Threonine metabolism in sheep

2. Threonine catabolism and gluconeogenesis in pregnant ewes

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1. The irreversible loss rate (ILR) of glucose, bicarbonate and threonine were determined in six twin- and triplet-bearing ewes on three occasions during the last 6 weeks of gestation.

2. Three of the ewes (group S) were given conventional rations of hay plus concentrates so that their blood ketone levels did not rise over this period. The other three ewes (group H) were given a fixed intake of hay throughout; their blood ketone levels rose, but remained at subclinical levels.

3. The results are presented in the form of three-pool open-compartment models for each period. There was an increase in the glucose ILR for both groups over the 6-week period, but the over-all increase (mean $\pm$ SE) was much greater ($P < 0{\cdot}001$) in group S ($35{\cdot}1 \pm 2{\cdot}43$ g carbon/d) than in group H ($11{\cdot}3 \pm 1{\cdot}28$ g C/d). Similarly, increases in bicarbonate ILR were also higher in group S ($161 \pm 11{\cdot}2$ g C/d) than in group H ($63 \pm 21{\cdot}7$ g C/d). However, whereas with group S ewes, receiving supplementary feed, this increase was progressive throughout the 6 weeks, with the group H ewes the increase which occurred over the last 3 weeks of gestation ($56 \pm 26{\cdot}3$ g C/d) was much greater than that which occurred over the preceding 3 weeks ($7 \pm 4{\cdot}7$ g C/d). This pattern was also evident in the oxidation of glucose to CO_2.

4. In contrast to the previously mentioned findings, neither threonine ILR nor the amount of threonine converted to glucose or catabolized to CO_2 changed significantly over the 6-week period.

5. The results are discussed in the light of findings presented in the previous paper that the amount of threonine used in catabolic processes can alter if a glucose-only sink is created in wether sheep.

Despite the fact that the ruminant absorbs very little glucose *per se* from its gastrointestinal tract, the glucose production rates of ruminants are similar, on a metabolic body-weight basis, to that of non-ruminants (see Armstrong, 1965). For this reason the glucose economy of ruminants has elicited considerable interest, particularly in situations such as pregnancy and lactation, where glucose utilization is high. Investigations of the ruminant's ability to derive glucose from precursors, such as propionic acid or the glucogenic amino acids, have been reviewed recently (see Lindsay, 1970; Elliot, 1980; Trenkle, 1980). However, as yet few studies have directly considered the consequences of a demand for glucose on the nitrogen economy of the animal.

It has been pointed out (Egan & MacRae, 1979; MacRae & Egan, 1980; Egan *et al.* 1983) that if essential amino acids are used extensively for gluconeogenesis this will have a serious consequence for the protein economy of the animal, but as yet little quantitative information is available on the extent to which the essential amino acids do donate carbon to glucose. The non-essential amino acids, alanine, glutamate, aspartate and glycine, appear to be the main glucogenic amino acids in ruminants (Wolff *et al.* 1972) but conversion of C from these amino acids to glucose may be merely reflecting an intermediary step in the net movement of C from other sources, including other amino acids, into glucose. In earlier experiments (Egan *et al.* 1983) the gluconeogenic contribution of the essential amino acid threonine, although only very small ($< 1\%$) in mature wethers, was increased significantly when the glucose demand on the animals was increased as a result of infusion of phloridzin and excretion of glucose in the urine. The transfer of threonine-C to glucose and carbon dioxide both increased, by 39 and 69% respectively, when the animals were infused with

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phloridzin, and it was calculated that the total amount of threonine catabolized daily in this way represented that contained in approximately 40 g protein (MacRae & Egan, 1980).

