
F879	1	1.11 ± 0.02^a	5.46 ± 0.04^a	3.05 ± 0.06^a
F879	2	0.98 ± 0.02^b	5.13 ± 0.06^b	2.80 ± 0.03^b

general trend was for the plasma hexose concentrations to decrease, but the foetal plasma glucose concentrations in sheep F816 and F847 remained constant throughout the study and the maternal plasma glucose concentrations in sheep F847 increased between experiments. The concentrations of plasma glucose were similar in foetuses A and B of sheep F816 but were significantly ($P < 0.001$) lower in foetus A than in foetus B of sheep F847.

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. These values were used to calculate the fraction of the total flow into the infused foetal pool of glucose derived directly from the maternal pool as described by Hodgson *et al.* (1980), and mean values of 0.69 ± 0.03 ($n = 11$) and 0.46 ± 0.03 ($n = 11$) were obtained for ^{14}C glucose and ^3H glucose results respectively (Table 2). When these ratios were

recalculated using the results from the 9 h samples alone as a measure of the sensitivity of this calculation to the variation observed in the plateau specific radioactivities (cf. Hodgson *et al.*, 1980), values of 0.71 ± 0.04 ($n = 11$) and 0.49 ± 0.03 ($n = 11$) were obtained for ^{14}C glucose and ^3H glucose results respectively, which were not significantly different ($P > 0.05$) from the original mean values.

Maternal-foetal glucose fluxes

The rates of gluconeogenesis, glucose recycling, net utilization and net transfer (as defined in Fig. 1), within and between mother and foetuses, are given in Table 3. The true values for the maternal rates F_{mo} , F'_{mo} and F_{om} were, on average, 96%, 90% and 76% respectively of the rates calculated using the two-pool model.

The mean rate of glucose utilized (F_{of}) by foetus A was 26.1 ± 2.6 mg/min, equivalent to 11.2 ± 1.3 mg/min per kg of foetus. Although the mean total flux of glucose from the mother to foetus A (F_{fm}) was 26.4 ± 3.4 mg/min, sufficient to meet the foetal rate of utilization, there was a substantial

Table 2. Specific radioactivities (corrected for residual activity and to an infusion rate of $1 \mu\text{Ci}/\text{min}$) of foetal and maternal plasma glucose and the proportion of foetal glucose derived from the mother

SR denotes specific radioactivity, with the superscript denoting the circulation infused and the subscript denoting the circulation sampled (m = maternal; f = foetal). Results are means $\pm$ S.E.M.

