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Kinetics of Glucose Metabolism in Sheep

M. E. Wastney,[A,B] *J. E. Wolff,*[C] *R. Bickerstaffe,*[D] *C. F. Ramberg,*[E] *and the late M. Berman*[A]

[A] Laboratory of Mathematical Biology, DCBD, NCI, Bethesda, MD 20205, U.S.A.
[B] Current address: Center for Molecular Nutrition and Sensory Disorders, Georgetown University Hospital Medical Center, Washington, D.C. 200007, U.S.A.
[C] Ruakura Animal Research Station, Private Bag, Hamilton, N.Z.
[D] Department of Biochemistry, Lincoln College, Canterbury, N.Z.
[E] School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348, U.S.A.

Abstract

The kinetics of glucose cycling in 24 ewes bearing twins were studied 1 month before term by bolus injections of [6-^{3}H]- and [U-^{14}C]glucose. The function representing glucose carbon recycling was determined by deconvolution of the [^{3}H]glucose from the [^{14}C]glucose decay curves in plasma by using the SAAM and CONSAM programs, and a model for kinetics of glucose cycling was developed.

The [^{3}H]glucose data were fitted by four compartments, and an additional three compartments were required to explain recycling. The results show that labelled carbon was still recycling to plasma 2 days after the injection of tracer. By contrast, a similar analysis on a non-pregnant sheep, with data taken from the literature, showed that no more material was recycled after 1 day. It appears that a larger fraction (20 *v.* 5%) of the carbon 6 of glucose recycles in pregnant than in non-pregnant sheep. This presumably reflects the metabolism by the feto-placental unit and the increased rate of glucose metabolism during pregnancy.

Introduction

Ruminants rely on gluconeogenesis for the supply of glucose, and differ therefore from non-ruminants in which glucose is mainly absorbed from the diet. Some glucose is synthesized from products of glucose catabolism, such as lactate, pyruvate and alanine and this process can be defined as recycling of the glucose carbon. It occurs via several pathways and may be important in the conservation of carbon in gluconeogenic compounds when glucose demand is high.

The different synthetic pathways may be studied by using glucose labelled with different tracers (Dunn *et al.* 1967; Katz and Dunn 1967). Since ^{3}H labels are generally not recycled, but lost from the system on water, they trace the movement of the glucose molecule as a whole; carbon labels, on the other hand, are variously incorporated back into glucose and are used to assess the recycling pathways. The differences in tracer loss can be ascribed to differences in metabolism of the molecule versus the carbon atoms.

In the past, glucose tracer studies have been undertaken in sheep with ^{14}C and ^{3}H labels (for a review see Leng 1970). Glucose production rates have been determined from the areas under the specific activity curves and the fraction of glucose carbon recycling has generally been determined from the ratios of these areas determined with the two tracers (Brockman *et al.* 1975; Wilson *et al.* 1981). This method has limitations as it assumes that a plateau specific activity has been reached within the experimental time and provides no information on the processes involved. Additional information, such as rate constants and pool sizes, can only be obtained by modelling the data (Berman 1979, 1982) and a compartmental model has been proposed, based on the data of one pregnant sheep, by Hodgson and Mellor (1977).

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The aim of our studies was to examine the contribution of recycling in pregnant ewes following the injection of [6-^{3}H]- and [U-^{14}C]glucose, by compartmental analysis of the kinetics of tracer recycling and deconvolution. Deconvolution has been used previously to assess the fraction of [^{14}C]glucose that recycles (Katz *et al.* 1974*a*, 1974*b*). In the present study it was used to generate the function for the fraction of glucose carbon recycling back to glucose and this input function was used in the development of a compartmental model for the kinetics of glucose metabolism in pregnant sheep. As data were available for a non-pregnant ewe studied with the same tracers (Judson and Leng 1972), the parameters of the model for the non-pregnant ewe were determined and compared with the values obtained for the pregnant ewes.

Materials and Methods

Animals

The sheep, mature Perendale Romney cross-bred ewes, were mated on known dates and then X-rayed at 80 days of gestation. Those carrying twins ($n = 12$) were moved indoors to individual pens. They were fed on pellets (40 g dry matter $kg^{-0.75}$ daily) containing, on an air-dried basis, lucerne (600 g kg^{-1}), barley (300 g kg^{-1}), linseed meal (50 g kg^{-1}), molasses (50 g kg^{-1}) and at least 17% by weight crude protein in one-hourly rations.