The glucose production rates of ewes increased substantially more during pregnancy and lactation, to meet the demands of foetal metabolism and lactose synthesis respectively, than occurred in the wethers infused with phloridzin. The effect of this additional demand for glucose on the threonine metabolism of the pregnant ewe was therefore studied. The present paper reports the results obtained with six twin- or triplet-bearing ewes, from measurements made on three occasions during the latter stages of pregnancy. Three of the ewes were given conventional amounts of hay plus concentrates, the latter being progressively increased during the last 6 weeks of gestation to hold plasma 3-hydroxybutyrate levels between 0·4 and 0·8 mM (Russel *et al.* 1967) taken as adequacy of supply of glycogenic precursors. The other three ewes were kept on a constant intake of roughage throughout the period. Elevation of blood ketone body concentration was evidence of energy and gluconeogenic precursor inadequacy in the non-supplemented group and so it was possible to examine threonine catabolism and gluconeogenesis from threonine under two sets of supply and demand conditions.

Parts of this study have been reported briefly elsewhere (Egan & MacRae, 1979; MacRae & Reeds, 1980; MacRae & Egan, 1980).

EXPERIMENTAL

Sheep and diets

Six pregnant Greyface ewes were obtained from Dr J. Robinson at the Rowett Research Institute, Aberdeen. They were at approximately day 80 of gestation and had been X-rayed to determine that they were all twin or triplet bearing. They were kept in metabolism crates and received their rations by means of continuous belt-type feeders. Ewes 1, 2 and 3, which will be subsequently designated group H, received daily 880 g dry matter (DM) of a chopped medium quality hay (in vitro digestibility 0·67; N content 23·5 g/kg; cell wall carbohydrate content 52·2 g/kg) throughout the experiments. Ewes 4, 5 and 6, subsequently designated group S, received the same intake of hay for the first period of gestation but then the hay was supplemented with 450 g Ewemax pellets/d (DM content 850 g/kg; N content 22 g, oil 35 g, fibre 40 g, metabolizable energy (ME) 12·5 MJ/kg DM) during the second period and this amount was increased to 600 g/d during the final period. Details of the individual feed intakes of each ewe, their plasma ketone body levels at each stage of the experiment and the number and weight of lambs subsequently born to them are given in Table 1. Decisions on the level of Ewemax pellets to be given were based on the level and rate of change of plasma 3-hydroxybutyrate and non-esterified fatty acid concentrations (Russel *et al.* 1967).

Experimental design

The metabolism of each ewe was examined on three occasions, i.e. between days 100 and 105 (period 1), 120 and 125 (period 2) and 135 and 142 (period 3) of gestation. On each occasion separate infusions of D-[U-^{14}C]glucose, L-[U-^{14}C]threonine and $NaH^{14}CO_3$ (obtained from Amersham International, Amersham, Bucks) were administered and irreversible loss rates (ILR) of the respective metabolites and transfer quotients relating the movement of C between the three metabolic pools were determined using the procedures described by Egan *et al.* (1983). In this study, however, unlike the previous one (Egan *et al.* 1983), it was found that on a number of occasions when $NaH^{14}CO_3$ was infused, the specific radioactivity (SR) of bicarbonate measured in blood was significantly lower than that measured in urine (on nine out of the eighteen occasions the mean SR in blood was lower ($11 \pm 1{\cdot}5\%$) than the SR in the corresponding urine samples taken over the plateau period). Prieto *et al.* (1983) recently reported this problem with blood SR values during

Table 1. *Daily feed intakes and plasma ketone body levels of each ewe during the three stages of the experiment, plus the birth weights of the lambs subsequently produced*

(Values given without standard errors indicate all the diet was consumed, SE values indicate variance of the mean intake value over the experimental period)

	Period of gestation*	Ewe no. 1	2	3	4	5	6
Dry matter intakes (g/d)							
Hay	1	880	880	880	880	880	843 (SE 11·7)
Hay	2	880	880	880	880	880 (SE 15·4)	777 (SE 29·1)
Concentrates†					385	385	385
Hay	3	880	880	880	880	880	590 (SE 20·4)
Concentrates†					510	510	510
Plasma	1	1·05	0·52	0·56	0·77	0·38	0·87
D-3-hydroxybutyrate	2	1·56	1·15	1·15	0·75	0·70	0·82
(mM)	3	2·35	1·00	0·91	0·65	0·61	1·25
Lamb birth wt (kg)		5·2	5·0	3·2	4·5	2·6	6·0
		5·2	4·3	Still born	3·7	5·2	5·8
					3·7	2·7	

* 1, 100–105 d; 2, 120–125 d; 3, 135–142 d.
† Concentrates, Ewemax pellets.

