Whole-body metabolism of glucose and lactate in productive sheep and cows

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1. Constant infusions of D-[U-^{14}C]glucose, D-[6-^{3}H]glucose and L-[U-^{14}C]lactate were used to determine rates of apparent turnover, *de novo* production, disposal and interconversions of glucose and lactate, together with total recycling of glucose-C, in ewes and dairy cows during late pregnancy and early lactation. The cows were also examined while being fasted. In the fed animals, infusions were made within 5 h after the morning meal when steady-state conditions appeared to exist.

2. In the ewes, circulating concentrations of glucose and lactate, and magnitudes of apparent turnovers of glucose and lactate, tended to be higher during lactation than during pregnancy, while the extent of interconversions of glucose and lactate tended to be lower.

3. Although the metabolic pattern seen in the cows appeared to be similar to that of the ewes during pregnancy, there were clear differences during lactation. Thus, in the lactating cows, as compared with the lactating ewes, circulating concentrations of glucose and lactate were lower, as was apparent lactate turnover related to metabolic body-weight. Furthermore, the percentage of lactate turnover converted to glucose was higher.

4. In the cows, fasting was characterized by low rates of apparent turnover of glucose and lactate and relatively high rates of interconversion of the two compounds.

5. The results indicated that, under the conditions used in this study and when feeding is to recommended levels, carbohydrate metabolism in ewes is more precarious during late pregnancy than during early lactation, while in dairy cows it is more or less equally precarious in both physiological states.

6. A further conclusion is that the extent of glucose–lactate interconversions, and thus Cori cycle activity, seems to be lower in ruminants than in other species.

The major portion of glucose available to the ruminant has to be supplied by gluconeogenesis, since little glucose is normally absorbed from the gut (Lindsay, 1970; Bergman *et al.* 1974). The provision of glucose is therefore an energetically expensive process and the ruminant animal may be expected to have evolved a variety of mechanisms for conserving glucose-carbon. These mechanisms must play a particularly important role in situations where glucose demand is high. Two such situations are late pregnancy and early lactation (Linzell, 1974; Battaglia & Meschia, 1981) and it is, in fact, during these periods that ruminants are highly susceptible to hypoglycaemia and ketosis. Mechanisms for conserving glucose are also likely to be prominent during fasting, when gluconeogenic precursors have to be provided entirely from endogenous sources.

One way in which glucose can be conserved is by operation of the Cori and alanine cycles, i.e. by recycling of glucose-C as lactate, pyruvate and alanine, which are subsequently reconverted to glucose by gluconeogenesis (Cori & Cori, 1929; Brockman & Bergman, 1975; Bergman & Heitmann, 1978; Foster *et al.* 1980). Experiments conducted with appropriately catheterized dairy cows, in which net exchanges of glucose and lactate were measured across gut and liver, suggested that Cori cycle activity might be greater in lactating or in fasted animals (Baird *et al.* 1980). By contrast, determination of total glucose recycling by

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comparison of glucose turnover rates obtained with [U-^{14}C]- and [6-^{3}H]glucose indicated that activities of both the Cori and alanine cycles were uniformly low in either fed or fasted sheep (Bergman *et al.* 1974; Brockman *et al.* 1975).

Because of these possible differences between the two ruminant species, it was decided to investigate the extent of glucose-C recycling in ewes and dairy cows during late pregnancy, early lactation and fasting. The need for comprehensive data in this area has recently been emphasized by the finding of Wilson *et al.* (1981), using [U-^{14}C]- and [6-^{3}H]glucose, that Cori cycle activity can be extensive in ewes during late pregnancy and early lactation. This finding would appear to be in conflict with the earlier observations of Bergman *et al.* (1974) and Brockman *et al.* (1975) outlined above.

In the present study, one experimental approach was to compare the whole-body metabolism of [U-^{14}C]- and [6-^{3}H]glucose (Dunn *et al.* 1967; Brockman *et al.* 1975). The other approach was similar to that first used for androgenic hormones (van de Wiele *et al.* 1963) and volatile fatty acids (Bergman *et al.* 1965) and later used for glucose itself (Depocas & de Freitas, 1970; Reilly & Chandrasena, 1978). In this latter approach, each experiment consists of infusions on separate days of [U-^{14}C]glucose and [U-^{14}C]lactate, with determination of the specific activities of circulating glucose and lactate during each of the infusions. The specific activities are then fitted into a two-pool model of whole-body glucose and lactate metabolism that also yields values for rates of interconversions of glucose and lactate.

METHODS

Animals and diet

Four mature crossbred ewes (A, B, C and D) were used (Table 1) weighing 61, 51, 52 and 70 kg respectively at midpregnancy. Two mature Holstein cows (E and F), each pregnant with one calf, were also used (Table 2) and weighed 712 and 595 kg respectively.

All ewes were fed on a diet of lucerne (*Medicago sativa*) hay and closed-formula lamb pellets (Agway, Syracuse, NY), while cows were fed on a diet of lucerne hay and mixed-grain concentrate (Agway). The lamb pellets contained approximately 500 g ground hay/kg and 500 g mixed cereal grains/kg, and consisted of (g/kg fresh weight) crude protein (nitrogen $\times 6{\cdot}25$) 140, crude fat 20, crude fibre 180. The corresponding values for the mixed-grain concentrate for cows were 160, 33 and 80. Both the lamb pellets and mixed-grain concentrate contained added trace minerals. In determining feed requirements (Ministry of Agriculture, Fisheries and Food, 1975) the dry weights (g/kg) and metabolizable energy contents (MJ/kg dry matter) respectively of the feed were calculated as follows: hay 850 and 8, lamb pellets 850 and 10, concentrate 900 and 12·5. One-third of the energy intake was always in the form of hay. Total metabolizable energy intakes were approximately 0·55, 1·1 and 1·4 MJ/kg$^{0{\cdot}75}$ per d for the ewes in late pregnancy, lactating with one lamb and lactating with twin lambs respectively. Corresponding values for the cows were 0·7 and 1·4 MJ/kg$^{0{\cdot}75}$ per d during late pregnancy and early lactation respectively. All animals were fed twice daily, except when being fasted (see p. 252), at 08.00 and 15.00 hours. Water and mineral-licks were available at all times. When lactating, the cows were milked immediately after the feed was presented.