One week prior to the experiment polyvinyl catheters (1·0 mm i.d., 2·0 mm o.d., Dural Plastics, Australia) were inserted into a jugular vein for blood sampling. The patency was maintained by flushing daily with saline (sodium chloride, 9 g l^{-1}) (Travenol, Australia) containing 10 units ml^{-1} of heparin (Evans, Liverpool) and 0·5 ml l^{-1} of neomycin sulfate, Neobiotic, (200 mg ml^{-1}, Upjohn, New Zealand). One day before the experiment an Intracath catheter (Deseret Pharmaceuticals, Utah, U.S.A.) was inserted into the other jugular vein for injection of the tracers.

Tracers

Stock solutions of 370 MBq of D-[6-^{3}H]- or 112 MBq of D-[U-^{14}C]glucose (Amersham International, United Kingdom), each in 100 ml of sterile saline (sodium chloride, 9 g l^{-1}), were stored at −20°C. On the day of the experiment 10 ml of each solution were combined, Seitz-filtered and weighed into sterile syringes. Approximately 37 MBq [6-^{3}H]- and 12 MBq [U-^{14}C]glucose were injected into each animal. The actual doses were determined by weight difference.

Experimental Procedure

The experiments were undertaken at about 115 days of gestation. The tracers were injected via one catheter over a period of 10 s and sampling was begun immediately through the other catheter. Thirteen samples (10 ml) were drawn over the first hour and four (15 ml) over the next 2 h. A total of 24 samples were taken over 48 h.

The samples were stored for up to 2 h on ice in tubes containing 25 mg sodium fluoride and 20 mg potassium oxalate and then centrifuged at 2700 *g* for 15 min at 4°C. Plasma was deproteinized by the method of Somogyi (1945) and stored at −20°C pending further analysis.

Chemical Analyses

Plasma glucose

Plasma glucose was analysed on a Technicon Autoanalyzer II by the method of Trinder (1969) with the modifications previously described (Wastney *et al.* 1982).

Glucose specific activity

The specific activity of glucose was determined in diluted aliquots of the injectate and in the protein-free filtrate of plasma. Glucose was isolated as potassium gluconate by the method of Blair and Segal (1960) and the samples were counted in a Beckman liquid scintillation counter, as previously described (Wastney *et al.* 1983).

Kinetic Analysis

The plasma glucose radioactivities of the 12 pregnant ewes were averaged (Figs 1*a*,1*b*), and the data fitted to series models using the SAAM and CONSAM programs