$NaH^{14}CO_3$ infusions and suggested that urine or saliva bicarbonate SR values are preferable to blood values in that they provide CO_2 entry rates which agree closely with gaseous exchange values. In this study, urine bicarbonate SR values were used to calculate the CO_2 ILR. There were no differences between blood and urine bicarbonate SR values ($P > 0{\cdot}05$, n 36) when glucose or threonine was infused and so blood values were used to determine transfer quotients. As in the previous paper (Egan *et al.* 1983) D-[2-^{3}H]glucose was infused along with each ^{14}C-labelled tracer as a covariant in order to obtain 'adjusted glucose ILR values' where day-to-day variation in glucose ILR was > 5% between the different infusions (i.e. in five of the eighteen sets of infusions performed).

Calculations

Values given on the three-pool open-compartment models in Fig. 2 (see p. 390) were determined by deriving the complete model for each sheep in groups H and S before calculating the mean values for each group. Thus for each measurement, values are means with their standard errors for three animals.

RESULTS

The feed intakes and circulating plasma 3-hydroxybutyrate levels for each animal during each period of gestation are given in Table 1. Apart from sheep no. 6, who refused some hay throughout the experiment, all sheep consumed their offered rations.

Fig. 1 illustrates the various plateau SR values obtained when the three infusion procedures were carried out on sheep no. 2 during period 2. This particular set of results was chosen because it was one of the five occasions during the eighteen sets of infusions where the glucose ILR measured using [2-^{3}H]glucose during the D-[U-^{14}C]threonine infusion and during the D-[U-^{14}C]glucose infusion did not agree to within 5%; taken over the eighteen sets of infusions, the [2-^{3}H]glucose values obtained during the D-[U-^{14}C]glucose infusions were $99{\cdot}97 \pm 2{\cdot}67\%$ of that obtained during the D-[U-^{14}C]threonine infusions.

Table 2. *Measurements of the irreversible loss rates (ILR) of glucose-C, threonine-C and bicarbonate-C and their transfer quotients in non-supplemented (ewe nos. 1–3) and supplemented (ewe nos. 4–5) ewes during the last 6 weeks of pregnancy*

Period of gestation...	1 (100–105 d)						2 (120–125 d)						3 (140–145 d)					
Ewe no...	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
ILR (g C/d)																		
Glucose	63·6	60·7	61·1	70·8	64·8	70·2	66·7	63·4	69·7	73·8	82·2	97·4	73·2	71·2	74·9	103·3	97·9	110·2
Threonine	4·65	4·73	5·36	4·58	4·64	5·19	4·43	4·24	5·05	3·20	3·00	5·09	4·28	3·76	3·50	4·19	5·78	5·73
CO_2	332	337	338	335	318	302	375	353	355	287	304	409	481	389	396	501	435	470
Threonine-C converted to glucose (%)	3·6	4·0	3·1	3·5	3·5	4·4	3·6	3·8	4·9	4·0	4·1	6·0	4·1	2·4	3·6	4·4	3·5	3·7
Threonine-C oxidized to CO_2 (%)	7·6	9·2	8·5	7·8	8·3	7·4	6·9	6·9	8·0	4·2	5·7	6·6	7·4	7·3	8·4	8·6	10·1	6·6
CO_2-C derived from glucose (%)	9·6	11·2	11·0	11·9	12·0	10·5	10·0	9·8	11·0	8·8	9·8	12·8	10·5	12·0	12·0	12·3	12·6	15·0
Glucose-C drived from CO_2 (%)	18·6	21·8	14·7	20·1	18·9	17·8	16·1	16·5	19·1	14·7	11·4	20·6	10·6	12·7	14·8	13·9	14·6	13·6