Calves were removed from the cows 3 d after birth, but lambs were allowed to suckle the ewes until the end of all the experiments. All animals bore healthy offspring, except that one lamb born to ewe A was suffocated at birth (Table 1).

Surgical preparation

Polyvinyl catheters were implanted in the caudal vena cava and aorta, the portal vein, a mesenteric vein and an hepatic vein in each pregnant ewe using techniques reported previously (Katz & Bergman, 1969). The surgery was carried out under general anaesthesia,

Table 1. *Schedule of experiments on the ewes*

Expt no.	Ewe	Status	Day of ^{14}C infusion†	
			Glucose	Lactate
1	A	Pregnant, two fetuses	−35	−33
2	A	Pregnant, two fetuses	−20	−14
3	B	Pregnant, one fetus	−13	−10
4	D, B	Pregnant, two fetuses (D), one fetus (B)	−12 (D)	−4 (B)
5	C	Pregnant, two fetuses	−4	−2
6	C	Lactating, two lambs	+10	+15
7	A	Lactating, one lamb*	+10	+15
8	B	Lactating, one lamb	+15	+18

* One lamb suffocated at birth.
† No. of days before (−) or after (+) parturition.

induced and maintained with sodium pentobarbital, and took place between 19 and 49 d prepartum. The ewes were not used for an experiment until at least 2 weeks after the operation. At least 24 h before each experiment a further catheter, for infusion purposes, was inserted approximately 100 mm into a jugular vein. The ends of all catheters were secured to the wool, filled with heparinized physiological saline (9 g sodium chloride/l; 100 units heparin/ml) and flushed twice weekly.

In the cows, a polyvinyl infusion catheter was inserted approximately 150 mm into a jugular vein. Another catheter was then inserted about 800 mm into the other jugular vein to reach the right atrium, as ascertained by pressure measurements. This catheter was later used for withdrawal of samples of mixed blood, which was thus similar, in regard to dilution of infusate, to the arterial blood sampled in the ewes. The ends of the catheters were then glued to the animal, filled with heparinized saline and flushed every 2 d.

Experimental procedures

Each experiment consisted of two primed continuous infusions into the same animal, with each infusion taking place on a separate day (Tables 1 and 2). Different ewes were employed in Expt 4 (Table 1). The first infusion was either a mixture of D-(U-^{14}C]glucose and D-[6-^{3}H]glucose, or D-[U-^{14}C)glucose alone. The second infusion was L-[U-^{14}C]lactate. All labelled compounds were obtained from New England Nuclear Corp., Boston, MA. In each case the isotope was dissolved in sterile saline and milligram quantities of carrier were added. The rate of infusion was always 46·3 ml/h and the priming dose volume was 60 ml for ewes and 80 ml for cows.

In the ewes, infusions were given via the jugular catheter after the morning feed and lasted for 4·5 h, from approximately 08.30 to 13.00 hours. The rates of infusion were approximately 15, 21 and 9 μCi/h for D-[U-^{14}C]glucose, D-[6-^{3}H]glucose and L-[U-^{14}C]lactate respectively. Four blood samples, each of 21 ml, were withdrawn from the arterial catheter at 30 min intervals for the last 1·5 h of each infusion, and used for determination of the concentrations and specific activities of glucose and lactate. The concentrations of D-3-hydroxybutyrate, pyruvate and alanine were also measured in all or most of the blood samples. Specific activities of pyruvate were determined in a number of experiments. Emphasis was placed on slow withdrawal of blood (2–3 min/sample). Samples were also withdrawn simultaneously from the portal vein, hepatic vein and vena-caval catheters. Values obtained from these samples are discussed in the following paper (van der Walt *et al.* 1983).

Table 2. *Schedule of experiments on the cows*

Expt no.	Cow	Status	Day of ^{14}C infusion*		Daily milk yield (kg)
			Glucose	Lactate	
1	E	Pregnant	−30	−26	—
2	E	Pregnant	−23	−19	—
3	F	Pregnant	−10	−7	—
4	E	Pregnant	−6	−3	—
5	E	Lactating	+9	+12	24·5
6	F	Lactating	+11	+16	21·8
7	F	Lactating	+29	+31	22·5
8	E	Lactating	+30	+33	21·0
9	E	Lactating	+39	+44	22·8
10	F	Lactating, fasting†	+43	+45	1·3
11	E	Lactating, fasting†	+52	+54	5·5

* No. of days before (−) or after (+) parturition.
† Infusions into fasted cows took place after 4 and 6 d of fasting.
The milk yields are in each case means of those recorded on the two infusion days.

In the cows too, infusions were begun after the morning feed, except when fasting (see below). The infusions were given via the short jugular catheter and in this case lasted for 5 h, from about 08.30 to 13.30 hours. The rates of infusion were approximately 60, 300 and 30 μCi/h for D-[U-^{14}C]glucose, D-[6-^{3}H]glucose and L-[U-^{14}C]lactate respectively. Five blood samples (60 ml each) were withdrawn via the right-atrial catheter at 30 min intervals for the last 2 h of infusion and, as before, used for the determination of the concentrations and specific activities of blood metabolites. Alanine concentration was not measured, however. As indicated in Table 2, the two cows were also infused while being fasted during lactation. While fasting, the cows received no food but had access to water. They were milked as usual. At the time of the [^{14}C]glucose infusion each cow had received its last feed 96 h previously, and at the time of the [^{14}C]lactate infusion, 144 h previously. Blood acetoacetate concentration was measured after 144 h of fasting.