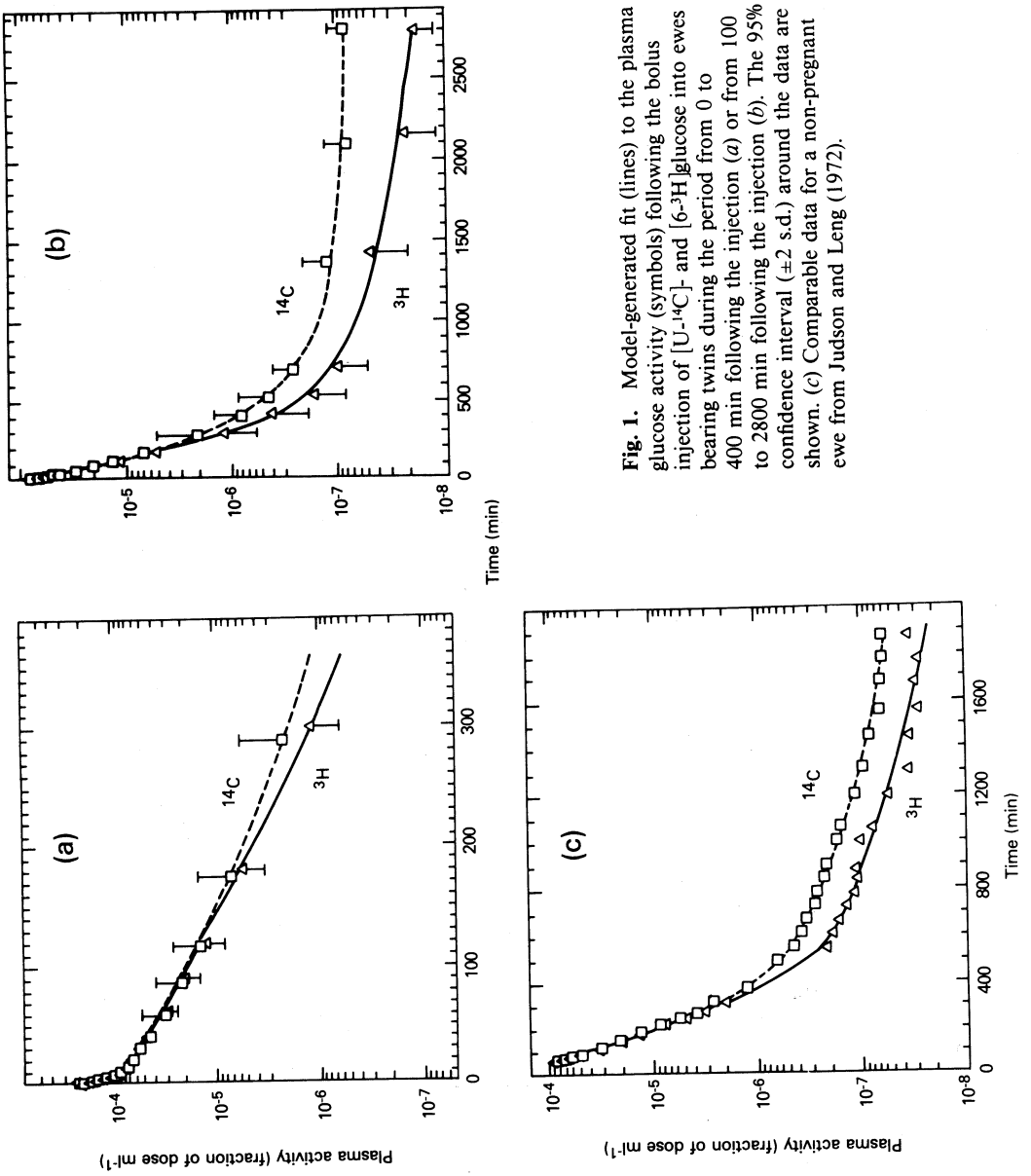


Fig. 1. Model-generated fit (lines) to the plasma glucose activity (symbols) following the bolus injection of [U-^{14}C]- and [6-^{3}H]glucose into ewes bearing twins during the period from 0 to 400 min following the injection (*a*) or from 100 to 2800 min following the injection (*b*). The 95% confidence interval (±2 s.d.) around the data are shown. (*c*) Comparable data for a non-pregnant ewe from Judson and Leng (1972).

(Berman and Weiss 1978; Boston *et al.* 1981). The data from a non-pregnant Merino ewe from Judson and Leng (1972), (Fig. 1*c*), was fitted in the same way. The function representing glucose carbon recycled was generated by deconvolution of the [6-^{3}H]- from the [U-^{14}C]glucose data.

Deconvolution may be defined as follows: given that the response, $r(t)$, (using the notation of Table 1) of a linear† system with transfer function (response to a unit impulse) $w(t)$ to an input, $u(t)$, is given by the convolution of $u(t)$ with $w(t)$, viz:

$$r(t) = u(t) * w(t) , \tag{1a}$$

where $*$ denotes convolution, or

$$r(t) = \int_0^t \mathrm{u}(\theta)\, w(t-\theta)\mathrm{d}\theta , \tag{1b}$$

then the input $u(t)$ giving rise to a response $r(t)$ in a system with transfer function $w(t)$ is given by the deconvolution of $r(t)$ with $w(t)$. Berman (1978) has shown that this is given by

$$u(t) = [1/w(0)][\dot{r}(t) - u(t) * \dot{w}(t)] , \tag{2}$$

where the overhead dot denotes the first derivative. For a unit injection at the site of observation [$w(0) = 1$] this reduces to:

$$u(t) = \dot{r}(t) - \int_0^t \mathrm{u}(\theta)\, \dot{w}(t-\theta)\mathrm{d}\theta . \tag{3}$$

Table 1. Notation

Parameter	Name	Definition
$r(t)$	System response	Response of system at time t to an input $u(t)$
$u(t)$	Input function	Input at time t
$w(t)$	Weighting function (transfer function)	Response of a system to a unit impulse
$F(\mathrm{I},t)$	Compartment response	Tracer content of compartment I at time t
IC(I)	Initial condition	The tracer in compartment I at $t(0)$
$L(\mathrm{I,J})$	Rate coefficient	Fractional flow per unit time into compartment I from compartment J
$M(\mathrm{I})$	Mass	Mass of tracee in compartment I
$R(\mathrm{I,J})$	Flow rate	Flow rate of tracee into compartment I from compartment J per unit time
$S(\mathrm{I,J})$	Summing coefficient	The fraction of compartment I that is summed in compartment J
$U(\mathrm{I})$	Tracee input	The steady rate tracee input into compartment I
$UF(\mathrm{I})$	Constant input	An input of tracer into compartment I

Deconvolution is readily applied to compartmental systems as the derivatives of the responses are functions of the rate coefficients (Berman 1978). For our data the four-compartment system that fitted the ^{3}H data constituted the weighting function, $w(t)$ (i.e. the response to a unit input), while the system that fitted the ^{14}C data constituted the response function, $r(t)$ (i.e. the weighting function convolved with an input). To generate this input function the ^{3}H data was deconvolved from the ^{14}C data according to equation (3) by simulation in SAAM (Berman and Weiss 1978) using the schema shown in Fig. 2. Because the deconvolution procedure is sensitive to the early data points (Berman

† Given that $r(t)$ is the response of a system to input $u(t)$, then, if the response of the system to independent inputs $a_1u_1(t)+a_2u_2(t)$ is $a_1r_1(t)+a_2r_2(t)$, the system is said to be 'linear'.