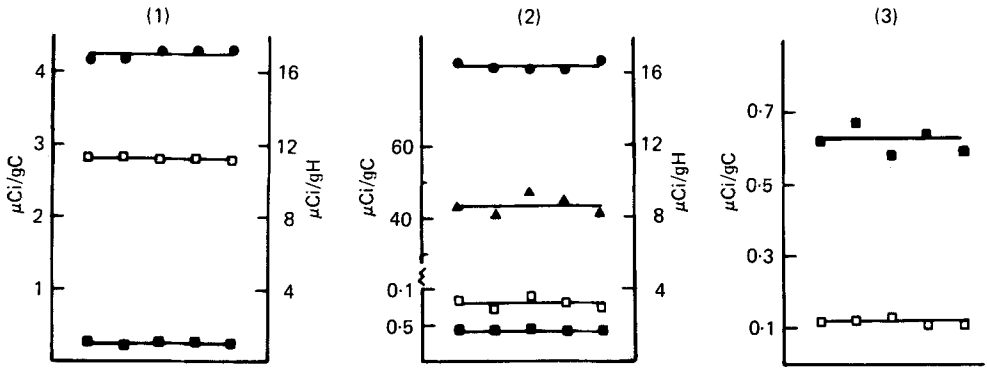

Fig. 1. Plateau specific activities of (*a*) [^{14}C]glucose (□), (*b*) [2-^{3}H]glucose (●), (*c*) [^{14}C]threonine (▲) and (*d*) $NaH^{14}CO_3$ (■) during infusions of (1) a+b, (2) c+b and (3) d in ewe no. 2 during period 2.

Individual values obtained for ILRs of threonine, glucose and CO_2, for the percentages of threonine-C which were converted to glucose or oxidized to CO_2, and for the relationships between the CO_2 and glucose SR plateaux during both glucose and CO_2 infusions for each sheep on each occasion are given in Table 2. These values were used to compute the six three-pool open-compartment models shown in Fig. 2.

Changes in glucose kinetics

Although the ewes were allocated at random to groups H and S, the mean glucose ILR of the three animals in group S (68·6 g C/d) was higher ($P < 0·05$) than that for group H (61·8 g C/d) during period 1 when they all received the same feed at the same level. However, by period 2 when ewes in group S were offered 385 g/d of Ewemax pellets, their glucose ILR had increased by 15·9±7·02 g C/d, while ewes in group H, which were still receiving the standard amount of hay, had increased their glucose ILR by only 4·8±1·90 g C/d. By period 3, immediately before full term when group S were then being offered 510 g Ewemax pellets/d, their glucose ILR had increased by 35·1±2·43 g C/d over period 1, which was considerably more ($P < 0·001$) than the increase of 11·3±1·28 g C/d of group H.

There was little change in the amount of glucose-C which was oxidized to CO_2 in the group H ewes until the last 3 weeks of gestation, when an extra 10±2·25 g C/d was oxidized. This was reflected in the CO_2 ILR values from this group which showed little change between periods 1 and 2 (7±4·7 g C/d) but which were 56±26·3 g C/d higher in period 3 than in period 2. In the group S ewes there was slightly more glucose oxidized in period 2 than in period 1 (6±8·2 g C/d) but substantially more oxidized in period 3 than in period 2 (22±1·4 g C/d). In these ewes, which received supplementary feed, the CO_2 ILR values increased steadily throughout the experiment (i.e. CO_2 ILR in period 2 was 86±14·3 g C/d higher than in period 1; in period 3 it was 76±3·2 g C/d higher than in period 2).