In both the ewes and cows a sample of blood was taken immediately before starting each isotope infusion for measurement of any residual radioactivity from a previous infusion.

Chemical methods

Glucose concentrations in whole blood were determined colorimetrically with glucose oxidase (*EC* 1.1.3.4; Sigma, St Louis, MO). Glucose specific activities were determined by the method of Jones (1965), which involved preparation of the pentaacetate derivative and counting in scintillation fluid.

Whole-blood samples for determination of concentrations of lactate and other metabolites, and of specific activities of lactate and pyruvate, were collected in ice-cold 0·6 M-perchloric acid. Following neutralization of the extracts with potassium hydroxide, metabolite concentrations were measured fluorimetrically: lactate and pyruvate with lactate dehydrogenase (*EC* 1.1.1.27), alanine with alanine dehydrogenase (*EC* 1.4.1.1), and D-3-hydroxybutyrate and acetoacetate with D-3-hydroxybutyrate dehydrogenase (*EC* 1.1.1.30) (see Bergmeyer, 1974). Enzymes used in all assays were obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN.

Specific activities of lactate and pyruvate were determined by the method of Reilly (1975).

This method involves the conversion of the lactate to pyruvate, which is then counted as the phenylhydrazone derivative. Counting took place in scintillation fluid consisting of toluene–Triton X-100 (2:1, v/v), 0·5 g dimethyl-POPOP/l and 5 g PPO/l.

Calculations and theoretical aspects

The apparent turnover of a metabolite (mg atoms C/h) was calculated from the equation:

$$\text{apparent turnover} = I/SA \quad (1)$$

where I is the rate of infusion of isotope (μCi/h) into a peripheral vein and SA is the equilibrium specific activity (μCi/mg atom C) of the metabolite after being mixed in the blood (either arterial (A) in ewes or mixed venous (V) in cows) and before entering peripheral tissues, i.e. the V–A mode of Katz (1982). As explained by Katz (1982), however, the value obtained for apparent turnover will differ if blood is sampled before, rather than after, being mixed with the infused metabolite; for example, if infusion takes place into the aorta and sampling is from the right heart chamber (A–V mode). In the case of glucose, little or no difference in results is obtained between these two modes. With rapidly-turning-over metabolites such as lactate, however, a difference of 30% or more has been observed in starved rats (Katz, 1982). In the sheep of the present study, with a blood lactate concentration of approximately 0·5 mM (Table 5), a cardiac output of 7–10 l/min (Arnold, 1979) and a [^{14}C]lactate infusion rate of 0·15 μCi/min (see p. 251), the sampled lactate radioactivity would be increased by 10–15%. Lactate turnover would thus be decreased by this amount. Therefore, if only turnover rates are being measured, the A–V mode may be preferred. For interconversions between metabolites, however, the V–A mode is preferable, since information is needed on the specific activity of precursor before entering the peripheral tissues and on that of the product after leaving them. This information is provided by the V–A mode of infusion and sampling.

It also must be emphasized that previously mentioned calculations do not take into account that quantity of metabolite which is absorbed from the gut and then immediately taken up by the liver. Again, taking this quantity into account will have only little effect in the case of glucose in ruminants, but will increase lactate turnover by 6–10% (Bergman *et al.* 1974; Bergman, 1975; van der Walt *et al.* 1983).

In calculating rates of turnover of glucose and lactate, and transfer of C between these two compounds in the whole body, we nevertheless made the assumption that there are single pools of glucose and lactate. Our major objective was to compare ewes and cows during both pregnancy and lactation. The theoretical error for our measurements of glucose turnover is, of course, negligible, but that for lactate turnover could be 15–25%. Additionally, the actual transfer rate of C from lactate to glucose would tend to be underestimated because of the potential crossover of ^{14}C and ^{12}C atoms in the liver at the level of oxaloacetate (Wolff & Bergman, 1972).

The two-pool model is illustrated in Figs. 1 and 2 (p. 258) and its solution, as described earlier (Bergman *et al.* 1965; Depocas & de Freitas, 1970), was obtained by the use of the following simultaneous equations:

$$R_1 + R_4 = R_3 + R_5 \quad (2)$$

$$R_2 + R_3 = R_4 + R_6 \quad (3)$$

$$F_g + A_g^l R_4 = A_g^g (R_3 + R_5) \quad (4)$$

$$F_l + A_l^g R_3 = A_l^l (R_4 + R_6) \quad (5)$$

$$A_l^l R_4 = A_l^g (R_3 + R_5) \quad (6)$$

$$A_g^g R_3 = A_g^l (R_4 + R_6) \quad (7)$$

where R_1, R_2, R_3, etc., are the fluxes of metabolite C (mg atoms C/h), as shown in Fig. 1; F_g and F_l are the rates of infusion (μCi/h) of [^{14}C]glucose and [^{14}C]lactate respectively; and A_g^g and A_l^l are the specific activities (μCi/mg atom C) of glucose when [^{14}C]glucose or [^{14}C]lactate is infused respectively, etc.

Statistics

Comparison of values obtained during pregnancy and lactation. Mean values obtained for ewes A, B and C during pregnancy were compared with the corresponding mean values obtained during lactation using a paired t test with 2 degrees of freedom. Similarly, the pregnant and lactating values for cows E and F were compared with 1 df. In view of the small numbers of degrees of freedom available, probability values have been given up to the 10%, rather than the more usual 5%, level.

Analysis of change in values of measurements with time during sampling. Regression analyses were carried out for each group of sampling values, obtained during pregnancy, lactation or fasting, to calculate the average percentage change with time over the 1·5–2 h sampling period. The statistical significance of the change was determined by Student's t test.