1979), the parameter $L(2,1)$, the fractional movement into compartment 2 from compartment 1, was set equal to $L(12,11)$ as both were considered to represent distribution of the glucose molecules from plasma.

The derivative of the first compartment, $\dot{w}(t)$, can be written in terms of the rate coefficients

$$\dot{w}(t) = \mathrm{d}F(1)/\mathrm{d}t = -L(2,1)F(1)+L(1,2)F(2)\ . \tag{4a}$$

where $F(1)$ is the activity in compartment 1, and $L(\mathrm{I,J})$ is the fraction of material transferred from compartment J into compartment I per unit time. Similarly, the derivative of compartment 11 (the plasma activity of ^{14}C data) gives $\dot{r}(t)$,

$$\dot{r}(t) = \mathrm{d}F(11)/\mathrm{d}t = -L(12,11)F(11)+L(11,12)F(12)\ . \tag{4b}$$

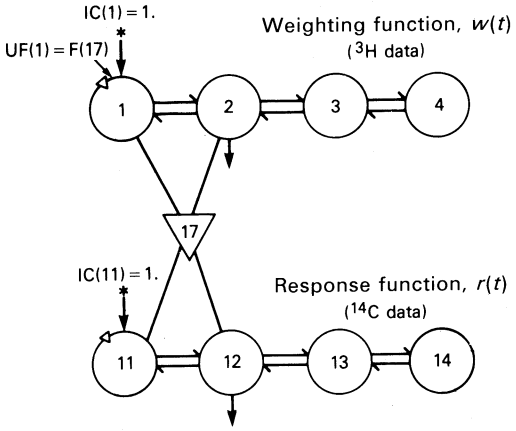


Fig. 2. Schematic representation of the system used to generate the input function. Compartments 1–4 represent the [^{3}H]glucose system while compartments 11–14 are the glucose system determined from injection of [U-^{14}C]glucose. The notation is described on Table 1. Compartment 17 is a 'summing' one and generates the input function (see text for details).

The input function, $u(t)$, representing the recycled carbon, was generated from the convolution of an input at arbitrary t, with the derivative of the weighting function subtracted from the derivative of the response (equation 3). The input was calculated in SAAM by starting with $u(0)$, and feeding it back into compartment 1. The next $u(t)$ was generated in compartment 17, a 'summing' compartment that summed the convolution of $u(t)$ with $\dot{w}(t)$ and subtracted it from $\dot{r}(t)$. Thus,

$$F(17) = L(2,1)F(1)-L(1,2)F(2)-L(12,11)F(11)+L(11,12)F(12) \tag{5a}$$
$$= -\dot{w}(t)+\dot{r}(t) \tag{5b}$$
$$= -\dot{F}(1)+\dot{F}(11) \tag{5c}$$
$$= \text{recycling rate.} \tag{5d}$$

Using certain assumptions (see Model Development section following) the whole function was thus determined by iteratively calculating $u(t)$ and feeding it back into the weighting system (represented by $UF(1)$ in Fig. 2).

Model Development

The model, shown in Fig. 3, was developed by first fitting the ^3H data to compartments 1–4 and then developing the recycling system from the input function.

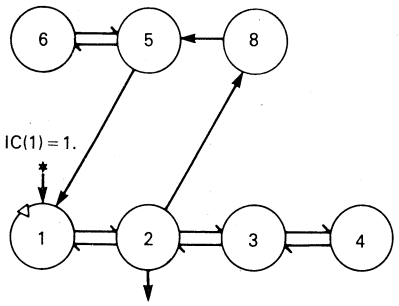


Fig. 3. Proposed model for glucose metabolism in sheep. Compartments 1–4 were defined by the ^3H data, while compartments 5, 6 and 8 account for glucose carbon recycling through products such as pyruvate, lactate and alanine.