Changes in threonine kinetics

Any small changes in the threonine ILR values between groups or between periods in Fig. 2 probably reflect more the limitation of analytical techniques than any significant biological change. Table 3 gives the proportion of the threonine-C ILR which was converted to glucose or oxidized directly to CO_2. There were no significant differences between the

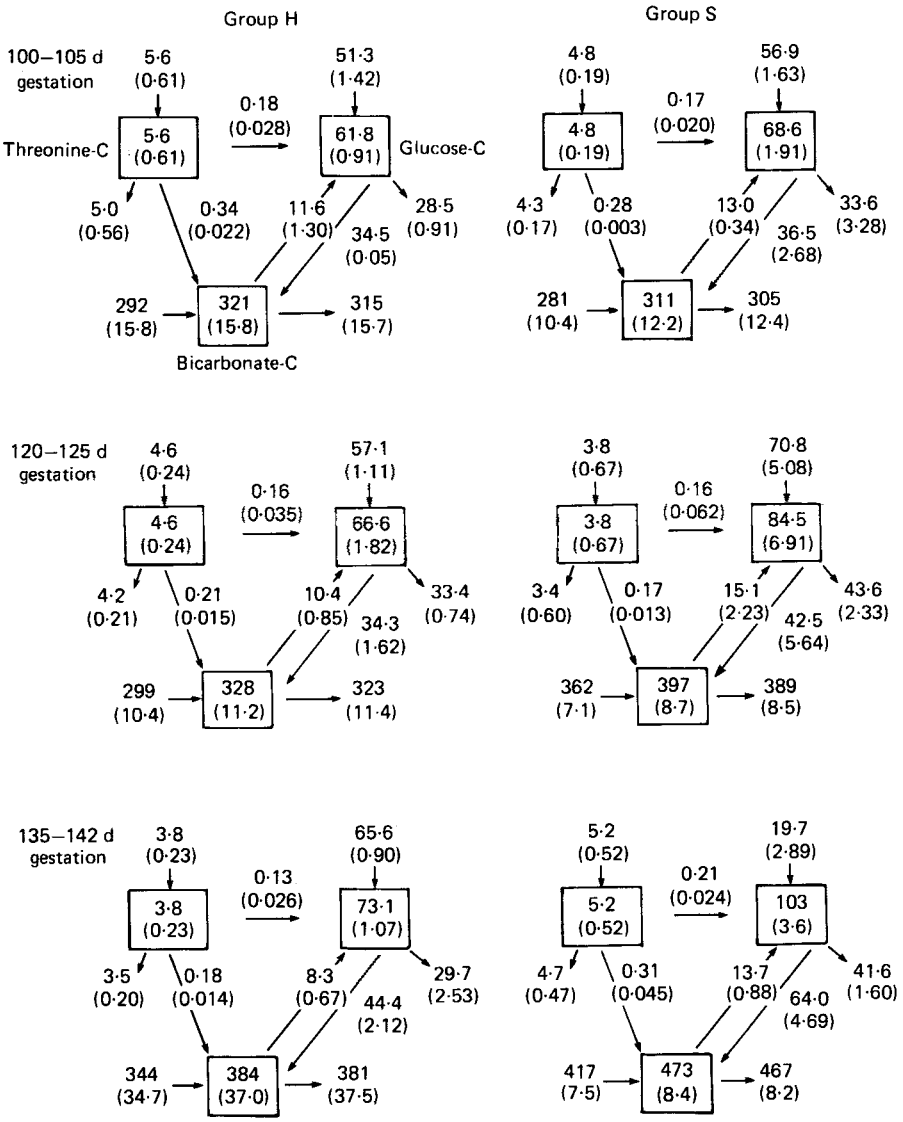

Fig. 2. The relationship between threonine-carbon, glucose-C and bicarbonate-C (mean values, standard errors in parentheses; g C/d) in group H (ewes nos. 1–3) and group S (ewes nos. 4–6) ewes over the last 6 weeks of pregnancy.

two groups in the amounts of threonine-C which was converted to glucose or oxidized to CO_2. Neither was there any significant increase as pregnancy progressed in the proportion of the threonine-C being used for gluconeogenic or oxidative processes.