RESULTS

Validation of the experimental approach

The regression analysis results given in Tables 3 and 4 indicate that the majority of the average changes in the magnitude of the various criteria over the sampling periods were less than 10% in both the ewes and the cows, and that in general there were no consistent trends with time. It therefore seemed justified to apply steady-state equations to the sampling means for each criterion. Nevertheless, it must be emphasized that in the fed animals the sampling period chosen was probably one of maximum absorption. It follows that the kinetic data calculated from these criteria means cannot be taken to apply throughout the whole 24-h period in animals that are fed twice daily.

Metabolite concentrations

In the ewes, the blood concentrations of glucose and alanine were significantly higher during lactation than during pregnancy. Furthermore, the mean concentration of hydroxybutyrate was only half that during pregnancy. Significance was not obtained for this latter difference, however (Table 5). In the cows, in contrast to the ewes, the concentrations of glucose, lactate and pyruvate in the blood were all somewhat lower during lactation than during pregnancy, while that of hydroxybutyrate seemed higher (Table 5). Additionally, the blood concentration of glucose was lower during fasting than during pregnancy, while that of hydroxybutyrate was higher.

Because the concentration of pyruvate tended to alter in parallel with changes in lactate concentration in both species, lactate: pyruvate varied only within narrow limits, i.e. between 20 and 22 in ewes, and 12 and 14 in cows.

Specific activities

Table 6 lists values for specific activities of glucose and lactate measured in the same blood samples as those used for measurement of metabolite concentrations. As previously indicated, all the values were obtained over the last 1·5–2 h of infusion, when plateau, and thus presumed steady-state, conditions existed, and were those used for subsequent calculations of flux and interconversions. Specific activities of pyruvate were also measured in a number of experiments and were consistently found to be similar to those of lactate. It could be concluded, therefore, that pyruvate-C was usually in equilibrium with lactate-C. For this reason, and because pyruvate was quantitatively insignificant as a metabolite as

Table 3. *Regression analysis of change in the values of glucose and lactate specific activities and concentrations with time during sampling in the ewes*

Infusate	Measurement	Units	Physiological status	Mean	Standard error of mean	Slope of regression line (change/0·5 h)	Change during sampling period (%)	Statistical significance (*P*)
[^{14}C]glucose	Glucose s.a.	nCi/mg atom C	P	49·2	3·0	0·6	3·5	NS
			L	32·9	2·1	0·7	5·3	< 0·05
	Lactate s.a.	nCi/mg atom C	P	38·1	1·8	−0·1	−0·2	NS
			L	20·0	1·6	0·2	2·9	NS
	Glucose concn.	mM	P	2·60	0·08	0·03	3·5	< 0·05
			L	3·81	0·12	0·09	7·3	< 0·01
	Lactate concn.	mM	P	0·48	0·02	−0·02	−9·6	NS
			L	0·63	0·03	−0·01	−4·8	NS
[^{14}C]lactate	Glucose s.a.	nCi/mg atom C	P	8·8	0·5	0	0	NS
			L	3·3	0·3	0	−0·5	NS
	Lactate s.a.	nCi/mg atom C	P	83·5	4·8	−6·4	−20·6	< 0·05
			L	65·2	1·7	−0·1	−0·5	NS
	Glucose concn.	mM	P	2·85	0·12	0·02	2·0	NS
			L	3·79	0·04	0·05	3·7	NS
	Lactate concn.	mM	P	0·57	0·03	−0·01	−2·8	NS
			L	0·65	0·03	−0·01	−3·3	NS
[^{3}H] glucose	Glucose s.a.	nCi/mg atom C	P	64·2	4·1	1·0	4·9	NS
			L	44·8	3·1	1·2	8·3	< 0·05

Abbreviations: s.a., specific activity; concn., concentration; P, pregnancy; L, lactation; NS, non-significant.
For description of statistical analysis, see p. 254. Statistical significance taken to be achieved at $P < 0·05$.

Table 4. *Regression analysis of change in the values of glucose and lactate specific activities and concentrations with time during sampling in the cows*

Infusate	Measurement	Units	Physiological status	Mean	Standard error of mean	Slope of regression line (change/0·5 h)	Change during sampling period (%)	Statistical significance (*P*)
[^{14}C]glucose	Glucose s.a.	nCi/mg atom C	P	31·4	0·7	0	−0·6	NS
			L	20·9	0·5	0·2	4·4	NS
			F	63·5	2·8	1·5	9·9	<0.05
	Lactate s.a.	nCi/mg atom C	P	16·2	1·0	0·9	25·0	NS
			L	10·0	0·5	0·3	13·6	NS
			F	36·0	3·7	−1·4	−14·7	NS
	Glucose concn.	mM	P	2·84	0·04	0·03	3·8	NS
			L	2·58	0·07	0	0·5	NS
			F	1·91	0·03	0·01	1·7	NS
	Lactate concn.	mM	P	0·35	0·01	−0·01	−7·4	NS
			L	0·26	0·01	−0·01	−15·8	< 0·05
			F	0·51	0·10	0·02	14·7	NS
[^{14}C]lactate	Glucose s.a.	nCi/mg atom C	P	4·1	0·3	0·1	9·4	< 0·05
			L	3·3	0·1	0·1	7·0	< 0·05
			F	10·5	0·4	0·4	17·7	< 0·01
	Lactate s.a.	nCi/mg atom C	P	32·4	1·4	0·5	8·2	NS
			L	52·7	2·4	0·5	4·0	NS
			F	79·5	5·2	2·6	14·4	NS
	Glucose concn.	mM	P	2·81	0·01	0·02	1·7	NS
			L	2·71	0·06	−0·02	−3·3	NS
			F	2·22	0·09	0	0·7	NS
	Lactate concn.	mM	P	0·42	0·01	−0·01	−8·4	< 0·05
			L	0·23	0·01	−0·01	−9·7	< 0·05
			F	0·42	0·05	−0·01	−11·3	NS
[^{3}H]glucose	Glucose s.a.	nCi/mg atom C	P	153·5	5·7	0·4	1·0	NS
			L	95·3	1·2	0·9	4·0	NS
			F	267·3	5·3	3·5	5·4	NS

Abbreviations: s.a., specific activity; concn., concentration; P, pregnancy; L, lactation; F, fasting; NS, not significant.
For description of statistical analysis, see p. 254. Statistical significance taken to be achieved at $P < 0·05$.