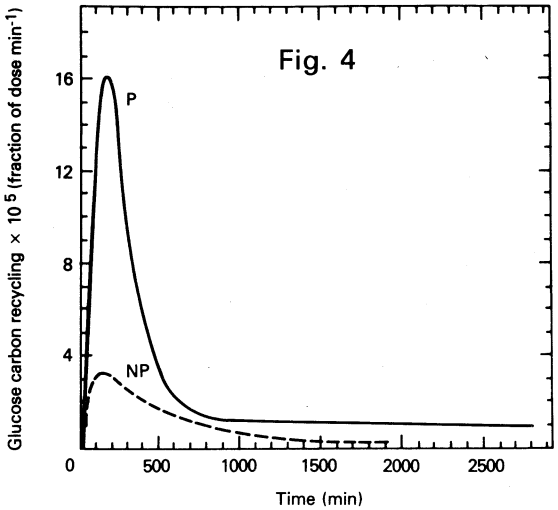
Because features of the systems were expected to be similar, the ^3H data for the pregnant and non-pregnant ewes were fitted simultaneously, using the minimum change postulate (Berman 1963). A good fit to the data was obtained (Fig. 1) by allowing only three parameters to vary (Table 2). Four compartments were required to fit the [^3H]glucose data,

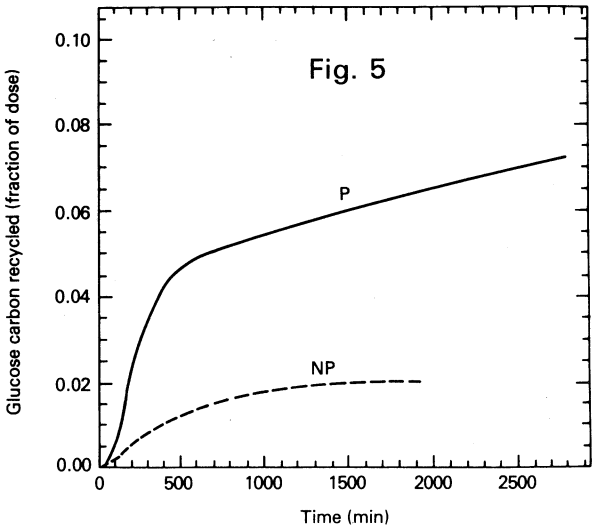
Table 2. Parameters used in the model

Data for the non-pregnant ewe were taken from Judson and Leng (1972). The fractional standard deviation of the estimate (s.d. of parameter/parameter), is given in parentheses

Parameter	Pregnant ewes	Non-pregnant ewe
Rate coefficient (h^{-1})		
$L(0,2)$	1·217 (0·04)	1·451 (0·04)
$L(1,2)$	6·490 (0·19)	6·490 (0·19)
$L(2,1)$	9·887 (0·13)	9·887 (0·13)
$L(2,3)$	0·109 (0·12)	0·109 (0·12)
$L(3,2)$	0·071 (0·10)	0·051 (0·09)
$L(3,4)$	0·051 (0·33)	0·019 (0·24)
$L(4,3)$	0·061 (0·15)	0·061 (0·15)
$L(1,5)$	0·180	1·200
$L(5,6)$	0·016	0·011
$L(5,8)$	0·553 (0·35)	0·189 (0·15)
$L(6,5)$	0·674	2·010
$L(8,2)$	0·308	0·075
Ratio		
$L(1,5)/L(6,5)$	0·267 (0·17)	0·597 (0·13)
$L(8,2)/(L(5,6)$	19·2 (0·15)	6·82 (0·21)
Volume of distribution		
(L)	4·89 (0·02)	2·38 (0·02)
(L kg^{-1})	0·094 (0·02)	0·070 (0·02)
Fraction recycling		
$L(8,2)/[L(8,2)+L(0,2)]$	0·202 (0·07)	0·049 (0·03)

and this agrees with a previously published observation for a pregnant ewe (Hodgson and Mellor 1977). The initial space of distribution was determined from the intercept of the fraction of dose ml^{-1} curve, at $t(0)$. The sampled compartment had a space of 4·9 litres, or 9% of liveweight, and so included plasma plus some extracellular fluid. Compartment 2 had a space of 10% of liveweight, and this approximated the interstitial fluid volume. Compartments 1 and 2 therefore had a combined space of 18%, which was approximately the extracellular fluid volume (20%) calculated for sheep by Holmes and English (1969).





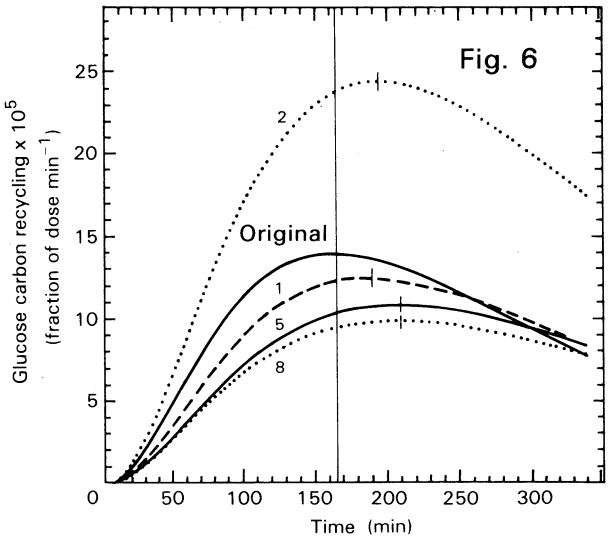


Fig. 4. Input function representing glucose carbon recycling to plasma in pregnant (P) and a non-pregnant (NP) sheep. Data for the non-pregnant ewe were from Judson and Leng (1972).