	Foetal age (days)	Food intake (kg/day)	Label	SR ^f Foetus A	Plateau specific radioactivities ($\mu\text{Ci}/\text{g}$ of glucose carbon)				Proportion of foetal glucose derived from mother	
					SR ^f Foetus A		SR ^m Foetus B		Foetus A	Foetus B
					SR ^f _m	SR ^m _m	SR ^f _f	SR ^m _f		
Sheep										
H308	126-127	1.4	U- ^{14}C	57.6 ± 5.9	8.2 ± 0.1	26.1 ± 0.7	16.2 ± 1.7	—	0.62	—
			2- ^3H	27.1 ± 2.1	9.0 ± 0.1	26.0 ± 0.3	12.0 ± 0.5	—	0.46	—
H308	133-134	1.4	U- ^{14}C	90.3^*	7.7 ± 0.4	27.0 ± 0.3	14.5^*	—	0.53	—
			2- ^3H	39.7^*	5.3 ± 0.0	29.2 ± 1.1	10.5^*	—	0.36	—
H308	140-141	1.4	U- ^{14}C	138.3 ± 13.9	3.0 ± 0.1	18.6 ± 0.2	11.8 ± 3.0	—	0.64	—
			2- ^3H	34.3 ± 5.2	3.0 ± 0.2	19.3 ± 0.8	7.6 ± 0.5	—	0.39	—
H310	128-129	1.4	U- ^{14}C	52.5 ± 2.9	5.5 ± 0.1	24.8 ± 1.1	18.4 ± 1.2	—	0.74	—
			2- ^3H	33.9 ± 0.8	3.8 ± 0.2	21.0 ± 0.6	13.1 ± 0.4	—	0.62	—
F816	124-125	1.6	U- ^{14}C	71.2 ± 1.8	8.3 ± 0.2	26.4 ± 0.8	17.1 ± 1.7	16.5^*	0.65	0.62
			2- ^3H	60.3 ± 1.8	6.8 ± 0.3	24.4 ± 0.7	10.0 ± 1.5	17.6^*	0.41	0.72
F816	131-132	1.7	U- ^{14}C	52.1 ± 1.6	10.0 ± 0.5	26.2 ± 0.4	21.7 ± 2.9	23.1^*	0.83	0.88
			2- ^3H	43.4 ± 1.6	8.0 ± 0.2	24.0 ± 0.8	12.0 ± 1.6	11.6^*	0.50	0.48
F816	138-139	1.7	U- ^{14}C	74.9 ± 5.2	7.4 ± 0.4	19.9 ± 1.1	16.4 ± 0.6	—	0.82	—
			2- ^3H	54.8 ± 3.7	5.9 ± 0.3	17.8 ± 1.4	11.1 ± 0.9	—	0.62	—
F847	125-126	1.6	U- ^{14}C	47.8^*	7.6 ± 0.4	24.0 ± 0.4	18.6 ± 0.4	18.3^*	0.78	0.76
			2- ^3H	62.4 ± 1.1	4.8 ± 0.2	19.0 ± 0.1	7.1 ± 1.1	9.5^*	0.37	0.50
F847	132-133	1.7	U- ^{14}C	62.7^*	4.5 ± 0.1	18.0 ± 1.8	12.1^*	10.0^*	0.67	0.56
			2- ^3H	28.0^*	3.6 ± 0.1	15.0 ± 1.2	7.5^*	10.2^*	0.50	0.68
F879	124-125	1.6	U- ^{14}C	89.4^*	7.9 ± 0.2	29.4 ± 1.3	17.8^*	—	0.61	—
			2- ^3H	55.0^*	7.4^*	24.9 ± 1.1	11.9^*	—	0.48	—
F879	131-132	1.6	U- ^{14}C	145.0^*	9.3 ± 0.3	25.6 ± 2.2	17.8^*	—	0.70	—
			2- ^3H	101.6^*	8.5 ± 0.1	25.1 ± 2.9	11.5^*	—	0.46	—

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

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

Sheep	Foetal age (days)	Food intake (kg/day)	Ewe live weight (kg)	Estimated weight of foetus (kg)		Metabolic or transfer process (mg of glucose/min)									
						Mother					Foetus				
				A	B	Input		Output		Net foetal uptake	Input		Output		Net utilization
						Gluconeogenesis*	Recycling*				Gluconeogenesis	Recycling			
						F_{mo}	$(F_{mo} - F_{mo})$			$(F_{fm} - F_{mt})$	A	B	A	B	
											F_{fo}	R_{fo}	$(F'_{fo} - F_{fo})$	$(R'_{fo} - R_{fo})$	F_{of}
H308	126-127	1.4	58.5	1.83	2.08	83.6	-30.8			14.6	18.1	20.6	40.6	46.1	32.7
H308	133-134	1.4	60.0	2.30	2.61	84.5	-15.3			7.3	13.4	15.2	29.0	32.9	20.7
H308	140-141	1.4	61.0	2.81	3.19	132.1	-17.8			8.6	6.7	7.6	39.1	44.4	15.3
H310	128-129	1.4	62.0	2.72	2.36	95.3	13.7			26.8	13.3	11.5	16.5	14.4	40.1
F816	124-125	1.6	72.0	1.83	1.91	86.1	1.8			12.7	13.3	14.0	12.3	12.8	26.1
F816	131-132	1.7	74.0	2.31	2.41	87.8	-5.2			25.5	9.8	10.2	21.9	22.9	35.3
F816	138-139	1.7	75.0	2.84	2.97	120.8	7.2			16.5	6.4	6.7	12.0	12.5	22.9
F847	125-126	1.6	65.5	2.45	2.90	97.3	15.6			20.3	10.0	11.8	24.0	28.4	30.3
F847	132-133	1.7	66.5	3.09	3.65	131.4	12.5			17.7	13.7	16.2	33.7	39.8	31.4
F879	124-125	1.6	54.0	1.91	2.22	78.3	5.9			10.0	11.7	13.6	13.7	15.9	21.7
F879	131-132	1.6	56.0	2.42	2.81	93.4	-4.0			6.0	5.5	6.4	8.4	9.7	11.5

