SECTION 5

TOTAL ENTRY RATE AND RESYNTHESIS RATE OF GLUCOSE

Introduction

The use of tritiated glucose as a tracer for measuring glucose synthesis rate has been investigated with monogastric animals. Results obtained from these studies (see literature survey) indicate that [6-^{3}H]glucose corrects for resynthesis of glucose carbon from lactate and other precursors which give rise to hepatic oxaloacetate, whereas [2-^{3}H]glucose corrects for resynthesis of glucose carbon from all precursors. The use of the latter tracer may however, overestimate the total gluconeogenic rate if plasma glucose equilibrates extensively with liver glycogen.

Combinations of [6-^{3}H]glucose and [^{14}C]substrates have been used to study gluconeogenesis in the intact rat (Dunn *et al.*, 1969; Dunn, Chenoweth and Hemington, 1971) and, in the studies reported in Section 4, to measure effects of raising substrate availability on the gluconeogenic rate in sheep. In these studies with sheep it was found that estimates of the irreversible loss of glucose obtained with [6-^{3}H]glucose were only slightly greater than those obtained with [U-^{14}C]glucose. These results were interpreted to indicate that the extent of resynthesis of glucose

carbon in the fed sheep was quantitatively unimportant. In contrast, multiexponential analysis of the disappearance curve of carbon-14 from plasma glucose of sheep (White et al., 1969) and cattle (Kronfeld et al., 1971) given injections of [U-^{14}C]glucose indicate that glucose resynthesis may be a major process.

In view of the potential of the dual isotope technique, employing tritiated glucose as one of the tracers as a tool for studying gluconeogenesis in the intact animal, it was decided to investigate further the metabolism of tritiated glucoses in sheep. In the studies now presented [6-^{3}H]-, [3-^{3}H]- and [2-^{3}H]glucose were administered to sheep in order to establish whether these tracers could be used to measure total entry rate of glucose, and, in association with [U-^{14}C]glucose, the extent of glucose resynthesis. Formation of water appears to be a predominant early fate of the hydrogen from metabolised glucose (Shreeve, 1965; Katz and Dunn, 1967; Shreeve, Lamdin, Oji and Slavinski, 1967) and the dilution of tritium from glucose in a large pool of body water probably minimizes recycling of this tracer to the glucose pool. In order to assess the extent of this cycle, two sheep were also given injections of tritiated water.

Experimental

Sheep used in this study were each given 800 g lucerne chaff daily. At least 5 days before administration of radioactive tracers, the animals received their ration in 24 equal amounts at hourly intervals.

Four sheep were each given single injections of a mixture of [U-^{14}C]glucose (228 μCi) and [3-^{3}H]glucose (427 μCi) and three months later the same sheep were injected with a mixture of [U-^{14}C]glucose (194 μCi) and [2-^{3}H]glucose (207 μCi). After a further two months two of these sheep were given a single injection of a mixture of [U-^{14}C]glucose (218 μCi) and [6-^{3}H]-glucose (711 μCi) and the other two sheep received single injections of titriated water (30 mCi). Tracers were injected between 09.00 h and 10.00 h and samples of blood were usually taken at intervals of 5 or 10 min for the first 70 min, followed by samples at 30 min intervals for the next 5 h. Hourly samples were then taken up to 16 h post-injection and thereafter at 2 to 4 h intervals for the next 16 h.

A further six sheep received constant infusions of mixtures of [U-^{14}C]glucose and [6-^{3}H]glucose (see Section 4). These infusions were commenced between 05.00 h and 07.00 h and blood samples were taken at frequent intervals for up to 10 h during the infusion of tracers.

Formulae used in this study to calculate kinetic variable of glucose metabolism by multiexponential analysis of the SR-time curve of plasma glucose, following injections of labelled glucoses, have been summarized in Section 1 (i.e. equations 1-5, 1-6 and 1-10). The log (SR)-time curve of plasma glucose was also analyzed by fitting a straight line, by the method of least squares, to the initial rectilinear portion of the curve, which was apparent from about 20 min post-injection of labelled glucoses. Kinetic variables of glucose metabolism were calculated by assuming that recycling of tracer was negligible during this rectilinear phase and that a first-order dilution process applied (i.e. equations 1-1 and 1-2).