DISCUSSION

Glucose kinetics

As in experiments previously reported (Egan *et al.* 1983) D-[2-^{3}H]glucose was infused along with D-[U-^{14}C]glucose and L-[U-^{14}C]threonine to provide a covariant basis for correcting any variation in glucose ILR between the days over which the successive isotope tracer studies

Table 3. *The proportion of threonine-C converted to glucose or oxidized directly to carbon dioxide in group H* and S* ewes during the three periods of the experiment*

(Mean values with their standard errors)

Period of gestation†	Threonine-C converted to glucose (%)				Threonine-C oxidized to CO_2 (%)			
	Group H		Group S		Group H		Group S	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	3·2	0·19	3·7	0·39	6·2	0·29	5·9	0·27
2	3·6	0·57	4·0	0·83	4·6	0·17	4·8	0·83
3	3·2	0·55	4·0	0·39	4·7	0·20	5·9	0·42

* For details, see p. 386.

† 1, 100–105 d; 2, 120–125 d; 3, 135–142 d.

were made on each period (Wilson *et al.* 1981). D-[2-^{3}H]glucose has been used in many experiments to measure total entry rate of glucose, because it is thought not to re-cycle in any way and indeed it has been used simultaneously with D-[U-^{14}C]glucose to calculate the re-cycling rate of glucose in many species, e.g. in sheep (Judson & Leng, 1972), rats (Katz *et al.* 1974; Freminet *et al.* 1976), dogs (Issekutz *et al.* 1972; Belo *et al.* 1976), rabbits (Dunn *et al.* 1976) and young chickens (Brady *et al.* 1977). The re-cycling rate reported ranged from 13 to 14% in sheep (Judson & Leng, 1972; Wilson *et al.* 1981) to 57% in young chickens (Brady *et al.* 1977). In the present experiment with twin- and triplet-bearing ewes in late pregnancy, the [2-^{3}H]glucose ILR values were $12{\cdot}3 \pm 2{\cdot}45$% higher than the corresponding values simultaneously obtained using D-[U-^{14}C]glucose.

The changes in glucose kinetics in groups H and S and indeed the possible relevance or otherwise of these measurements to the glucose requirements of the ewes have already been referred to by MacRae & Egan (1980). Clearly, the results suggest that a considerable proportion of the increased glucose ILR which occurs in conventionally fed pregnant ewes is associated more with the extra metabolizable energy intake of the animal than with any absolute physiological adaptation to produce more glucose to meet the needs of foetal metabolism. Though Steele & Leng (1973) identified the two components in the rise in glucose production, they emphasized the influence of physiological need to provide glucose for foetal metabolism, whereas Lindsay (1970) has stressed the confounding influence of rising energy intake under good nutritional management. Our findings have shown the magnitude of each type of contribution, using 3-hydroxybutyrate as the index of adequacy or degree of inadequacy of the energy supply. Thus, in group H ewes the glucose ILR increased only by $11{\cdot}3 \pm 1{\cdot}28$ g C/d over the last 6 weeks of pregnancy whereas in group S ewes it increased by $35{\cdot}2 \pm 2{\cdot}41$ g C/d. Kronfeld (1958) suggested that a single foetus drains some 32 g/d of hexose during the advanced stages of pregnancy and this value (14·5 g C/d) would be reasonably well supplied by the extra glucose produced by the non-supplemented ewes. Indeed, two of the group H ewes, though clearly approaching ketotic instability, successfully carried twin lambs which at birth had a combined live weight of 9·3 and 10·4 kg respectively, not very much less than the 10·6, 11·8 and 11·9 kg produced by the three group S ewes.