* Corrected for the estimated fraction of R_{fo} (and R'_{fo}) of foetus B which enters the maternal pool.
† Corrected for the fraction of F_{om} calculated from the two-pool model estimated to be lost via R_{of} of foetus B.

backflow of glucose from foetus A to the mother (F_{mf}) of 11.3 ± 1.5 mg/min. This indicated that the efficiency of uptake of maternal glucose by foetus A was only 57%. Consequently, the net uptake of maternal glucose by foetus A (15.1 ± 2.1 mg/min) supplied only $56 \pm 4\%$ of the glucose used by foetus A. The discrepancy was met by the input of glucose to the infused foetal pool via F_{fo} , i.e. foetal gluconeogenesis (see Hodgson *et al.*, 1980). The mean rate of gluconeogenesis in foetus A was 4.9 ± 0.7 mg/min per kg of foetus, equivalent to $44 \pm 4\%$ of its rate of glucose utilization. The results from repeat experiments in the same sheep indicated that the estimate of the rate of glucose utilization by the infused foetus expressed per kg of foetal weight decreased with gestational age.

The mean rate of glucose production by the mother (F_{mo}) was 99.1 ± 5.9 mg/min, of which $32 \pm 5\%$ was accounted for by the combined net glucose uptakes of foetuses A and B. The mean rate of glucose utilization by the mother (F_{om}) was 67.8 ± 7.0 mg/min or 1.1 ± 0.1 mg/min per kg of ewe live weight, which was only 0.10 of the rate observed in foetus A (F_{of} , 11.2 ± 1.3 mg/min per kg of foetus). The absolute rate increased in individual sheep with stage of gestation and there were corresponding increases in the rate of maternal glucose production.

The mean ($\pm$ S.E.M.) rates of glucose recycling within mother and infused foetus, when expressed as a fraction of the glucose-utilization rates, were -0.02 ± 0.08 ($n = 11$) and 0.95 ± 0.19 ($n = 11$) respectively. The low negative maternal ratio was due to the high negative rates of glucose recycling calculated for sheep H308, which in turn were due to the [3 H]glucose SR_m^m values being equal to or higher than the [14 C]glucose SR_m^m values, and the apparent high rates of foetal glucose recycling in this sheep. The mean maternal ratio excluding values from sheep H308 was 0.09 ± 0.05 ($n = 8$).

Discussion

The plateau specific radioactivities in plasma glucose were lower in the foetus than in the mother during infusions of radioactively labelled glucose to the mother, thus confirming the observation first made in monotocous ewes (Hodgson *et al.*, 1980) that in sheep only a proportion of foetal glucose is of direct maternal origin. The ratio of the foetal to maternal [14 C]glucose plateau specific radioactivities indicated that on average the proportion in ditocous ewes was only 0.69 and that in terms of a three-pool model representing mother and foetuses an input of non-maternal glucose (F_{fo}) equivalent to 7.1 g/day per kg of foetus was required to meet the deficit. The corresponding input in monotocous ewes was 3.4 g/day per kg of foetus, and since this rate was of sufficient magnitude to largely exclude the

possibility that unlabelled glucose was entering the foetal pool via recycling through foetal glycogen, it was attributed to foetal gluconeogenesis (Hodgson *et al.*, 1980). The significantly higher ($P < 0.01$) value of F_{fo} observed in the twin foetuses verifies this interpretation.