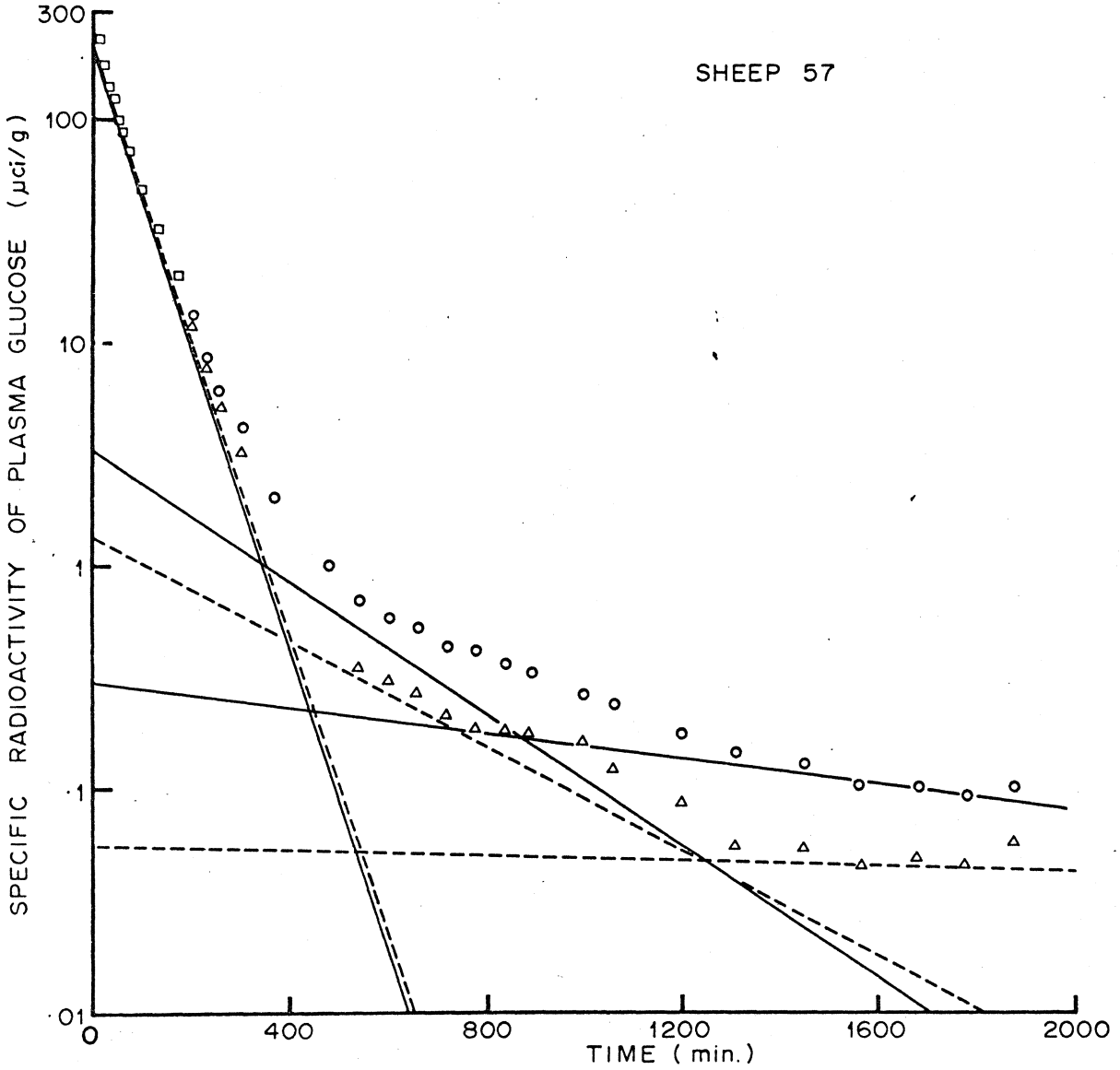
Results

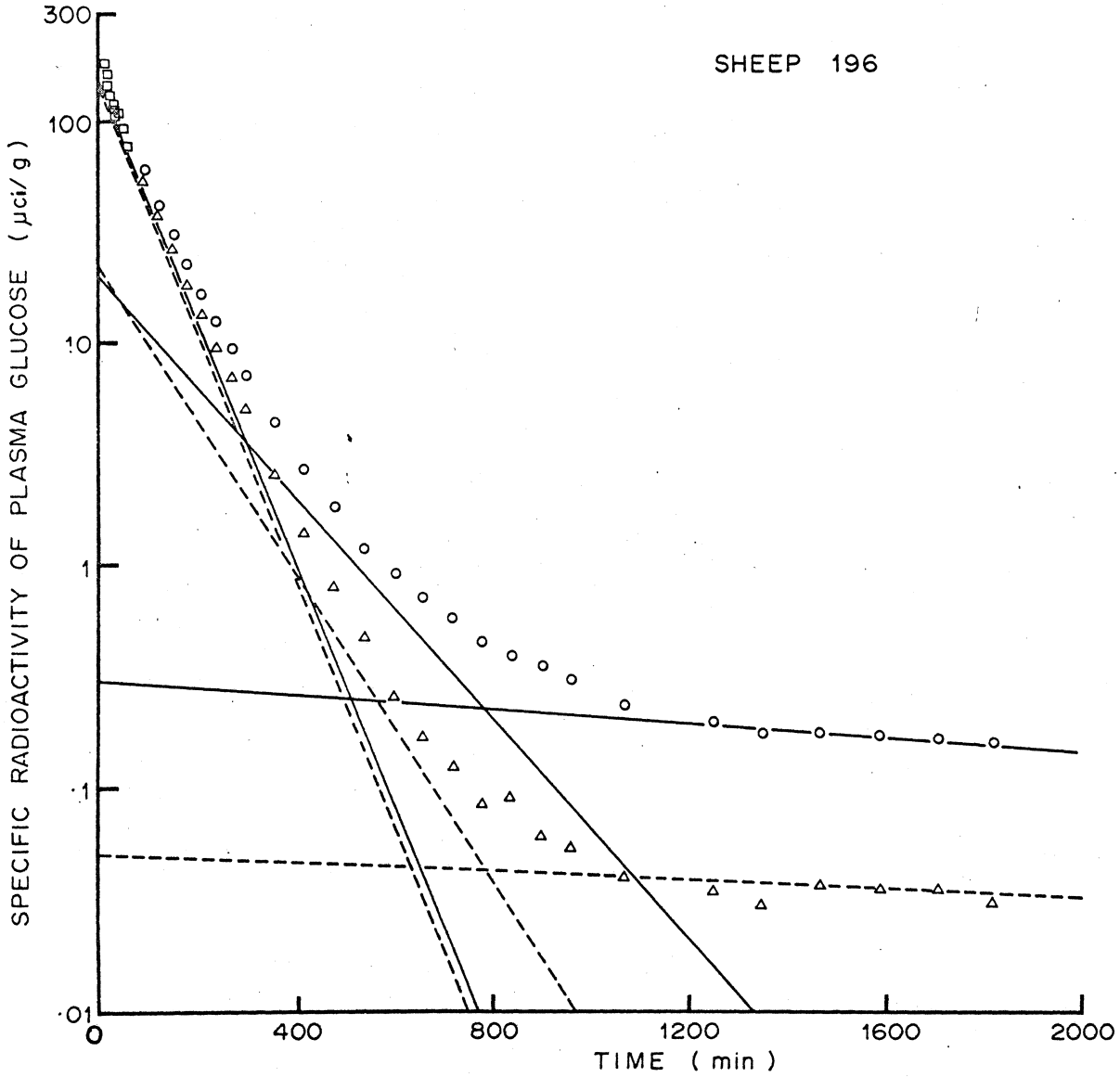
Description of SR-time Curves of Plasma Glucose

In the studies reported here glucose concentrations in plasma were found to be almost constant throughout any experimental period. The coefficient of variation of glucose concentration was less than 4%.

Figure 5-1 shows on semi-logarithmic co-ordinates examples of typical SR-time curves of plasma glucose following single injections of the mixtures of [U-^{14}C]glucose and [6-^{3}H-, [3-^{3}H]- or [2-^{3}H]glucose. These curves show an initial rapid decline,

Figure 5-1. Examples of the relationship between the log SR values of plasma glucose and time after injection of mixtures of [U-^{14}C]glucose and [6-^{3}H]glucose (a), [3-^{3}H]glucose (b), or [2-^{3}H]glucose (c). Where the SR of [^{14}C]glucose (o) and of [^{3}H]glucose (Δ) differ by less than 5% of the former value a common symbol (□) is used for clarity. Symbol (▽) indicates no tritium counts were detected in sample. The straight lines (——) and (– – –) represent the exponential functions that when summed give the line of best fit to the [^{14}C]glucose and [^{3}H]glucose dilution curves in plasma glucose respectively.


SHEEP 57
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci/g)
300
100
10
1
·1
·01
0
400
800
1200
1600
2000
TIME (min.)


SHEEP 196
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (µci/ g)
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100
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0
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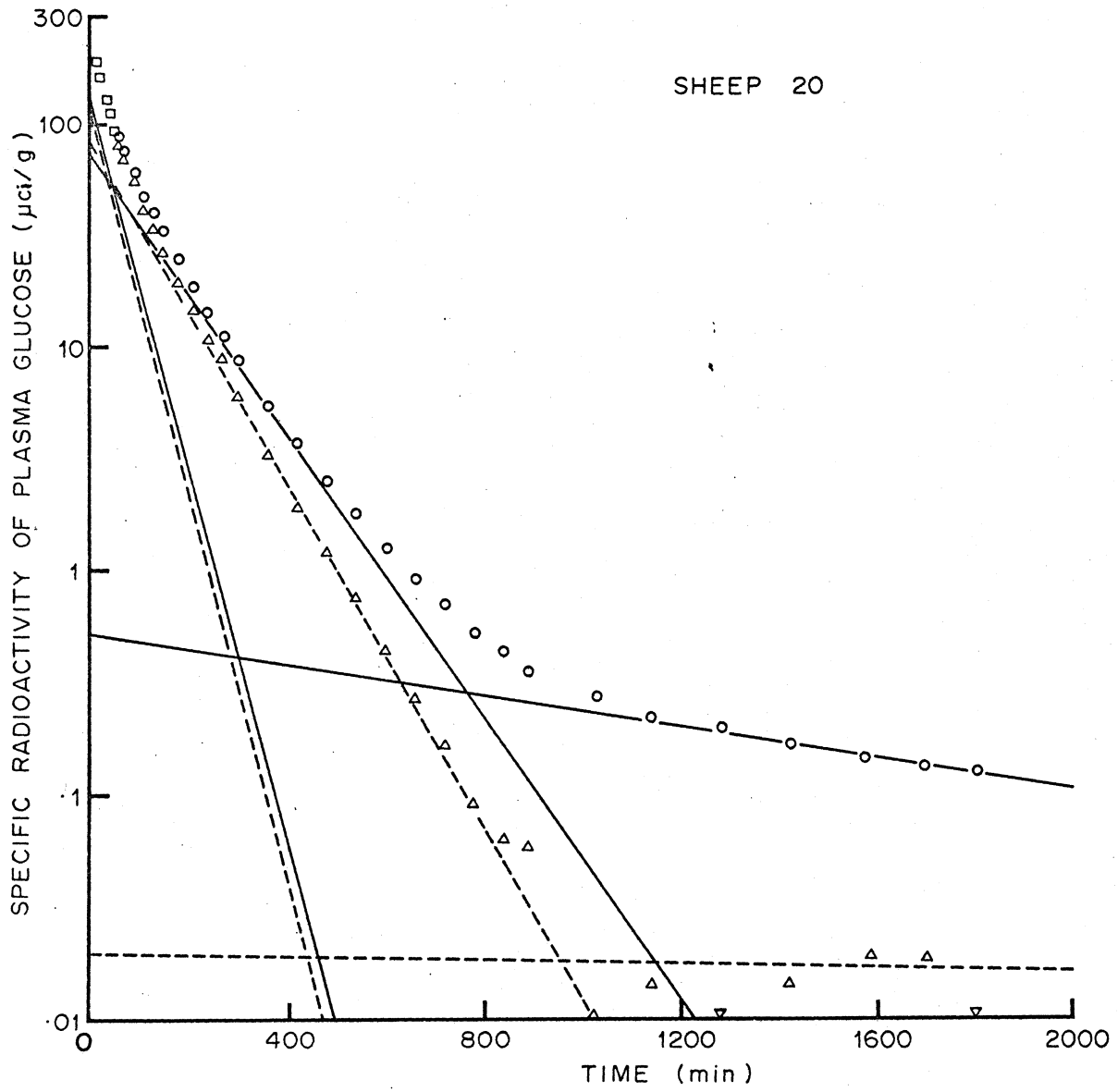
1200
1600
2000
TIME (min)


SHEEP 20
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (µci/g)
300
100
10
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·1
·01
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1200
1600
2000
TIME (min)

followed after about 20 min by a linear decay which gives way to a curvilinear change before a final, more horizontal component, which is evident approximately 17 h after the injection of tracer. The linear decay was apparent for up to about 1 h post-injection of [U-^{14}C]glucose and [6-^{3}H]glucose and for up to 2.2 h and 3.5 h post-injection of [3-^{3}H]glucose and [2-^{3}H]glucose respectively. The rapid component which preceded it was probably a result of physical mixing of tracers in the sampled pool and was excluded in the analysis of these curves.

All curves required at least three exponential components to eliminate systematic deviations between observed and calculated values. The calculated curve was generally found to be a good fit, except for the final observed component after injections of [2-^{3}H]glucose where the SR of plasma glucose was erratic with occasional samples having no detectable tritium activity. This variation account for a relatively large residual standard deviation of the calculated curve for injections of this tracer. Table 5-1 gives an example of these data for sheep. In general, it was found that the rate constants for the first and second exponential components were faster for tritium than for carbon-14 and that the zero-time intercepts for the third or slowest exponential component for tritium was less than one-third of the value recorded for carbon-14. This faster loss of tritium than

carbon-14 from plasma glucose was particularly evident following injections of [U-^{14}C]glucose with [2-^{3}H]- or [3-^{3}H]glucose but to a lesser extent with [6-^{3}H]glucose. However, the rate of loss of tritium and carbon-14 from plasma glucose was similar during the apparent mixing phase and part or whole of the rectilinear change of the log (SR)-time curve of plasma glucose. This rectilinear change was well described by a monoexponential component (see examples in Table 5-1).

Figure 5-2 shows a typical example of the SR-time curves of plasma glucose after the start of a constant infusion of a mixture of [6-^{3}H]glucose and [U-^{14}C]glucose. The SR of plasma glucose plateaued at similar times, between 2 and 4 h after the start of the infusion of these tracers. In all experiments the SR values recorded during the plateau period were, after correction for differences in the rate of infusion of tracers, consistently greater for [6-^{3}H]glucose than the corresponding SR values for [U-^{14}C]glucose.