One interesting aspect of the present results is that in the group H ewes, it was not until the last 3 weeks of gestation that the proportion of the glucose which was oxidized to CO_2, or indeed the CO_2 production rates themselves, increased substantially. In current

recommendations of nutrient requirements (Agricultural Research Council, 1980) the energy requirements of the pregnant ewe are increased linearly from mid-pregnancy through to full term.

Threonine kinetics

The aim of the present study was to determine whether, when the ewe would appear to need more glucose for foetal metabolism, glucose is obtained to any extent from essential amino acids, so possibly penalizing its protein economy. In previous studies (Egan *et al.* 1983), when wethers were infused with phloridzin, reabsorption of glucose across the kidney tubules was reduced and this loss of glucose in the urine (22–28 g/d) imposed an apparent increase in glucose demand, so that glucose ILR values were increased by 10–11 g C/d (i.e. 30%) over the basal glucose ILR value. Although the absolute amounts of threonine-C which appeared in glucose and CO_2 (0·125 and 0·405 g C/d, representing 4 and 13% of the threonine flux respectively) were small in these animals, and made little contribution to the total glucose of CO_2 ILR values, the wethers did demonstrate an ability to alter their C metabolism. They increased the amount of threonine-C going to glucose by 39% and the amount of threonine-C oxidized to CO_2 by 69%. This adaptation meant that, whereas before phloridzin these combined processes had accounted for approximately 10% of the threonine resource, during phloridzin infusion they accounted for 17% of the threonine produced. If expressed as a percentage of threonine apparently absorbed from the small intestine rather than of threonine ILR (see Egan & MacRae, 1979) this represented an apparent change in efficiency of utilization of absorbed threonine for protein deposition from 70% before phloridzin infusion to 52% during phloridzin infusion.

In contrast, in the present experiment, where the glucose ILR values were increased by approximately the same amount as occurred in the phloridzin-infused wethers (approximately 40 g glucose/d) in the group H ewes but by considerably more in the group S ewes (approximately 90 g glucose/d), there was no indication of an increase in the catabolism of threonine through the last 6 weeks of pregnancy (see Table 3). This presumably suggests that during pregnancy when the ewes' foetal requirements for protein are no doubt increased at a rate similar to her requirements for glucose and energy, the ewe is capable of regulating her metabolism so that essential amino acids such as threonine are not drawn from the plasma pool more extensively into glucose production, but continue to be used predominantly for the anabolic processes associated with protein deposition.

This of course poses a problem in relation to the source of the extra glucose produced. Ways in which threonine-^{14}C may appear in either glucose or CO_2 outside the limited time-scale of the tracer experiment have been discussed previously (Egan *et al.* 1983) but whatever the mechanism of threonine-C conservation there is a clear contrast to the response observed in phloridzin-infused wethers. When wethers were infused with phloridzin it was likely that the only extra demand on their metabolism over the short-term of the infusion was to provide extra glucose. In that situation the animal appeared to exhibit an ability to obtain some glucose from threonine.

The co-ordinated regulation of metabolic processes in order to maintain both N and C economies in optimum balance, with respect to physiologically essential substrates, is not a surprising capability, but these studies make clear the need for more detailed work on both essential and non-essential glycogenic amino acids in the sheep. Threonine was chosen for this study because (*a*) it is a glucogenic essential amino acid, (*b*) once deaminated or cleaved it is destined for catabolism, and (*c*) it has been considered to be an essential amino acid often in short supply (limiting or next to limiting for protein deposition) in sheep. The extent to which the pregnant ewe conserves or protects other essential amino acids also deserves attention since conservation of one essential amino acid for protein deposition is

not possible without parallel changes in retention of other essential and non-essential amino acids. It remains possible that part of the irreversible loss of threonine-C not accounted for in the glucose or CO_2 transfers is related not only to protein deposition but to entry of C into pools or compartments in maternal or foetal metabolism which introduce a time lag before any of that C appears in the CO_2 pool in excess of the 16 h used for infusion.

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