Table 5. *Concentrations of metabolites in the blood of the ewes and cows*

(Mean values with their standard errors obtained during the number of separate infusions indicated. Extra values included in the pregnant and lactating groups of ewes were obtained in a parallel study using the same animals (J. G. van der Walt, E. N. Bergman and G. D. Baird, unpublished results))

		Whole-blood concentration (mM)														
		Glucose			Lactate			Pyruvate			Alanine			Hydroxybutyrate			Acetoacetate
Species	Status	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean
Ewe	Pregnant	2·67	0·14	11	0·50	0·04	11	0·025	0·003	8	0·059	0·003	5	1·18	0·25	11	—
	Lactating	3·64**	0·15	7	0·58	0·04	7	0·027	0·002	6	0·126*	0·012	4	0·65	0·06	7	—
Cow	Pregnant	2·81	0·11	8	0·38	0·02	8	0·031	0·003	6	—			0·57	0·09	8	—
	Lactating	2·63	0·10	10	0·26†	0·02	10	0·020	0·002	6	—			0·85	0·11	10	—
	Fasting	2·05†	0·15	4	0·47	0·14	4	0·032‡			—			2·88	0·78	4	0·36‡
								0·033									0·81

† $P < 0·10$, * $P < 0·05$ and ** $P < 0·01$ as compared with the corresponding pregnant group.
‡ Individual values.

Table 6. *Specific activities of metabolites in the blood of the ewes and cows*

(Mean values with their standard errors obtained during the number of separate infusions indicated. In the ewes, all values are adjusted for infusion rates of 15, 9 and 21 μCi/h for [^{14}C]glucose, [^{14}C]lactate and [^{3}H]glucose respectively. In the cows, all values are adjusted for infusion rates of 60, 30 and 300 μCi/h for [^{14}C]glucose, [^{14}C]lactate and [^{3}H]glucose respectively)

		Specific activity (nCi/mg atom carbon)														
Compound infused...		D-[^{14}C]glucose						L-[^{14}C]lactate						D-[6-^{3}H]glucose		
		Glucose			Lactate			Glucose			Lactate			Glucose		
Species	Status	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Ewe	Pregnant	49·4	6·4	5	38·2	3·2	5	8·7	0·9	5	82·5	7·6	5	65·4	8·7	5
	Lactating	32·9†	4·8	3	20·1*	3·4	3	3·3*	0·6	3	65·2	1·4	3	45·3†	6·5	3
Cow	Pregnant	31·0	1·6	4	17·7	2·1	4	4·0	0·8	4	34·6	1·7	4	144·0	8·4	4
	Lactating	20·9	1·1	5	10·2	0·9	5	3·3	0·3	5	51·3	4·4	5	94·9	2·7	3
	Fasting‡	55·3			20·2			9·5			66·6			—		
		71·4			42·2			11·3			88·2			—		

† $P < 0·10$ and * $P < 0·05$ as compared with the corresponding pregnant group.
‡ Individual values.

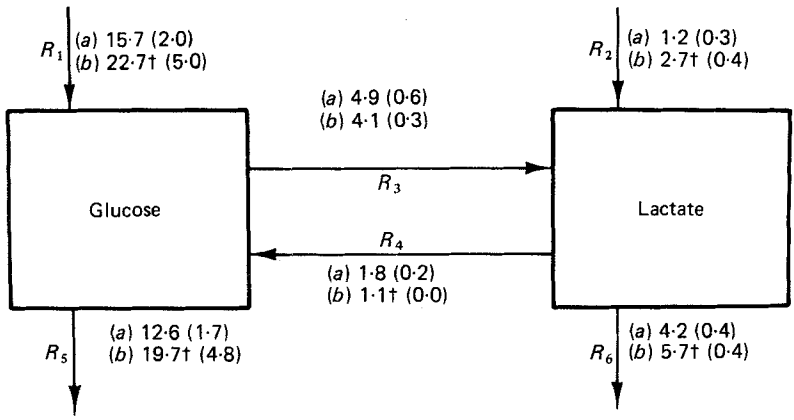

Fig. 1. Two-pool model of rates of carbon transfer in sheep. R_1–R_6 are fluxes of metabolite C (mg atoms C transferred/h per kg body-weight$^{0.75}$); mean values, with their standard errors in parentheses, for (*a*) pregnant and (*b*) lactating ewes. †$P < 0.10$, as compared with corresponding pregnant group.

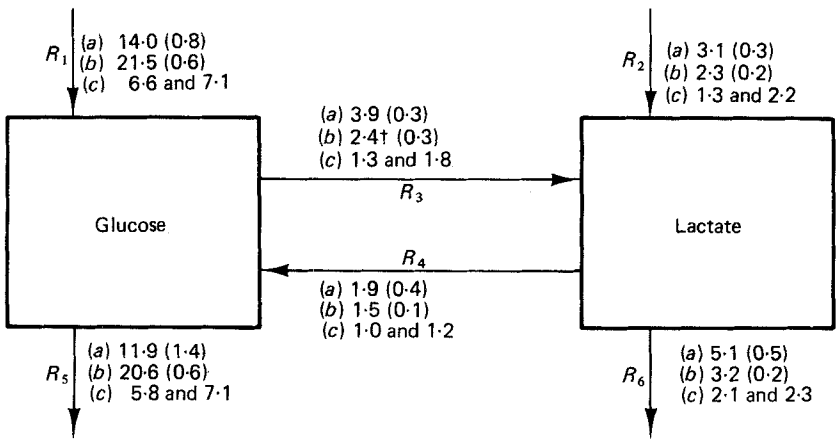

Fig. 2. Two-pool model of rates of carbon transfer in cows. R_1–R_6 are fluxes of metabolite C (mg atoms C transferred/h per kg body-weight$^{0.75}$); mean values, with their standard errors in parentheses, for (*a*) pregnant and (*b*) lactating cows. †$P < 0.10$, as compared with corresponding pregnant group. Individual values are given for fasting cows (*c*).

compared with lactate, values relating to pyruvate metabolism that were derived from the isotope infusions have been omitted.