Parameters of Glucose Metabolism

Table 5-2 gives the estimates of pool size and space of distribution of glucose in sheep injected with mixtures of [U-^{14}C]-glucose and [^{3}H]glucose. Examination of the data by analysis of variance and a Duncan's test of the means (Duncan, 1955) revealed that pool size and space were not significantly different ($P > 0.05$)

Table 5-1. Zero-time intercepts and rate constants of mono- and multiexponential components describing the specific radioactivity - time curve in plasma glucose of sheep 57 following intravenous injections of mixtures of [U-^{14}C]glucose and [6-^{3}H]-, [3-^{3}H]- or [2-^{3}H]glucose

Glucose injected	Multiexponential analysis								Monoexponential analysis				
	Time interval* (h)	Zero-time intercepts (μCi/g glucose) a_1	a_2	a_3	Rate constants (min^{-1}) ($x10^1$)	($x10^2$)	($x10^3$)	Residual standard deviation	Time interval* (h)	Zero-time intercept (μCi/g glucose) a_s	Rate constant (min^{-1}) m_s ($x10^1$)	Correlation coefficient	Residual standard deviation
[U-^{14}C]	.3-31 (31)	219	3.35	.301	.149	.354	.66	.075	.3-1.0 (5)	241	.168	.996	.0125
[6-^{3}H]	.3-31 (31)	221	1.38	.056	.150	.284	.17	.127	.3-1.0 (5)	238	.163	.996	.0113
[U-^{14}C]	.4-30 (35)	208	13.1	.329	.145	.590	.58	.064	.4-1.0 (8)	219	.140	.983	.0155
[3-^{3}H]	.4-30 (35)	172	49.4	.075	.172	1.02	.59	.099	.4-1.5 (9)	212	.147	.996	.0120
[U-^{14}C]	.5-32 (32)	98.2	81.1	.463	.135	.737	.57	.072	.5-1.0 (4)	171	.096	.983	.0121
[2-^{3}H]	.5-32 (32)	27.8	159	.111	.496	1.01	.24	.406	.5-3.5 (11)	170	.108	.999	.0072

* The number of observations is given in parenthesis.

Figure 5-2. Relationship between SR values of plasma glucose and time during a constant infusion of a mixture of [U-^{14}C]glucose and [6-^{3}H]glucose. o, SR of [^{14}C]glucose; Δ, SR of [^{3}H]glucose. The relationship between the SR of plasma [^{14}C]glucose (SR_c, ——) or plasma [^{3}H]glucose (SR_T, ---) and time (t, min) between the 160th and 670th min of the infusion had no significant slope ($P > 0.05$), as tested by least-squares regression analysis. These relationships were:

$$SR_c = 21.2 - 0.0026t$$

$$SR_T = 20.3 - 0.0023t$$

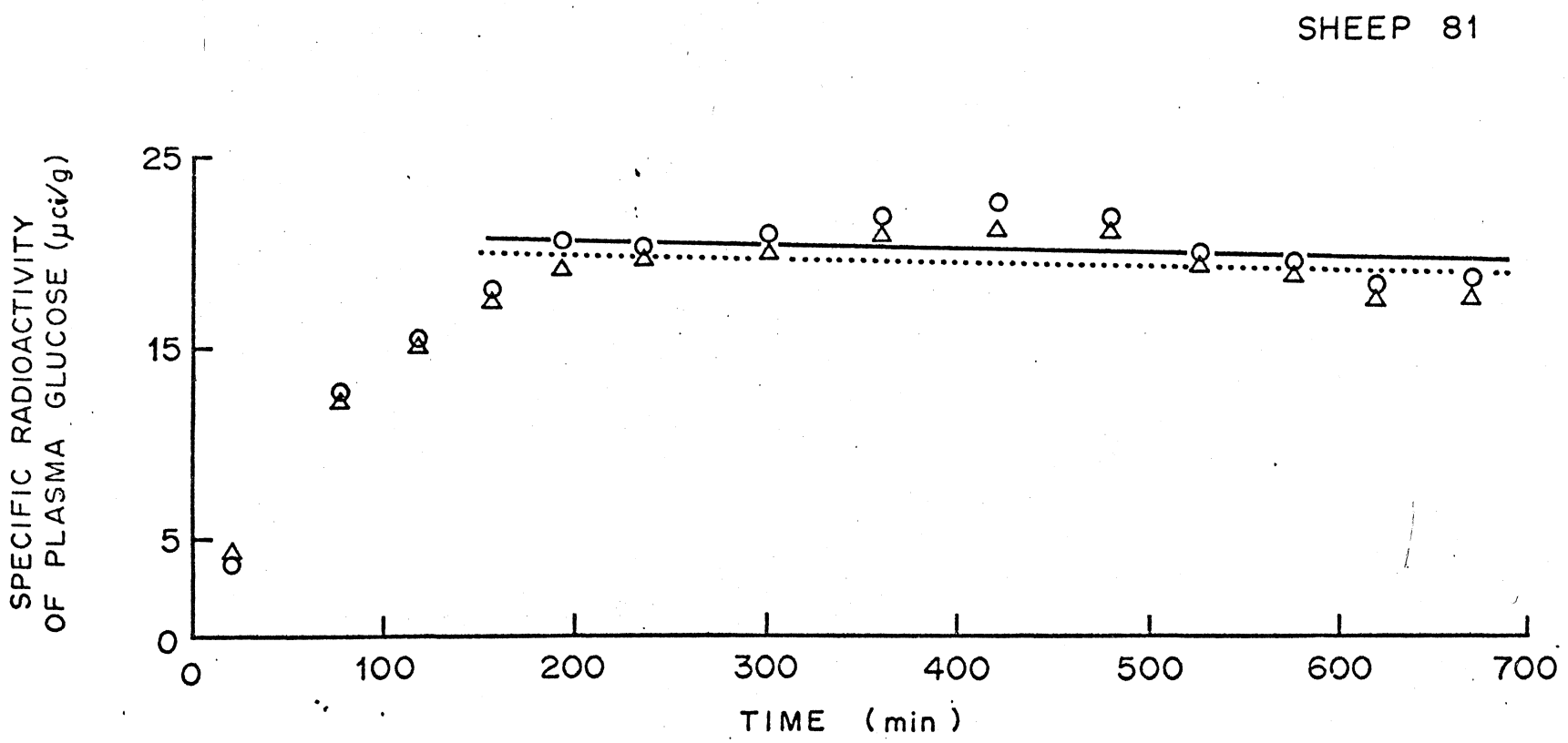

SHEEP 81
SPECIFIC RADIOACTIVITY
OF PLASMA GLUCOSE (μci/g)
25
15
5
0
0
100
200
300
400
500
600
700
TIME (min)

Table 5-2. Parameters of glucose metabolism in sheep estimated by using single intravenous injection of mixtures of [U-^{14}C]glucose and [6-^{3}H]-, [3-^{3}H]- or [2-^{3}H]glucose

Values are given as means with their standard errors

No. of sheep	Sheep wt (kg)	Plasma glucose (mg/100 ml)	Glucose injected	Multiexponential analysis				Monoexponential analysis			
				Pool size (g)	Space* (% of body wt)	Irreversible loss (mg/min)	Total entry rate (mg/min)	Pool size (g)	Space* (% of body wt)	Half-time (min)	Total entry rate (mg/min)
2	34±0.7	62±0.0	[U-^{14}C]	4.2±.34	20±1.0	58±4.2	62±4.3	4.1±.07	20±0.5	47±5.4	62±8.2
			[6-^{3}H]	4.1±.42	20±1.5	59±5.0		4.1±.12	20±0.5	48±5.2	60±8.3
4	32±1.1	65±1.9	[U-^{14}C]	4.9±.40	23±0.9	60±1.0	70±3.2	4.9±.36	23±0.6	49±2.9	69±2.1
			[3-^{3}H]	5.1±.43	24±0.6	68±1.9		5.0±.35	24±0.5	47±3.5	74±1.7
4	34±1.5	71±2.1	[U-^{14}C]	4.7±.11	21±1.0	55±3.0	65±4.0	5.0±.38	21±1.1	57±6.4	62±3.6
			[2-^{3}H]	4.5±.39	19±1.2	62±2.8		5.1±.35	21±1.0	54±5.1	67±3.3

* Space is defined as pool size (mg) x 100/plasma glucose concentration (mg/1) x body wt (kg).

when calculated by mono- or multi-exponential analysis of carbon-14 and tritium disappearance curves from plasma glucose following injections of [6-^{3}H]- or [3-^{3}H]glucose with [U-^{14}C]glucose. However, the measurement of these variables by multiexponential analysis of the tritium decay curve following injections of [2-^{3}H]glucose with [U-^{14}C]glucose was significantly less ($P < 0.05$) than when estimated by monoexponential analysis of the tritium and carbon-14 decay curves in plasma glucose but not when estimated by multiexponential analysis of the carbon-14 decay curve.