Fig. 5. Cumulative fraction of glucose carbon recycled to plasma in pregnant (P) and non-pregnant (NP) sheep. Data for the non-pregnant ewe were from Judson and Leng (1972).

Fig. 6. Effect of the turnover times of compartments 1, 2, 5 and 8 on the maxima of the glucose carbon recycling function in pregnant sheep. Reducing the turnover of each of these compartments by 50% delayed the peak by the amount shown. The turnover times of compartments 3, 4 and 6 did not affect the location of the peak.

Loss of glucose and recycling of glucose carbon were considered to occur from compartment 2. The other two compartments (3 and 4) were intracellular. Since glucose is not considered to be released after entering the peripheral cells, these exchange compartments probably include compounds such as glycogen, glycerol and glycolytic intermediates that are both formed from glucose and retain the ^{3}H label (Dunn *et al.* 1967).

The input functions, generated for the pregnant sheep and for one non-pregnant sheep from data taken from Judson and Leng (1972), are shown in Fig. 4. In pregnant sheep some ^{14}C label reappeared in plasma after only a few minutes and this implied that there was a rapid breakdown and resynthesis of glucose. The maximum recycling, however, was not observed until 150 min after the tracer injection (Fig. 4) and this suggested that the rapid pathways were augmented by pathways of slower breakdown and resynthesis. The type of response between the sheep differed and the maximum in pregnant sheep was five times higher than the non-pregnant sheep.

The cumulative functions are shown in Fig. 5. In pregnant ewes the curve was still rising after 32 h (1920 min) and so material was still being recycled back to plasma. After 24 h 6% of the label had recycled in pregnant animals compared with 2% in the one non-pregnant animal studied with [6-^{3}H]glucose.

A subsystem, that was the simplest consistent with the data, was developed for the recycling function. Three compartments (5, 6 and 8, Fig. 3) were required. Compartment 8 acted as a delay compartment since it did not exchange with other compartments and probably represents a transport process. Compartment 6 was large and slowly turning over (2·6 days) and was necessary to account for the final slope in the ^{14}C curve (Figs 1*b*, 1*c*) and the input curve (Fig. 4). About 20% of the material lost from compartment 2 entered the recycling pathway (Table 2) and this represented the fraction that recycled. The recycling function was regenerated by the product of $L(1,5)$ with $F(5)$ as this represented all the material returning to plasma.

For the non-pregnant sheep, less early data were reported and $L(2,1)$ was set equal to the value in pregnant sheep. The first compartment had a space of distribution of 2·4 litres (or 7% of liveweight) and therefore included plasma and some extracellular glucose. The exchange coefficients between the early compartments were similar to pregnant sheep, while the exchanges between compartments 3 and 4 were slower in the non-pregnant animal (Table 2). This may imply that there was less reappearance of label from compounds that exchange ^{3}H with glucose.

The recycling function showed that less glucose carbon recycled in non-pregnant animals (Fig. 4). It was assumed that features of the recycling system would be common to the experiments reported here and by Judson and Leng (1972) and the data for the non-pregnant and pregnant ewes were fitted to the same subsystem. The rate coefficients in the non-pregnant animal were generally faster and only 5% of the material lost from the system recycled in the non-pregnant sheep (Table 2).

The maxima recycling for the pregnant and non-pregnant ewes coincided (Fig. 4). The location of this maxima was influenced by the turnover times of compartments 1, 2, 5 and 8 (Fig. 6). While the turnover times of compartments 1 and 2 were the same in the pregnant and non-pregnant sheep (Table 2), non-pregnant sheep had a slower turnover of compartment 8 but a faster turnover of compartment 5. These two effects cancelled (Fig. 6), such that the recycling peaks occurred at about the same time. Further experiments are necessary to physiologically, or biochemically, define compartments 5 and 8.

Results

The final model is shown in Fig. 3 and the model-generated fits to the data are shown in Fig. 1. The rate coefficients of the model and their fractional standard deviations are given in Table 2. The parameters for the non-pregnant ewe were better determined than those of the pregnant ewe, as a result of the extra data that was available. The early (<5 min)

and late (>1 day) data, however, of the pregnant animals were important in describing the rapid and very slow components in the system.