Rates of flux of glucose and lactate-C

These rates across and between the putative pools of glucose and lactate were calculated from the values summarized in Table 6. Values for ewes are given in Fig. 1 and those for cows in Fig. 2. In the ewes, rates of C entry into and departure from both the glucose and lactate pools, i.e. R_1, R_5, R_2 and R_6 respectively, tended to be higher during lactation than during pregnancy. While R_4, the rate of transfer of lactate-C to the glucose pool, appeared to be somewhat lower during lactation than during pregnancy, there was little difference in the values of R_3, the rate of transfer of glucose-C to lactate, in the two physiological states.

In the cows, values of R_2 and R_6 were lower, rather than higher, in the lactating group than in the pregnant group (Fig. 2). Other changes that occurred in rate values with the transition from pregnancy to lactation were in the same direction as in the ewes. One difference was, however, that the decrease in R_4 was less in cows than it was in ewes, while the decrease in R_3 was greater. In the cows, values for all rates were lower during fasting than at other times.

Values for rates of total flux, i.e. (R_1+R_4) and (R_2+R_3), and for percentage interconversions of glucose and lactate, were calculated from the values summarized in Figs. 1 and 2. These derived values are presented in Table 7. In both species, the total flux of glucose was higher during lactation than during pregnancy. In the cows, but not in the ewes, the total flux of lactate was lower during lactation than during pregnancy. Also in the cows, total fluxes of lactate and glucose were lower during fasting than during either lactation or pregnancy.

In the ewes, the percentage of total glucose flux converted to or derived from lactate, and of lactate flux converted to or derived from glucose, all appeared to be lower during lactation than during pregnancy (Table 7). Clearly, therefore, interconversions of glucose and lactate were substantially lower during lactation in this species. The situation in the cows was similar as far as the percentages of glucose flux converted to or derived from lactate were concerned, since both values appeared lower during lactation. In contrast to the ewes, however, values for the percentages of lactate flux converted to or derived from glucose were of a similar order during lactation to those observed during pregnancy. Finally, in the cows, the values for the percentage interconversions tended to be rather high during fasting.

Whole-body turnover rates of metabolites

These values are given in Table 8. As expected, the whole-body apparent turnover rates of both glucose and lactate varied with physiological status in the same manner as did the total fluxes presented in Table 7. However, when expressed in the same units, i.e. mg atoms C/h per kg body-weight$^{0.75}$, the values in Table 8 are between 3 and 10% less than the corresponding values for the total flux in Table 7. This is due to the fact that the apparent turnover rates for glucose and lactate (F_g/A_g^g and F_l/A_l^l respectively, see p. 254) are equal to R_1+R_4 (R_2/R_2+R_3) and R_2+R_3 (R_1/R_1+R_4) respectively (see also Depocas & de Freitas, 1970).

Recycling of C through glucose and lactate

Table 9 compares the total percentage recycling of glucose-C with values for percentage contribution of lactate to glucose flux. Values for total recycled glucose-C were calculated from a comparison of apparent glucose turnover rates obtained with [6-^{3}H]glucose and [U-^{14}C]glucose in those experiments where both isotopes were infused (Bergman *et al.* 1974; Brockman *et al.* 1975). Values for the contribution of lactate to glucose were derived from the appropriate R values for the same sets of experiments. Surprisingly, in both pregnant and lactating ewes and cows values for the contribution of lactate, although small, were still somewhat greater than those values calculated for total glucose recycling. More important, however, is the fact that both the percentage of total glucose recycling and percentage contribution of lactate to glucose flux were lower during lactation than during pregnancy.

Finally, Table 10 shows there was evidence for a gradual and systematic increase in total flux of glucose during the last month of pregnancy in the cows, that was accompanied by a parallel increase in the quantity of lactate-C converted to glucose. The increase in glucose flux amounted to approximately 40%, while the increase in lactate conversion to glucose was about 190%. This increase in conversion of lactate to glucose occurred even though total lactate flux declined slightly. In the ewes, however, little evidence was obtained for

Table 7. *Values, derived from rates of flux of metabolite carbon* R_1–R_6, *for total flux and percentage interconversions of glucose and lactate in the ewes and cows*

(Mean values with their standard errors of the no. of experiments indicated)

Species	Status	No. of experiments	Total flux (mg atoms C/h per kg body-wt$^{0.75}$)				Percentage interconversion							
							Glucose				Lactate			
			Glucose (R_1+R_4)		Lactate (R_2+R_3)		To lactate $\left(\frac{R_3}{R_3+R_5}\times 100\right)$		From lactate $\left(\frac{R_4}{R_1+R_4}\times 100\right)$		To glucose $\left(\frac{R_4}{R_4+R_6}\times 100\right)$		From glucose $\left(\frac{R_3}{R_2+R_3}\times 100\right)$	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ewe	Pregnant	5	17·5	2·2	6·1	0·5	28	2	11	1	30	1	80	6
	Lactating	3	23·8†	5·0	6·8	0·4	18*	3	5**	1	16*	1	61†	5
Cow	Pregnant	4	15·8	1·2	7·0	0·1	25	3	11	2	27	7	56	5
	Lactating	5	23·0	0·6	4·7†	0·2	10	1	7	0	32	2	50	5
	Fasting‡	2	7·6		3·1		15		13		32		36	
			8·3		3·5		24		14		34		59	

R_1–R_6, for details see Figs. 1 and 2 and p. 254.