Kinetic parameters of glucose metabolism are given in Tables 5-2 and 5-3 for single injections and constant infusions of labelled glucose respectively. Irreversible loss of plasma glucose measured with single injections of [3-^{3}H]- or [2-^{3}H]glucose was significantly greater ($P < 0.05$) than with [U-^{14}C]glucose when tested by analysis of variance and Duncan's test of the means. Similarly, the irreversible loss of glucose when measured by using single injections or constant infusions of [6-^{3}H]glucose was significantly greater ($P < 0.01$, paired t-test) than when measured simultaneously with [U-^{14}C]glucose. The mean estimate (with standard error) of the irreversible loss of glucose for sheep given single injections of [U-^{14}C]glucose of 58 $\pm$ 1.0 mg/min and of 55 $\pm$ 1.4 for sheep given constant infusions of [U-^{14}C]glucose (see Tables 5-2 and 5-3) was not significantly different ($P > 0.05$,

Table 5-3. Irreversible loss of plasma glucose and its contribution to blood bicarbonate production of sheep estimated by using an intravenous infusion of a mixture of [U-^{14}C]glucose and [6-^{3}H]glucose

Sheep no.	Sheep wt (kg)	Plasma glucose* (mg/100 ml)	Irreversible loss of glucose (mg/min) [U-^{14}C]	[6-^{3}H]	Bicarbonate derived from glucose** (%)
81	33.4	62 ± 0.9	49.8	51.5	9
114	34.1	57 ± 0.2	53.2	56.8	-
191	40.1	58 ± 0.8	62.1	66.7	-
191	38.7	60 ± 0.3	54.6	57.5	11
175	31.1	67 ± 0.8	62.1	67.1	-
175	31.2	65 ± 1.0	50.8	62.9	13
143	30.2	62 ± 0.8	54.6	56.2	12
140	32.7	58 ± 1.0	58.8	60.6	14
Mean for sheep	33	61	55	58	12
Standard error	± 1.3	± 1.4	± 1.4	± 1.6	± 0.9

* Mean values with their standard errors for four to ten samples.

** Calculated by comparing the plateau specific radioactivity of bicarbonate carbon of jugular blood and plasma glucose carbon.

t-test).

Total entry rates of glucose calculated by monoexponential analysis of the tritium decay curves and mono- or multiexponential analysis of the carbon-14 decay curves following injections of [U-^{14}C]glucoses with [^{3}H]glucoses were not significantly different ($P > 0.05$). Further, the irreversible loss of plasma glucose estimated with single injections of [3-^{3}H]- or [2-^{3}H]glucose was not significantly different ($P > 0.05$) from estimates of the total entry rate of glucose by using mono- or multiexponential analysis of the carbon-14 data, obtained with simultaneous injections of [U-^{14}C]glucose. However, this irreversible loss was significantly less ($P < 0.05$) than the total entry rate of glucose when calculated by monoexponential analysis of the tritium decay curves. Half-times for the rate of loss of tritium from plasma glucose by mono-exponential analysis was consistently less than carbon-14 for [3-^{3}H]- or [2-^{3}H]glucose with [U-^{14}C]glucose, but these differences were not significant ($P > 0.05$, paired t-test).

Differences between the total entry rate of glucose as calculated by mono- and/or multiexponential analysis of tracer dilution curves in plasma glucose or the irreversible loss of glucose measured with [^{3}H]glucoses and the irreversible loss of glucose measured simultaneously with [U-^{14}C]glucose (see Tables

*Table 5-4. A summary of the estimates of glucose resynthesis in sheep**

Mean values for sheep with standard errors

Glucose administered	No. of sheep	Resynthesis rate of glucose (mg/min)** Multiexponential analysis	Monoexponential analysis
[U-^{14}C]	4	9 ± 2.9 (16)	8 ± 1.7 (13)
[6-^{3}H]	8	2 ± 0.4 (4)	5 ± 4.1 (4)†
[3-^{3}H]	4	8 ± 1.1 (13)	14 ± 1.3 (23)
[2-^{3}H]	4	7 ± 0.6 (13)	12 ± 0.5 (22)

* Resynthesis rate is calculated as the difference between the irreversible loss of glucose, measured with [U-^{14}C]-glucose and other kinetic parameters of glucose metabolism also determined with [U-^{14}C]glucose or simultaneously with [^{3}H]glucose (see Tables 5-2 and 5-3).

** Mean values for sheep for the resynthesis rate, expressed as a percentage of the irreversible loss of glucose measured with [U-^{14}C]glucose are given in parentheses.

† Mean values for two sheep.

5-2 and 5-3) provide estimates of the extent of glucose resynthesis, which are summarised in Table 5-4.

Transfer of Carbon-14 between Plasma Glucose and Blood Bicarbonate

The log (SR) of bicarbonate carbon of jugular blood increased rapidly to attain a peak value between 70 and 90 min following the intravenous injection of [U-^{14}C]glucose, before giving way to a multiexponential decline. The final exponential component of this decline was similar in slope and intercept to the slowest exponential component recorded simultaneously for plasma glucose carbon. The SR (bicarbonate carbon) - time curve was well described by an equation of the form:

$$SR_t = -a_i e^{-m_1 t} + a_2 e^{-m_2 t} + a_3 e^{-m_3 t} \qquad \ldots 5\text{-}1$$

Comparison of the integral, from time of injection of [U-^{14}C]glucose to time approaching infinity for the SR-time curves of glucose carbon and bicarbonate carbon allows calculation of the percentage of carbon in blood bicarbonate derived from glucose (see Kleiber, 1952). Estimates derived in this way were 13 to 18% which are similar to those of 9 to 14% obtained with constant infusions of [U-^{14}C]glucose by comparing the plateau SR values of carbon in blood bicarbonate and plasma glucose (Table 5-3). The SR of bicarbonate of jugular blood plateaued from 7 to 8 h after the start of an intravenous infusion of [U-^{14}C]glucose.

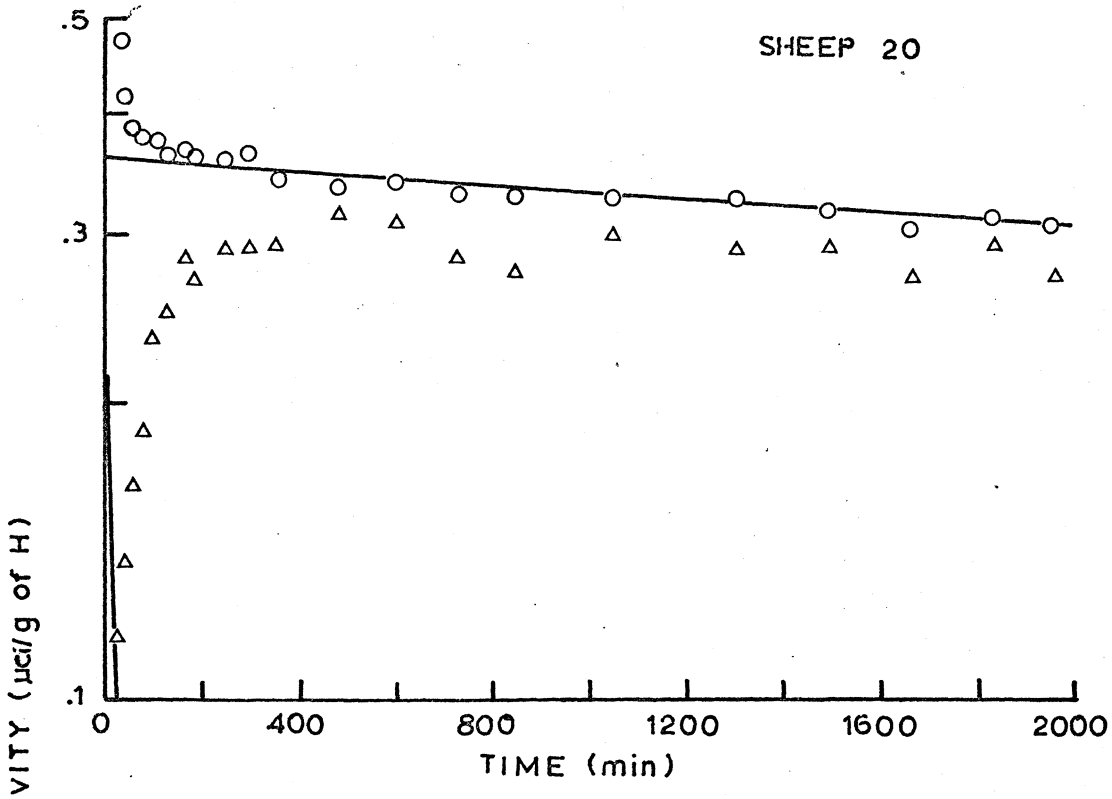
The mean percentage (with standard error) of carbon in bicarbonate arising from plasma glucose was 13 ± 1.1 for the seven experiments An estimate of the extent of reincorporation of this carbon into plasma glucose was calculated, according to the procedure of Depocas and De Freitas (1970), by using values for the fraction of carbon in plasma glucose derived from blood bicarbonate and the irreversible loss of blood bicarbonate of 14% and 144 mg carbon/min respectively, found for sheep given the lucerne diet (see Section 4, Part A). Figure 5-5 gives the calculated transport rates of direct formation, conversion and irreversible disposal of glucose and bicarbonate in the two pools considered. It is evident from these calculations that the magnitude of resynthesis of glucose carbon from bicarbonate was equivalent to about 2% of the irreversible loss of glucose carbon.