While the parameters describing the ^3H data were well determined, others, required to fit the carbon data, were highly correlated, and so the ratios of these parameters were

Table 3. Plasma concentration, compartment masses and flow rates of glucose

Data for the non-pregnant ewe were taken from Judson and Leng (1972). The fractional standard deviation of the estimate (s.d. of parameter/parameter), is given in brackets. The mass of compartment 1 was the plasma concentration × initial space of distribution. The masses of the recycling subsystem were calculated subject to the constraints in Table 2

Parameter	Pregnant ewes	Non-pregnant ewe
n	12	1
Liveweight (kg)	52·0 (0·05)	34·0
Plasma glucose (mmol l^{-1})	3·26 (0·12)	3·44
Mass (mmol kg^{-1})		
$M(1)$	0·306	0·241
$M(2)$	0·377 (0·04)	0·297 (0·04)
$M(3)$	0·247 (0·10)	0·139 (0·11)
$M(4)$	0·297 (0·30)	0·439 (0·30)
$M(5)$	0·645	0·018
$M(6)$	27·3	3·36
$M(8)$	0·210 (0·33)	0·117 (0·17)
Flow rates (mmol kg^{-1} h^{-1})		
$R(0,2)$	0·459 (0·17)	0·431 (0·02)
$R(1,2)$	2·45 (0·08)	1·93 (0·11)
$R(2,1)$	3·03 (0·08)	2·38 (0·09)
$R(2,3)$	0·027 (0·06)	0·015 (0·07)
$R(3,2)$	0·027 (0·06)	0·015 (0·07)
$R(3,4)$	0·015 (0·12)	0·008 (0·17)
$R(4,3)$	0·015 (0·12)	0·008 (0·19)
$R(1,5)$	0·116	0·022
$R(5,6)$	0·435	0·037
$R(5,8)$	0·116	0·022
$R(6,5)$	0·435	0·037
$R(8,2)$	0·116	0·022

Table 4. Synthesis and clearance rates (mean ± s.d.) of glucose

Data for the non-pregnant ewe were taken from Judson and Leng (1972)

Rate parameters	Pregnant ewes		Non-pregnant ewe	
	^3H	^{14}C	^3H	^{14}C
Synthesis rate (mmol kg^{-1} h^{-1})	0·575 ±0·011	0·459 ±0·018	0·454 ±0·022	0·431 ±0·021
Fractional catabolic rate (fraction kg^{-1} h^{-1})	0·036 ±0·001	0·028 ±0·001	0·055 ±0·002	0·052 ±0·002
Metabolic clearance rate (ml kg^{-1} h^{-1})	176·8 ±3·5	141·1 ±5·6	131·9 ±6·5	125·5 ±6·2

determined (Table 2). The first ratio, $L(1,5)/L(6,5)$, was invariant in that an increase of 10% in $L(6,5)$ caused a 10% increase in $L(1,5)$ to fit the data. This applied to both the pregnant and non-pregnant ewes. The value of the second ratio, $L(8,2)/L(5,6)$, however, was dependent on $L(5,6)$. Increasing $L(5,6)$ by 10% caused an increase by the same amount in the ratio and again this applied to both data sets. $L(8,2)$ was therefore invariant and so the fraction calculated for recycling was not affected by the value chosen for $L(6,5)$.

The masses and flow rates are given in Table 3 while the synthesis, fractional catabolic rates and metabolic clearance rates of plasma glucose, calculated by the two tracers and normalized for liveweight are given in Table 4. Subject to the assumptions of the model (see Model Development section) the results show that the pregnant sheep had synthesis and metabolic clearance rates that were larger than those of the non-pregnant animal. The fractional catabolic rates, however, of the pregnant ewes were considerably lower than those of the non-pregnant animal.

Discussion

The model differs from those proposed by others. White *et al.* (1969), on the basis of a sum-of-exponentials fit to the plasma activity data, suggested that a three-compartment model would be required for analysis of kinetics of glucose metabolism in sheep. Hodgson and Mellor (1977) proposed a four-compartment model from data following the injection of [2-^{3}H]glucose into a pregnant ewe bearing a single foetus in late gestation. They calculated a lower value for the fractional catabolic rate with ^{3}H (0·024 kg^{-1} h^{-1}); however, as the plasma glucose concentration was higher, (3·85 mmol l^{-1}), the synthesis rate (0·539 mmol kg^{-1} h^{-1}) was similar to our ^{3}H value (Table 4). They measured a slightly larger initial space of distribution, (11·5% versus 8% of liveweight). The sizes of three compartments in their model are similar to $M(1)$, $M(2)$ and $M(3)$ of Table 3. However, the mass of their other compartment is eight times larger than our $M(4)$. The reason for this difference is not clear and their data are not shown. The differences between our studies and those of Hodgson and Mellor (1977) are confounded by the different tracer, the stage of gestation and the presence of a single fetus. The production rate reported for sheep on a similar diet and at the same stage of gestation measured with ^{14}C (and assuming a liveweight of 40 kg) was 0·533 mmol kg^{-1} h^{-1} (Steel and Leng 1973).