† $P < 0{\cdot}10$, * $P < 0{\cdot}05$ and ** $P < 0{\cdot}01$ as compared with the corresponding pregnant group.

‡ Individual values.

Table 8. *Apparent whole-body turnover rates of glucose and lactate in the ewes and cows*

(Mean values with their standard errors of the number of observations indicated. Values were obtained by dividing the rate of ^{14}C infusions by the specific activities in whole blood (see Table 6))

		Apparent turnover rate											
		Glucose						Lactate					
		mmol/min			mg atoms carbon/h per kg body-$wt^{0.75}$			mmol/min			mg atoms carbon/h per kg body-$wt^{0.75}$		
Species	Status	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Ewe	Pregnant	0·90	0·11	5	16·0	2·0	5	0·63	0·06	5	5·5	0·4	5
	Lactating	1·33†	0·23	3	23·1†	5·0	3	0·77	0·02	3	6·6	0·4	3
Cow	Pregnant	5·43	0·30	4	14·8	1·04	4	4·85	0·24	4	6·6	0·2	4
	Lactating	8·07	0·39	5	22·2	0·62	5	3·33**	0·24	5	4·6*	0·2	5
	Fasting‡	2·3		2	7·0		2	1·9		2	2·8		2
		3·0			7·9			2·5			3·3		

†$P < 0·10$, * $P < 0·05$ and ** $P < 0·01$ as compared with corresponding pregnant group.
‡ Individual values.

Table 9. *Recycling of glucose-carbon and the contribution of lactate to glucose in the ewes and cows*

(Mean values with their standard errors of the number of observations indicated. The values for the sheep are summarized from van der Walt *et al.* 1983)

			Apparent turnover rate (mmol/min)				Total recycled glucose-C (%)		Contribution of lactate-C to glucose flux (%)	
			With [^3H]glucose		With [^{14}C]glucose		$\left(\frac{^3H - ^{14}C}{^3H} \times 100\right)$		$\left(\frac{R_4}{R_1 + R_4} \times 100\right)$	
Species	Status	No. of observations	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ewe	Pregnant	5	0·96	0·12	0·90	0·11	5·5	0·8	10·6	0·5
	Lactating	3	1·36†	0·23	1·33†	0·23	1·8	0·3	5·0**	0·9
Cow	Pregnant	4	5·85	0·33	5·43	0·30	7·0	2·4	11·4	1·9
	Lactating	3	8·79	0·24	8·62	0·21	1·9	0·9	6·5	0·7

R_1 and R_4, for details see Figs. 1 and 2 and p. 254.
† $P < 0·10$ and ** $P < 0·01$ as compared with the corresponding pregnant. group.

Table 10. *Increase in conversion of lactate to glucose with time during pregnancy in the cows*

Mean no. of d prepartum	Cow	Total flux*		Lactate to glucose* (R_4)
		Glucose (R_1+R_4)	Lactate (R_2+R_3)	
−28	E	13·1	7·3	0·89
−21	E	14·2	7·0	1·40
−9	F	18·1	6·9	2·57
−5	E	17·8	6·8	2·60

R_1–R_4, for details see Figs. 1 and 2 and p. 254.
* mg atoms carbon/h per kg body-$wt^{0.75}$.

any time-related systematic change in magnitude of lactate conversion to glucose either during pregnancy or during lactation. A major reason for lack of such evidence was the fact that the ewes bore varying numbers of fetuses, and this would tend to mask time-related changes in metabolism.

DISCUSSION

Metabolism in the ewes

Comparison of the blood metabolite concentrations indicates that the ewes exhibited a lower level of carbohydrate sufficiency during pregnancy than during lactation (see, for example, Baird, 1981). The apparent turnover rates of glucose obtained for the pregnant and lactating ewes in the present study compare well with corresponding values obtained previously in this laboratory, i.e. 0·9 and 1·3 mmol/min respectively (Table 8) *v.* 0·7 and 1·2 mmol/min as summarized for earlier work (Bergman *et al.* 1974). Turnover rates of lactate have not been measured previously in pregnant or lactating sheep, although values of 0·4 and 0·8 mmol/min were obtained by Reilly & Chandrasena (1978) and Annison *et al.* (1963) respectively, in non-pregnant, non-lactating animals. In line with the present results in the cows, Annison *et al.* (1963) found that the turnover of lactate declined during fasting.

Comparisons of glucose turnover rates measured with [6-^{3}H]glucose and [U-^{14}C]glucose is often considered to be the method of choice for determining the total contribution of the Cori, alanine and other C cycles to glucose production (Dunn *et al.* 1967; Brockman *et al.* 1975). This is because the ^{3}H should, according to theory, be lost when the recycled compound reaches the gluconeogenic pathway in the liver at the level of oxaloacetate. The low level of total recycling (2–6%) observed in the present study on ewes is consistent with previous observations (5%) made on fed and fasted sheep in this laboratory (Bergman *et al.* 1974; Brockman *et al.* 1975). It does, however, appear to conflict with the higher level of recycling found by Wilson *et al.* (1981) in their study of ewes in late pregnancy and early lactation. There are two possible reasons for this, i.e. the nature of the feeding regimen and the nature of the blood being sampled. In the present study, the ewes were fed twice daily and mixed (arterial) blood was sampled. By contrast, Wilson *et al.* (1981) fed their ewes continuously, and sampled jugular venous blood. That the difference in blood sampled may be important is suggested by the fact that the level of recycling in the fasted cows in the present study (13·5%), as computed from glucose and lactate specific activities (Table 7), was lower than the levels found by Wilson *et al.* (1981) in their fed ewes (15–21%). In the fasted cows, in which, of course, the sampled mixed blood was venous rather than arterial, it might have been expected both that steady-state conditions would have pertained

throughout a given 24-h period and that any glucose recycling that did occur would have been maximal.