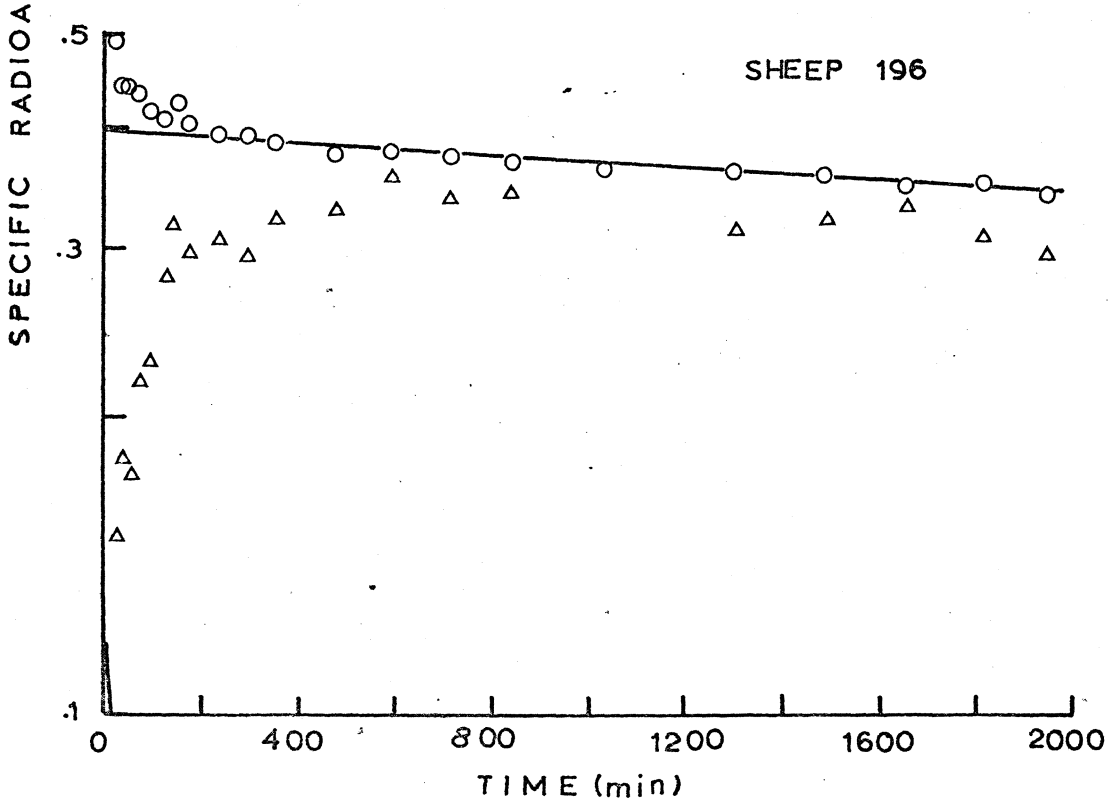
Transfer of Tritium between Body Water and Plasma Glucose

In order to estimate the percentage of tritium appearing in body water following injections of [^{3}H]glucose and the resynthesis of carbon-bound hydrogen of plasma glucose from body water, two sheep were given intravenous injections of tritiated water.

Figure 5-3 shows on semi-logarithmic co-ordinates the SR-time curve of hydrogen of blood water and plasma glucose for

Figure 5-3. Relationships between the log SR values of hydrogen of blood water (Δ) and plasma glucose (o) and time after an intravenous injection of tritiated water. The straight lines (——) represent the exponential functions that when summed give the line of best fit to the tritium dilution curve in blood water.





SPECIFIC RADIOACTIVITY (μci/g of H)

each sheep. The SR-time curve of water hydrogen showed an initial curvilinear component which gave way between 3 and 5 h post-injection of tracer, to a more rectilinear component, with a half-time of approximately 7.5 days. The curves were well described by two exponential functions and the respective a and m values were used to calculate the transport rates and pool sizes of water hydrogen (see Appendix, Section 5).

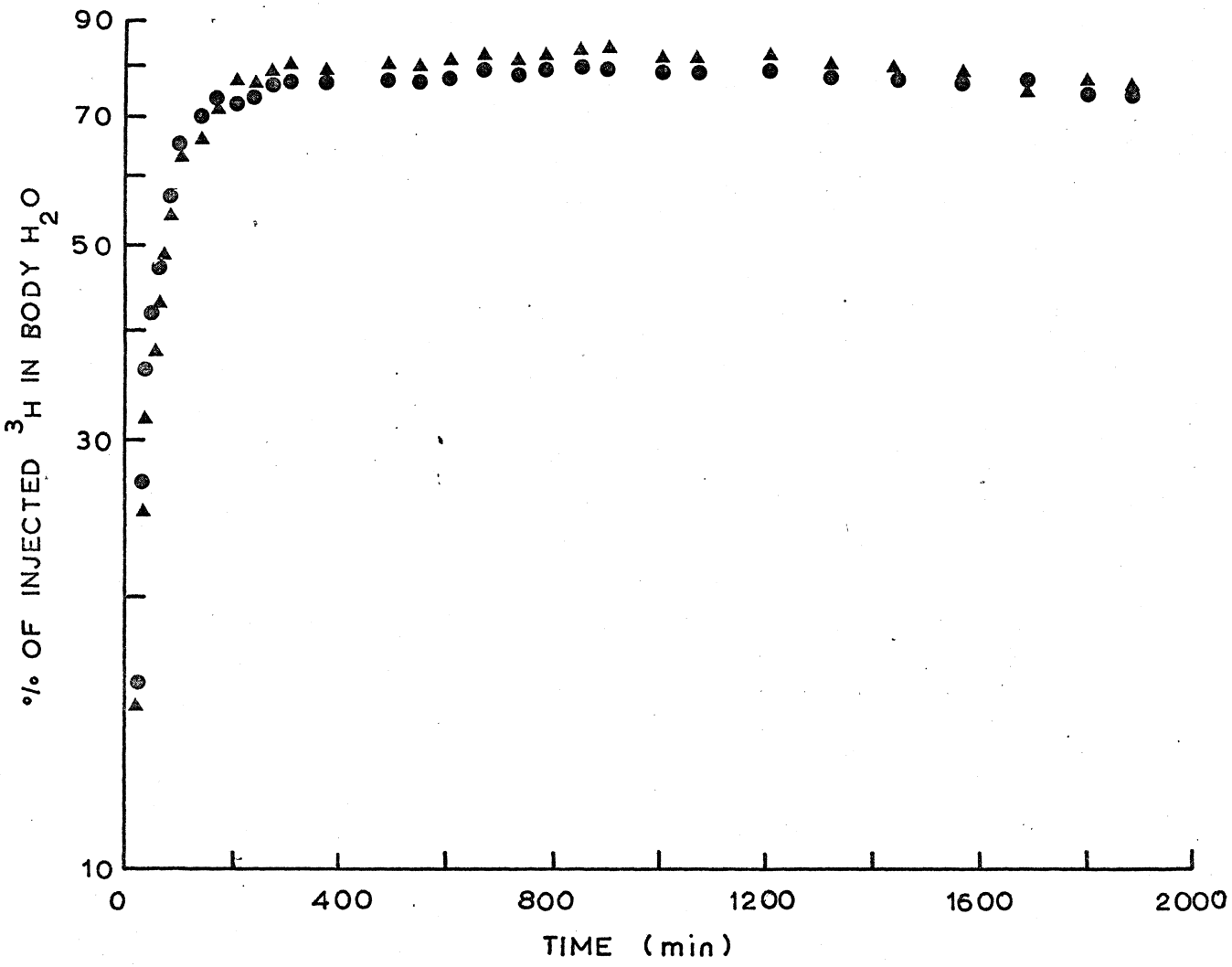
The SR of carbon-bound hydrogen of plasma glucose increased rapidly over the first 2 h and continued to rise for up to 6 to 7 h following intravenous injections of tritiated water (see Figure 5-3). Thereafter the change in the SR of hydrogen of glucose was similar to that of water and was, during this period, 89 $\pm$ 1.1% (mean with standard error for ten observations) and 92 $\pm$ 2.5% of the SR of water hydrogen for sheep 20 and 196 respectively.