A recent report by Anand *et al.* (1979) of work similar to our own gave results that showed no significant difference between the mean specific radioactivities in foetal and maternal plasma glucose during a maternal infusion of either [U- 14 C]- or [2- 3 H]-glucose, and it was concluded by them 'that virtually all of the foetal glucose was derived from the mother'. However, these authors attempted to isolate foetal plasma glucose as the penta-acetate derivative. Fructose also forms a penta-acetate (see Jones, 1965) and is known to become highly labelled during the administration of labelled glucose to foetal sheep (Setchell *et al.*, 1972; Warnes *et al.*, 1977; Hodgson *et al.*, 1980). Anand *et al.* (1979) will therefore have overestimated the specific radioactivity of foetal plasma glucose by an amount depending on the recovery of fructose during the derivatization process. The degree of overestimation may be assessed from the values presented by Anand *et al.* (1979) for the ratio of foetal to maternal glucose plateau specific radioactivities during a maternal infusion (SR_f^p/SR_m^m). The values ranged from 0.72 to 1.23, although the theoretical limit to the ratio is unity. No standard errors were presented with the results of plateau specific radioactivities but it was apparent from their published figures that specific radioactivities were remarkably stable with time, making it unlikely that the range of values observed was a consequence of experimental variation. It appears, therefore, that the ratios over the theoretical limit reflect the error in the analytical method, leading to overestimation of the SR_f^p/SR_m^m ratio by at least 23% that, when corrected for, resolves the apparent inconsistency between their data and ours.

Model parameters of glucose metabolism in ditocous compared with those in monotocous sheep

Model parameters of glucose metabolism in monotocous ewes and their foetuses for the period 124–134 days of gestation have been published previously (Hodgson *et al.*, 1980), and in this section are compared with model values from the present work restricted to the same gestational age range.

The absolute rate of maternal glucose production was significantly higher ($P < 0.05$) in ditocous than in monotocous ewes (93.1 and 72.5 mg/min respectively), the difference being accounted for almost entirely by the significantly higher ($P < 0.01$) combined glucose uptake of twin compared with single foetuses (32.5 and 14.1 mg/min respectively). However, when expressed on a body-weight basis (Table

Table 4. Rates of maternal glucose production and utilization (mg of glucose/min per kg of live weight) and of net foetal glucose uptake and foetal glucose production and utilization (mg of glucose/min per kg of foetus A) between days 124 and 134 of gestation

Results are means $\pm$ S.E.M. with the significance of the difference, indicated in parentheses, as follows: NS, not significant; **, $P < 0.01$.

Rate	Ditocous	Monotocous
F_{mo}	1.5 ± 0.1	1.3 ± 0.1 (NS)
F_{om}	1.0 ± 0.1	1.1 ± 0.1 (NS)
$F_{fm} - F_{mr}$	6.7 ± 1.0	5.3 ± 0.5 (NS)
F_{fo}	5.5 ± 0.7	2.4 ± 0.4 (**)
F_{of}	12.2 ± 1.3	7.6 ± 0.6 (**)

4) there were no significant differences between the ditocous and monotocous rates of maternal glucose production or utilization, which agrees with other work (Prior & Christenson, 1978). The net uptake of glucose by foetus A, expressed per kg of foetal weight (Table 4), was not significantly different to that in monotocous ewes, but the rate of glucose utilization by foetus A was significantly higher ($P < 0.01$) than the rate in single foetuses. The reason for this is not known. It may reflect a higher metabolic rate in twin foetuses, but O_2 -consumption values (James *et al.*, 1972) do not support this concept. However, an increased anaerobic metabolism of glucose to lactate cannot be excluded. An alternative explanation is that a greater proportion of the substrates used for metabolism by twin foetuses first passes through the glucose pool, and this is supported by the observation that the rate of gluconeogenesis by foetus A is significantly higher ($P < 0.01$) than in single foetuses, the difference contributing 70% of the increase in the rate of utilization. The rate of futile cycling between glucose, glucose 6-phosphate and fructose 6-phosphate (see Hodgson *et al.*, 1980) was similar in twin and single foetuses (9.8 ± 1.8 and 8.4 ± 1.8 mg/min per kg of foetus respectively).