The fact that total glucose recycling in the present study was even lower than that calculated from lactate alone was unexpected. The alanine cycle as shown by Foster *et al.* (1980) should surely account for an additional, albeit small, amount (Brockman & Bergman, 1975). This indicates, therefore, that the assumptions of the two methods are still imprecise. The retention of some ^3H in the glucose formed from recycled compounds remains a distinct possibility.

The present study is, nevertheless, in partial agreement with Wilson *et al.* (1981), in that the extent of total recycling of glucose-C was somewhat greater in the ewes during pregnancy than during lactation. In our experiments, this was the case whether interconversions were expressed as an absolute rate of transfer of C atoms or as a percentage of total flux of glucose (Fig. 1 and Table 9). One likely explanation for this difference is that it arose as a consequence of the patterns of metabolism across the productive organs. During lactation, there is a large net uptake by the mammary gland of both glucose and lactate for lactose, energy and milk production (Ling *et al.* 1961; Gardner & Hogue, 1964; Linzell, 1974). In this way, a large proportion of both compounds would be excluded from participation in whole-body metabolic interconversions. This, therefore, must be an important factor contributing to a diminution in the extent of interconversions of glucose and lactate during lactation. During pregnancy, there is a substantial net uptake of glucose by the placenta but, on the other hand, there is a net output of lactate (Battaglia & Meschia, 1981). The C of the placentally-released lactate could thus have taken part in the whole-body interconversions of glucose and lactate. It can be concluded from the previously mentioned considerations, therefore, that the peculiar metabolic requirements of the productive organs would have had a significant influence on the extent of interconversions during both lactation and pregnancy. In particular, lactate uptake by the lactating mammary gland would have tended to decrease interconversions, while output of lactate from the pregnant uterus would have tended to increase them.

Carbohydrate sufficiency (see, for example, Baird, 1981) is another factor that is likely to have influenced the extent of interconversions of glucose and lactate. Previous work with dairy cows has demonstrated that a decrease in blood and liver carbohydrate content is associated with an increase in the rate of net uptake of lactate by the liver and in the rate of net uptake of endogenously derived lactate by the splanchnic bed, i.e. by the liver and gut acting together as a functional organ (Baird *et al.* 1980). Both these changes are likely to be associated with an increase in glucose–lactate interconversions. Thus, an elevated rate of hepatic uptake of lactate increases the possibility of lactate acting as a gluconeogenic precursor, while an increase in the splanchnic uptake of endogenous lactate is indicative of an increase in lactate formation in peripheral tissues. In the present study, ewes showed clear signs of a lower carbohydrate sufficiency during pregnancy than during lactation (Table 5). Analogy with the dairy cow (Baird *et al.* 1980) would therefore suggest that rates of net hepatic uptake of lactate would have been higher during pregnancy than during lactation. This, in fact has been found to be the case (van der Walt *et al.* 1983).

Metabolism in the cows

Circulating concentrations of metabolites in the lactating and fasted cows of the present study were similar to those reported in earlier work (Baird *et al.* 1980). Corresponding measurements have not, however, been previously made during late pregnancy. The rates of apparent turnover of glucose also compare relatively well with previous observations, in that Paterson & Linzell (1974) reported a value of 3·7 mmol/min during late pregnancy, Bickerstaffe *et al.* (1974) a value of 8·0 mmol/min during early lactation and Lindsay (1970)

a value of 3·5 mmol/min during fasting. There are no previous reports of lactate turnover in dairy cows.

Of particular interest was the finding that the metabolic pattern pertaining to lactation differed in the cows as compared with the ewes. The key factor in producing these differences was probably carbohydrate sufficiency which, judged by circulating metabolite concentrations, was clearly lower in the lactating cows than in the lactating ewes. This difference in carbohydrate status during lactation occurred even though glucose turnover was quantitatively similar in both the cows and the ewes when expressed per unit of metabolic body size (Table 8). By contrast, lactate turnover, when expressed in the same way, was lower in the lactating cows than in the lactating ewes. Furthermore, while this factor was greater during lactation than during pregnancy in the ewes, the reverse was the case in the cows. It is likely that lactate turnover is another index of carbohydrate status, since it also decreased markedly in the cows during fasting (Table 8; see also Lindsay, 1970).

Another difference between cows and ewes was that while the precentage conversion of lactate flux to glucose was substantially lower during lactation than during pregnancy in the sheep, this was not so in the cows (Table 7). It is somewhat surprising, therefore, that the percentage of glucose converted to or derived from lactate was lower during lactation than during pregnancy in the cows, as it was in the ewes. One possible reason for this may have been that the proportion of the glucose turnover that did not participate in whole-body metabolism was greater in the cows. The average milk yield of the cows was 22·5 kg/d (Table 2) while that of the three lactating ewes can be assumed to have been about 2·3 kg/d (cf. Gardner & Hogue, 1964). Assuming that the lactose content of bovine milk is 4·6% (Ling *et al.* 1961) and that of ovine milk is 5·7% (Gardner & Hogue, 1964), then it is clear that while some 50% of glucose turnover was required to provide milk lactose in the cows, only 38% was required in the ewes.

Another surprising finding in the cows was that the apparent rate of recycling of glucose-C, as determined with [U-^{14}C]glucose and [6-^{3}H]glucose, was only about 2% during lactation (Table 9). The rate of hepatic uptake of lactate should, in fact, have been high and potentially capable of providing 20–25% of the hepatic glucose output (Baird *et al.* 1980). Presumably, a substantial portion of the lactate assimilated by the liver is not used for gluconeogenesis. Trans-organ measurements on ewes employed in the present study in fact revealed that up to 80% of the net lactate assimilated by the liver was indeed used for purposes other than gluconeogenesis (van der Walt *et al.* 1983).

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