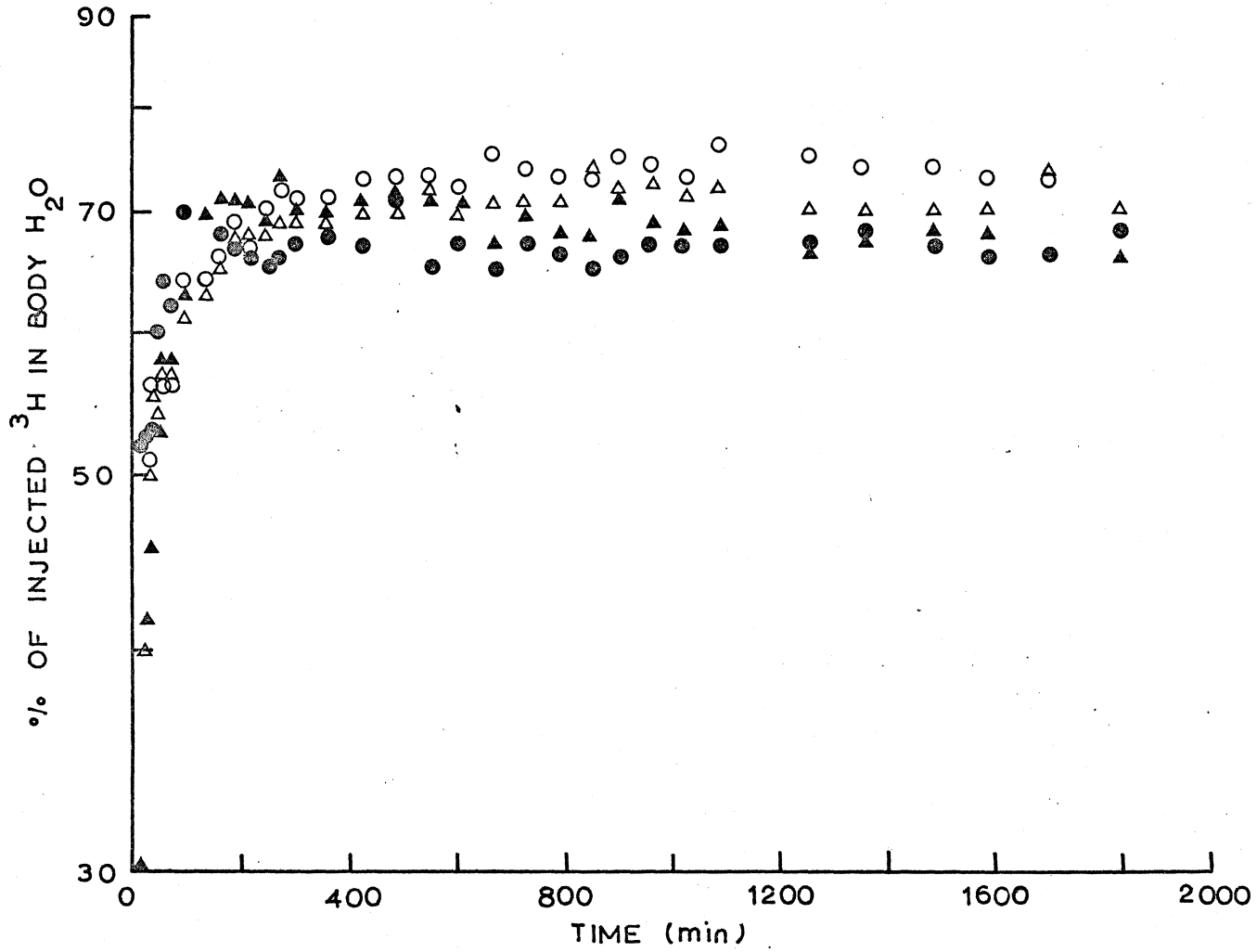
Similarly, the SR of water hydrogen increased rapidly over the first 1 to 2 h post-injection of [^{3}H]glucoses, especially following injections of [3-^{3}H]- and [2-^{3}H]glucose. Maximal SR in water was attained after 5 to 7 h and thereafter remained essentially constant for injections of [6-^{3}H]- and [3-^{3}H]glucose but declined slowly for injections of [2-^{3}H]glucose. The fraction of tritium injected as [6-^{3}H]-, [3-^{3}H]- or [2-^{3}H]glucose

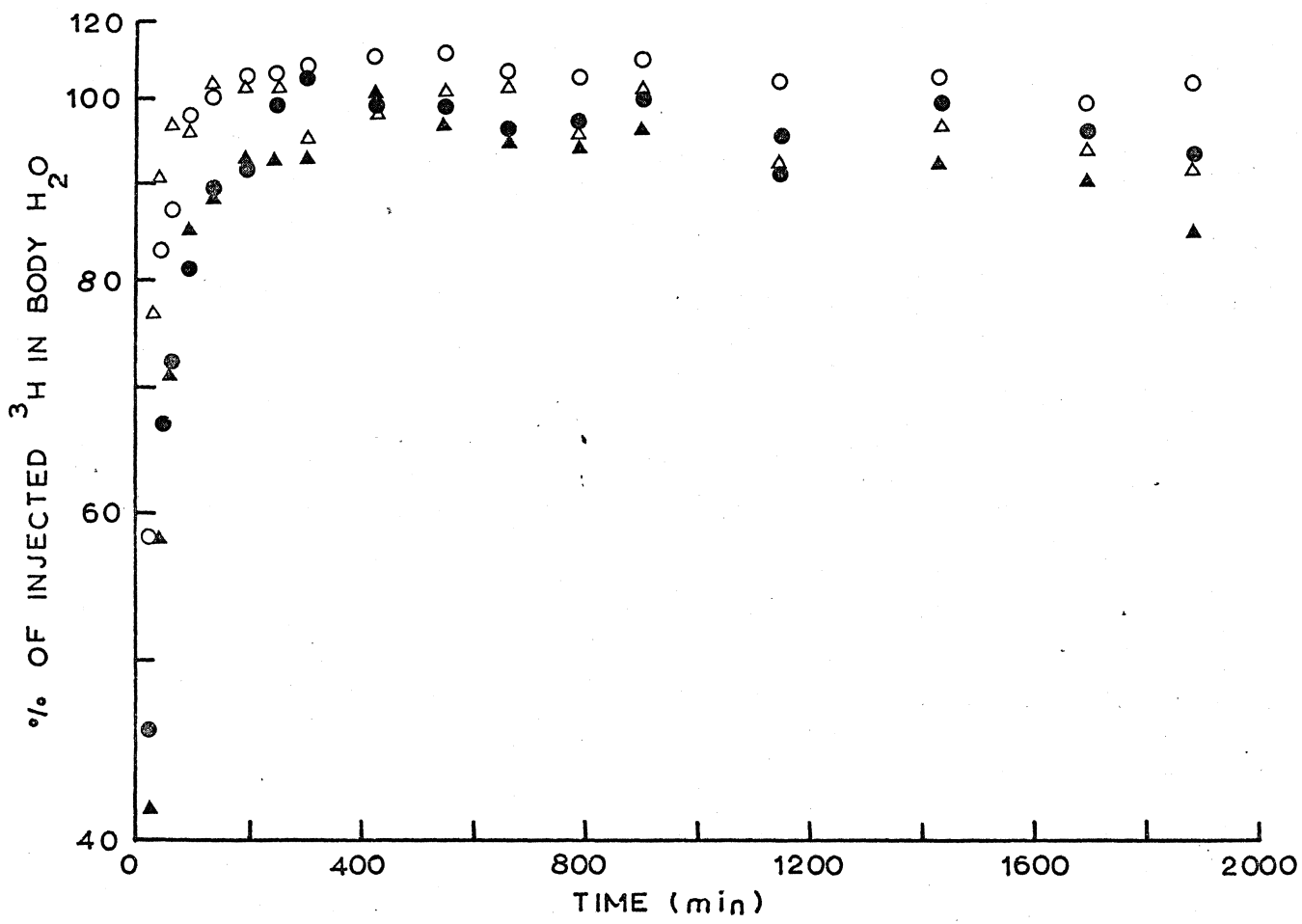
and appearing in water was calculated, and the results are presented in Figure 5-4. It was assumed that the tritium was distributed in the total pool of exchangeable water hydrogen equivalent to 7.4% of body weight (see Appendix, Section 5). The percentage of injected tritium in water at 7 h, when mixing of tritium in body water should have been completed, was approximately 79, 70 and 103 for injections of [6-^{3}H]-, [3-^{3}H]- and [2-^{3}H]glucose respectively, whereas tritium retained in the sampled pool at 7 h was usually less than 1% of the injected dose.

The effect of re-incorporation of tritium from body water on the disappearance curve of tritium from plasma glucose following injections of [^{3}H]glucose, was estimated by deducting 91% of the SR value of water hydrogen from the corresponding value of glucose hydrogen (see above). The resulting curve differed only slightly from the original curve; the exponential components were similar in slope and intercept except for the intercept of the slowest component, which was usually reduced by one-quarter to one-third of its original value. The percentage increase in the irreversible loss of plasma glucose determined with injections of [6-^{3}H]-, [3-^{3}H]- and [2-^{3}H]glucose after correction for this recycling of tritium through body water was 0.3 $\pm$ 1.5 (mean and standard error), 0.9 $\pm$ 0.2 and 0.6 $\pm$ 0.4 respectively.

Figure 5-4. Relationship between percentage of injected tritium in body water and time after injections of [6-^{3}H]glucose (a), [3-^{3}H]glucose (b) and [2-^{3}H]glucose (c). Symbols o, Δ, ● and ▲ represent sheep 20, 196, 170 and 57 respectively.


90
70
50
30
10
% OF INJECTED ^3H IN BODY H_2O
0
400
800
1200
1600
2000
TIME (min)


90
70
50
30
% OF INJECTED ^{3}H IN BODY H_2O
0
400
800
1200
1600
2000
TIME (min)


120
100
80
60
40
% OF INJECTED ^3H IN BODY H_2O
0
400
800
1200
1600
2000
TIME (min)