Kronfeld *et al.* (1971) and Kronfeld (1977), following an experiment involving the injection of [^{3}H]- and [^{14}C]glucose proposed a three-compartment model for lactating cows. They assumed that some ^{3}H label recycled, and fitted their data by representing recycling as one flow rate. In our study it was necessary to include three compartments to account for recycling (Fig. 3). This is a simplification of the processes *in vivo*, and compartments 5, 6 and 8 represent exchanges between tissue and liver pools of compounds such as lactate, alanine and pyruvate. Since compartment 8 does not exchange material, it acts as a delay and may represent a transport process into the cells.

One assumption of our model was that no ^{3}H recycled to glucose. The extent to which the [6-^{3}H] label is lost, however, is related to the enzyme activity in the conversion of pyruvate to oxaloacetate (Dunn *et al.* 1967). When [^{3}H]lactate was injected into rats some label appeared in glucose (Okajima *et al.* 1981). This suggests that some ^{3}H label may recycle. However, no comparable data are available for sheep.

Several differences were observed between the pregnant and non-pregnant sheep. Non-pregnant sheep had smaller compartment masses, and a smaller space of distribution in compartment 1. However, the liveweight of this sheep, was approximately one-half that of the pregnant sheep and, when normalized on the basis of liveweight, the space and compartment masses became comparable between animals (Tables 2 and 3). The differences in liveweight (18 kg) could be partially explained by the presence of a twin-pregnant uterus (estimated at 7–8 kg) and the rest was probably a breed difference.

Compartment 4, a pool of ^{3}H-labelled compounds, had a relatively larger mass. There is no evidence to suggest that glycogen stores are larger in non-pregnant sheep (Ford 1962) and the larger mass may be the result of lower enzyme activities. Higher activities of some enzymes of glucose metabolism have been observed in pregnant ewes (Mackie and Campbell 1972). The pools in the recycling pathway, representing carbon-containing compounds, were larger in the pregnant animals and may relate to differences in metabolism due to the presence of utero-placental and fetal tissues. Measurement of the incorporation

of ^{14}C into other compounds would be necessary to define these compartments experimentally, but they may include protein pools, which turnover slowly (Buttery *et al.* 1975). Compartment 6 appears quite large; however, it represents <0·5% of liveweight.

The rate coefficients were generally faster in the pregnant sheep and reflect the increased rate of metabolism that occurs in pregnancy. The fractional loss from compartment 2, $L(0,2)$, was comparable in both states and the lower FCR value of the pregnant animals results from the higher liveweight.

Recycling has been defined in various ways. Some workers (White *et al.* 1969; Steel and Leng 1973) calculate it as the difference between total glucose entry rate and irreversible disposal rate measured with one tracer. This, then, represents ^{14}C recycled from glucose pools in extravascular fluid and via other compounds. Other workers, including ourselves, define recycling as the difference in production rates (Brockman *et al.* 1975), or irreversible disposal rates (Judson and Leng 1972), estimated with ^{3}H and ^{14}C labels expressed as a fraction of the rate calculated with ^{3}H. This is equivalent to the ratio of the model parameters shown in Table 2.

In pregnant ewes 20% of the glucose carbon entered the recycling pathway while 5% entered in the non-pregnant animal (Table 2). This value is similar to the value of 4·4% calculated by Brockman *et al.* (1975) for non-pregnant sheep. The increased rate of recycling in the pregnant animals may be related to the presence of the feto-placental unit, as this is capable of taking up glucose and releasing lactate (Meschia *et al.* 1980).

The input functions (Fig. 5) showed that after 24 h only 6% of glucose had recycled to plasma in pregnant animals and 2% in the non-pregnant sheep. This meant that a large fraction was still in the recycling pathway in pools that turned-over in more than 1 day. These probably include proteins. Since glucose carbon is incorporated into these pools that are slowly turning-over, the fraction calculated to have recycled is affected by the length of the observations. We found that the fraction that recycled increased over 24 h in non-pregnant ewes, and over 48 h in pregnant sheep (Fig. 5). Experiments over shorter periods will underestimate the fraction recycled.

The model currently describes plasma glucose data from pregnant and non-pregnant sheep. In the future, feto-placental metabolism needs to be modelled separately and coupled to the maternal system. Preliminary models for the feto-placental system have been proposed by groups who have undertaken multiple-tracer experiments in the ewe and the fetus (Hodgson and Mellor 1977; Hodgson *et al.* 1980; Prior 1980). In addition, recycling needs to be analysed in terms of the compounds and pathways involved by incorporating data on the kinetics of the individual substrates, as has been done for alanine in humans (Hall *et al.* 1979).

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