Changes in model fluxes in ditocous ewes and their foetuses with stage of gestation

The assessment of any gestational trends in the kinetics of glucose metabolism was complicated by the failure to maintain the plasma glucose concentrations constant in all cases. Both the maternal rate of glucose irreversible loss (Steel & Leng, 1973a) and the umbilical uptake of glucose (James *et al.*, 1972) are known to be correlated with the maternal plasma glucose concentration. However, in all sheep the rates of maternal glucose production and utilization increased with stage of gestation, which agreed with observations made by Steel & Leng (1973a). On the other hand, the rates of foetal

glucose production and utilization, expressed per kg of foetus, decreased with stage of gestation. The decrease in the foetal rate of glucose utilization was greatest in the sheep, which, because of the decrease in maternal plasma glucose concentration (Table 1), also showed a marked decline in the net foetal uptake of glucose (cf. James *et al.*, 1972). There were no examples of increased rates of foetal gluconeogenesis or of consistent rises in the net foetal uptake of glucose in response to the decline in the foetal glucose utilization rate. Boyd *et al.* (1973) during their measurements of the umbilical uptake of glucose over a foetal age range of 117–145 days observed that 'glucose may be an important exception to the rule that the rate of transplacental exchange keeps pace with the growing metabolic need of the foetus'.

It is of interest to compare these observations with the likely pattern of foetal growth during this period of gestation. In pregnant sheep on a similar nutritional regimen to the animals examined here the rate of foetal growth decreased progressively between about 120 days and term (Mellor & Murray, 1981). The mean growth rate of each foetus was correlated positively with placental weight and it was suggested that there was some form of placental limitation to foetal growth. The present observations, although small in number, suggest that there was a limit to the placental transfer of glucose and that this limit was reached by at least 124 days of gestation (Table 3). The concurrent decrease in the rate of foetal gluconeogenesis presumably resulted from a decreased availability of glucose precursors, and since foetal amino acids are likely to be the major precursors (see Hodgson *et al.*, 1980) this suggests that either amino-acid transfer was also subjected to placental limitation or that the amino acids were used directly as energy substrates. Thus the decline in foetal growth rate may be through a shortage of energy in the form of glucose (which seems to be the energy substrate preferred by foetal tissues; see Boyd *et al.*, 1973) and a diversion of amino acids away from protein synthesis.

The observation that the rates of maternal glucose utilization increased, whereas those of the foetus decreased, agrees with the inverse relationship between these two parameters demonstrated for monotocous sheep (Hodgson *et al.*, 1980), but the results do not support the suggestion made by Steel & Leng (1973a) that the maternal rate of glucose production increased in response to an increased foetal demand for glucose as pregnancy progressed. However, the possibility that the increase in maternal production occurred in response to an increased glucose demand by the placenta cannot be excluded, as the present experimental technique does not differentiate between maternal and placental glucose utilization (see Hodgson *et al.*, 1980).

The rise in the rate of maternal glucose production while food intake remained virtually constant (cf. Steel & Leng, 1973a) may reflect an increase in the supply of glucose precursors through qualitative changes in the products of rumen fermentation (e.g. Kobayashi *et al.*, 1975). However, a more likely explanation is that a greater proportion of potential glucose precursors was diverted towards glucose synthesis. Steel & Leng (1973b) found that a significantly greater proportion of propionate was incorporated into glucose in pregnant than in non-pregnant sheep, although they could not demonstrate that this proportion increased with the stage of pregnancy.

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