Discussion

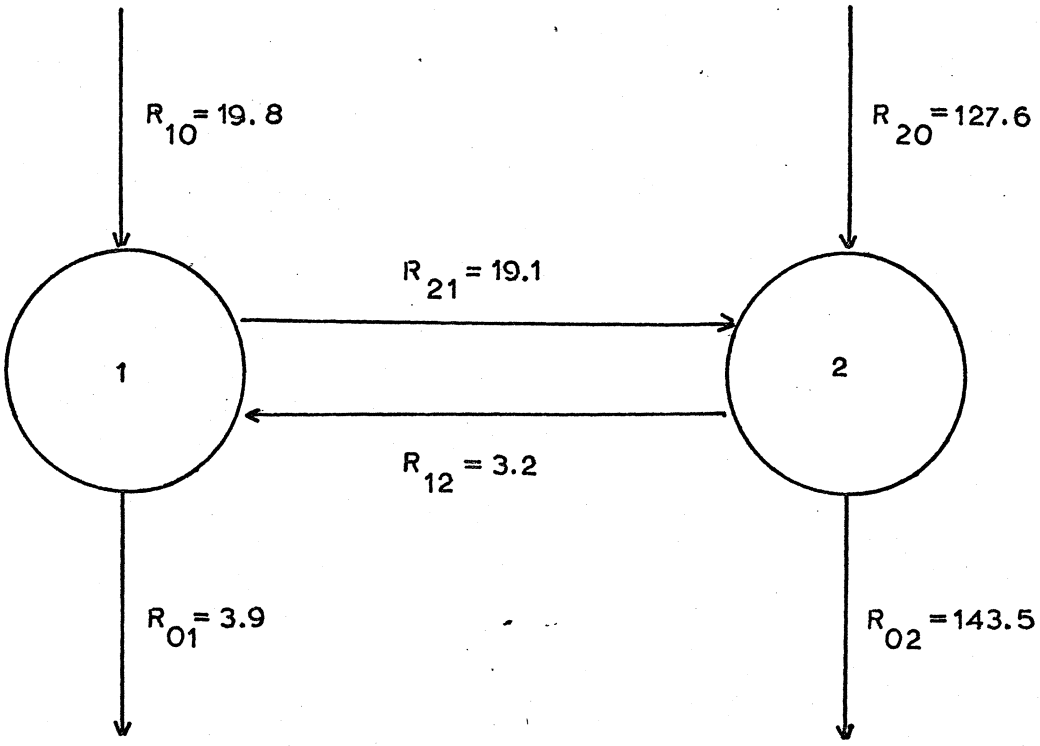
The irreversible loss of [U-^{14}C]glucose from plasma at steady state conditions apparently underestimates the total entry rate of glucose to the extent that glucose exchanges carbon reversibly with other substrates during the experimental period. Estimates of the irreversible loss of glucose with [6-^{3}H]-, [3-^{3}H]- or [2-^{3}H]glucose were found to be approximately 4, 13 and 13% respectively greater than when measured simultaneously with [U-^{14}C]glucose (see Table 5-4). The similarity in estimates of the irreversible loss of glucose obtained with [3-^{3}H]- and [2-^{3}H]-glucose suggests (see literature survey) that the interconversion of plasma glucose and liver glycogen contribute little to the recycling of glucose carbon, which is consistent with reports that the liver of ruminants has no net uptake of glucose and no glucokinase activity. Hence short-chain products of glucose metabolism appear to be the major sources for recycled glucose carbon. These compounds become labelled by and therefore also apparently return hydrogens from position 6 of glucose to the glucose pool.

Lactate is probably the most important product of glucose catabolism that is not exclusively intracellular and its importance as a source for recycling glucose carbon in monogastric animals has been demonstrated (see literature survey). In fed sheep,

lactate carbon enters the oxaloacetate pool in the liver along with substantial quantities of carbon from propionate and amino acids. However, it appears that resynthesis of glucose carbon from lactate may be a major process in these animals since Annison et al. (1963) reported that 15% of the glucose carbon equilibrated with 40% of the lactate carbon. In the present study it was shown that CO_2 from glucose oxidation can also be reincorporated into glucose. The magnitude of this process was, however, equivalent to only about 2% of the irreversible loss of glucose carbon (Figure 5-5).

Dunn et al. (1967, 1968) reported that the rate of loss of tritium from [6-^{3}H]glucose in the intact rat is a reflection of the physiological activity of the enzymes involved in the dicarboxylic acid shuttle. In the present studies it appears unlikely that the equilibration of oxaloacetate with malate and fumarate was not sufficiently extensive to minimize recycling of tritium from position 6 of glucose through lactate, pyruvate, alanine or other precursors of oxaloacetate since about 91% of the carbon-bound hydrogens of plasma glucose were derived from body water. Hydrogen exchange during the formation of glucose from phosphoenolpyruvate directly affects only the hydrogens on carbons 2, 3, 4 and 5 (see Saur et al., 1968a; Smith, 1969). Hydrogen exchange at position 6 may take place in part at the

Figure 5-5. Schematic presentation of the two-compartmental model used for the estimation of glucose oxidation and the reversible exchange of carbon between plasma glucose and blood bicarbonate in sheep. Compartments 1 and 2 are simplified representations of the glucose and bicarbonate pools respectively. R_{ij} denotes the transport rate of carbon (mg/min) from compartment j to compartment i. R_{10}, the net entry rate of glucose from substrates other than from bicarbonate; R_{20}, the net entry of bicarbonate from substrates other than from glucose; R_{21} and R_{12}, the conversion of glucose into bicarbonate and of bicarbonate into glucose respectively; R_{01}, the irreversible loss of glucose by pathways which do not involve bicarbonate; and R_{02}, the irreversible loss of bicarbonate by pathways which do not involve glucose.


$R_{10} = 19.8$
$R_{20} = 127.6$
$R_{21} = 19.1$
1
2
$R_{12} = 3.2$
$R_{01} = 3.9$
$R_{02} = 143.5$

hexose 6-phosphate level by recombination of the two isomerised triose phosphate and subsequent dephosphorylation to fructose 6-phosphate in which carbons 1 and 6 have been interchanged. However, replacement of hydrogens on carbons 1 and 6 can only occur with extensive equilibration of oxaloacetate with malate and fumarate. The remaining 9% of glucose hydrogens probably largely represent glucose absorbed intact from the digestive tract.

It is possible that the difference in estimates of the irreversible loss of glucose obtained in the present study with [6-^{3}H]- and [3-^{3}H]glucose reflects in part the resynthesis of glucose carbon from glycerol. This substrate enters the gluconeogenic pathway distal to oxaloacetate, giving rise to dihydroxyacetone 3-phosphate, and hence it would return tritium when [6-^{3}H]glucose was used as a tracer (see literature survey). Little glycerol is apparently absorbed from the digestive tract of ruminants (Bath and Hill, 1967) and endogenous precursors of plasma glycerol probably include glucose as shown in lactating cows for triglyceride-glycerol of milk fat (Luick and Kleiber, 1961; Tombropoulos and Kleiber, 1961). Bergman _et al._ (1968) reported that glycerol provides about 5% of the glucose carbon in the fed sheep, rising to about 23% during starvation. However, resynthesis of glucose from glycerol during the experimental

period may have provided a significant proportion of the carbon recycled in the fed animal. De Freitas and Depocas (1970) showed that in the postabsorptive and fasted rat the resynthesis of glucose carbon from circulating glycerol was equivalent to approximately 11 and 4% of the irreversible loss of glucose carbon respectively.

Pool sizes of glucose determined by mono- or multi-exponential analysis were similar and the space of distribution of glucose of 17 to 26% of body weight agrees closely with values of 16 to 25% for thiosulphate space of fed ewes (Holmes and English, 1969) indicating that the monoexponential analysis gives meaningful results (see also Leng, 1970a). In accord with the suggestions of Baker and Huebotter (1964), Shipley et al. (1967) and White et al. (1969) it is proposed that the sampled compartment of glucose represents a single well-mixed pool of glucose in plasma and interstitial fluid. Hence total entry rate of glucose into this pool is of physiological significance as it approximates the total rate of synthesis and absorption of glucose at steady-state conditions, which is contrary to the suggestions made by Kronfeld et al. (1971). It is unlikely that the sampled pool of glucose measured in the present study did not include most of the glucose in extracellular fluid since estimates of the total entry rate of glucose into this pool by multi- or monoexponential analysis were

similar to the irreversible loss of plasma glucose, measured simultaneously with [3-^{3}H]- or [2-^{3}H]glucose (Table 5-2). If the rate of disappearance of tracer from plasma glucose from about 20 min post-injection of the tracer was affected by mixing this would result in a major error in estimating total entry rate but not in estimating the irreversible loss which is inversely proportional to the total area under the SR curve and imprecise determination of the initial decline probably contributes little to the area. However, estimates of the total entry rate of glucose by monoexponential analysis of the early part of the [3-^{3}H]- and [2-^{3}H]glucose dilution curves were slightly, but consistently greater than for the [U-^{14}C]glucose dilution curves. This difference probably indicates that the initial loss of carbon-14 from plasma glucose (and tritium after injections of [6-^{3}H]glucose is affected to a small extent by rapid recycling of tracer through glucose derivatives.

The presence of three exponential components in the [^{14}C]glucose disappearance curve from plasma glucose indicates that substrates through which glucose carbon recycles in sheep can be represented by at least two compartments which differ in their rate to return glucose carbon to the sampled compartment (White _et al._, 1969). The first of these apparent precursor compartments may include substrate pools with high fractional

turnover rates such as plasma lactate, glycerol and carbon dioxide. The second apparent precursor compartment may represent a larger pool of carbon which slowly exchanges glucose carbon. In this respect Vrba (1964) and Shipley et al. (1967) have shown from whole-body analysis of mice and rats respectively, that glucose carbon was readily incorporated into many tissue components which could be regarded as 'sinks' because of their large size and slow turnover rate relative to plasma glucose.

The estimated percentage of injected tritium appearing in water approximately 7 h after injections of [6-^{3}H]-, [3-^{3}H]- and [2-^{3}H]glucose, when mixing of tritium with total water hydrogen in the two pools should have been completed, was about 79, 70 and 103 respectively. These estimates can only be regarded as approximate since large differences may have occurred in the water content of the intestinal tract of sheep but it is apparent that about 20 to 30% of the tritium injected as [6-^{3}H]- or [3-^{3}H]-glucose was not promptly released to body water, indicating a loss of intermediates of glucose catabolism between glucose and triose-phosphate. This proportion of the glucose carbon, apparently incorporated into substrate sinks, is in close agreement with the approximate estimate of the proportion of carbon irreversibly

lost from the glucose pool by pathways other than by oxidation of 15% (see Figure 5-5) and of estimates of 15 to 44% for fed sheep (Ford and Reilly, 1969). The latter values were based on measurement of $^{14}CO_2$ in respired air. It is possible that some carbon-bound tritium from positions 3 and 6 of glucose was also lost from the system in urine (see Shipley et al., 1967) and that tritium from carbon 3 was incorporated into products formed with reduced NADP, since in the catabolism of [3-^{3}H]glucose via the pentose phosphate pathway, the tritium is transferred to NADP (Katz et al., 1965). The pentose phosphate pathway is apparently operative in most tissues of the ruminant (Raggi, Hanson, Simesen, Kronfeld and Luick, 1961; Moss, 1964). However, much of the carbon-bound tritium from positions 3 and 6 of glucose was probably retained in muscle and liver glycogen, as shown by Dunn et al. (1967) for [6-^{3}H]glucose injections in rats, in triglyceride-glycerol of adipose tissue and in the carbohydrate moiety of structural components of cells. Annison et al. (1963) found little incorporation of carbon-14 into hepatic and muscle glycogen of sheep 24 h after feeding and given intravenous infusions of [U-^{14}C]glucose but these sheep were probably depleting glycogen reserves and/or the method of extraction of glycogen for analysis may have removed most of the carbon-14 incorporated into glycogen (see Lindsay, 1970). Katz and Rognstad (1966) have observed

retention of tritium from carbon 3 (as well as from carbon 6) of glucose in glycerol of adipose tissue *in vitro*, although the rates of triose isomerase were very high relative to glycerol synthesis. They suggested that the mechanisms of this enzyme in removing tritium from position 1 of dihydroxyacetone 3-phosphate may be different from its mechanisms in liver and muscle tissue. The continued release of injected tritium to body water late in the experimental period, as indicated by the slower rate of loss of tritium from body water for injections of [6-^{3}H]- or [3-^{3}H]glucose than for injections of [2-^{3}H]glucose (see Figure 5-4) or tritiated water, may have resulted from the turnover of these substrate sinks of glucose intermediates, some of which may have returned carbon-bound tritium to the glucose pool.

Recycling of tritium attached to carbons 2, 3 and 6 of glucose is the most likely explanation for the extraction of more than one exponential component from the disappearance curve of these labelled glucoses from plasma. Reincorporation of tritium into glucose from body water was shown to have little effect on the initial or fastest exponential components (see Figure 5-1) although it was partly responsible for the appearance of the slowest exponential component in these curves.

Recycling of tritium from position 2 of glucose may occur

if the tritium transferred from carbon 2 of glucose 6-phosphate to the 'iso' position of carbon 1 of fructose 6-phosphate was not completely labilised with the interconversion of these hexose phosphates or, if as discussed by Katz and Rognstad (1969), the glucose 6-phosphate was catabolised via the pentose phosphate pathway where the tritium can be distributed to carbon 3 and the 'glu' position of carbon 1 of fructose 6-phosphate. The subsequent fate of these tritiums on the hexose phosphates should be similar to that described for tritium on carbons 3 and 6 of glucose. Katz and Rognstad (1969) reported that [2-^{3}H]glucose metabolised in adipose tissue of rats, in contrast to liver (Bloom and Foster, 1964; Hoberman and D'Adamo, 1960) and muscle tissue (Rose and O'Connell, 1961), does not lose many of its protons to water because the phosphoglucoisomerase is less active in this tissue. However, the redistribution of tritium from carbon 2 of glucose to carbons 1 and 3 of hexose phosphates was probably not extensive as this tritium appeared rapidly and quantitatively in body water (Figure 5-4) as was also shown for rats injected with this tracer (Katz and Dunn, 1967). Hence it is doubtful whether this apparent recycling of tritium from carbon 2 of glucose was of sufficient magnitude to invalidate estimates of the total entry rate of glucose by monoexponential analysis, especially as the initial disappearance

rate of tritium from plasma glucose, which was also similar for [3-^{3}H]glucose, was maintained for up to 3.5 h post-injection of [2-^{3}H]glucose.

Total entry rate of glucose as measured by monoexponential analysis of the early part of the dilution curves of tracer in plasma glucose following injections of [3-^{3}H]- or [2-^{3}H]glucose was about 23% greater than irreversible loss of carbon-14 from glucose (Table 5-4), indicating that about 19% of the glucose carbon was resynthesised. This agrees with the estimates of the total rate of glucose resynthesis by compartmental analysis of the carbon-14 dilution curve in plasma glucose of fed sheep of 14% (present study) and 21% (White _et al._, 1969) and of 18% for fasted cows (Kronfeld _et al._, 1971).

The present studies suggest that tritiated glucose may be employed as a tracer for estimating parameters of glucose metabolism in sheep. Estimates of the total entry rate of glucose by monoexponential analysis of data obtained with single injections of [U-^{14}C]glucose or [6-^{3}H]glucose should be treated with caution since the initial disappearance curves of [^{14}C]glucose and [^{3}H]glucose may be affected to a small extent by recycling of tracer. Consideration of this portion of the curve following injections of [3-^{3}H]- and [2-^{3}H]glucose may be useful in the 'successive multiple

injection' technique of Wrenshall and Hetenyi (1959) to provide estimates of rapid changes in the total entry rate of glucose over short intervals of time, whereas the irreversible loss of glucose, measured with single injections or constant infusions of these tracers appear to under-estimate total entry rate, particularly with [6-^{3}H]glucose.

A combination of [3-^{3}H]- or [2-^{3}H]glucose with carbon-14 labelled substrates should be useful, particularly in quantitating precursor-product (glucose) transformations and to assess the possible importance of conserving glucose carbon by recycling (see Cahill and Owen, 1967) in sheep under different physiological and nutritional conditions. An added advantage of [2-^{3}H]glucose as a tracer is that the irreversible loss and body content of water may also be approximated since the tritium appeared rapidly and quantitatively in water following injections of this tracer. Black, Baker, Bartley, Chapman and Phillips (1964) calculated the irreversible loss of water from cattle with injections of [6-^{3}H]glucose. However, this tracer as well as [3-^{3}H]glucose appears unsuitable in sheep because of the slow release of tritium to body water.

GENERAL DISCUSSION AND CONCLUSIONS

The most significant conclusion which has been drawn from this investigation is that the quality and quantity of the feed eaten by sheep is a major determinant of the gluconeogenic rate.

In animals given roughage diets, glucose synthesis rate may simply be a product of substrate availability. It is possible that during pregnancy and lactation when glucose requirements are high, the effect is largely on appetite and that the secondary effect of an increased appetite is an increase in the availability of glucogenic products which stimulate glucose synthesis rate. In accord with this suggestion, Steel and Leng (1968) showed that glucose synthesis in sheep was determined more by diet than by the stage of pregnancy. Similarly, in the lactating ewe, the three-fold increase in the irreversible loss of plasma glucose, recorded by Bergman and Hogue (1967) was accompanied by a more than three-fold increase in feed intake. Annison and Linzell (1964) obtained a value as high as about 18 $mg/min/kg^{0.75}$ for the irreversible loss of plasma glucose

in a goat during peak lactation when hay was provided *ad libitum* and concentrates were fed according to milk yield. Part of this response however, may have been due to the absorption of dietary glucose. The results reported herein indicate that dietary glucose may also limit or control the gluconeogenic rate, particularly from substrates other than ruminal propionate.

Attempts to quantitate glucose absorption from the intestinal tract of ruminants have usually depended upon measurements of blood glucose concentrations in the portal vein and carotid artery in conjunction with estimates of portal blood flow rate (Bensadoun, Paladines and Reid, 1962; Roe, Bergman and Kon, 1966; Carr and Jacobson, 1969). This technique is unreliable for a number of reasons, including the possible incomplete mixing of blood from the gastrosplenic and mesenteric vein in the portal vein (Garner and Singleton, 1953) and the metabolism of circulating glucose in the splanchnic region. Bergman and Kaufman (1970) reported that the utilization rate of glucose in the splanchnic region of sheep was equivalent to about 20% of the irreversible loss of

plasma glucose. Two alternative techniques reported here for measuring glucose absorption in ruminants deserve further evaluation. The first involves the measurement of the proportion of glucose carbon derived from blood bicarbonate (see Section 4, Part A) whereas the second involves the determination of the proportion of carbon-bound hydrogens of plasma glucose which are not derived from body water (see Section 5). In conjunction with estimates of the total entry rate of glucose, these techniques may prove suitable for approximating the absorption rate of dietary glucose.

Some of the lines of investigation which suggest themselves as extensions to the work reported in this thesis include:

1. The means by which glucose, propionate and amino acids can alter the gluconeogenic rate. Of interest is whether hyperglycaemia can suppress the conversion of propionate into glucose, as opposed to glucogenic products arising from propionate metabolism. The relative contribution of these products and propionate to glucose synthesis during an increased availability of propionate also warrants further investigation.

2. The possible inhibitory effect of VFA on the fermentation rate by ruminal organisms. In studies of the short-term control of feeding in ruminants it has been shown that intraruminal injections of VFA can decrease feed intake (see Bailey and Mayer, 1970). In the discussion of these results it has generally been overlooked that part of this effect of VFA on appetite may have been due to an inhibition of ruminal fermentation and the resulting decrease in the rate of disappearance of digesta inhibiting voluntary feed intake (Balch and Campling, 1962).

3. Identification of the substrates retaining tritium from positions 3 and 6 of plasma glucose. Such studies may assist in establishing the glucose requirements for ruminants.