CHAPTER 6

QUANTIFICATION OF METABOLIC CROSSOVER

6.1 INTRODUCTION

In studies using isotope dilution techniques, the transfer quotient is often interpreted as the proportion of the product pool provided by the tracee pool. However, as discussed in Section 2.2, where intermediates in anabolic pathways form common pools with those from the tricarboxylic acid cycle, the pattern of tracer flow differs from the pattern of net tracee flow, due to metabolic crossover. This results in the transfer quotient underestimating the proportion of the product pool provided by the tracee pool.

In the studies presented here, attempts were made to calculate the extent by which the propionate to glucose transfer quotient underestimates the contribution of propionate to glucose due to metabolic crossover. The effects of metabolic crossover are related to the percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle, as indicated by the equations in Chapter 4. Therefore, this parameter was estimated and, in the later sections, used to correct the propionate to glucose transfer quotient for the effects of metabolic crossover. Thus, an estimate of the true contribution of propionate to glucose was

obtained.

6.2 ESTIMATE OF OXALOACETATE IN GLUCONEOGENIC TISSUES ARISING FROM CYCLING OF THE TRICARBOXYLIC ACID CYCLE, USING [1-^{14}C] AND [2-^{14}C]PROPIONATE

6.2.1 Introduction

When ^{14}C-propionate is infused, the incorporation of tracer into glucose is decreased due to the dilution of radioactivity in the dicarboxylic acid pools caused by molecules derived from propionate mixing with molecules derived from cycling of the tricarboxylic acid cycle i.e. metabolic crossover. However, tracer on the middle carbons of oxaloacetate remains in the intermediates of the tricarboxylic acid cycle for several turns of the cycle. Therefore, when [2-^{14}C]propionate is infused, tracer is returned to the oxaloacetate pool from the tricarboxylic acid cycle, thus, raising the specific radioactivity of the oxaloacetate pool and cancelling part of the initial dilution caused by the mixing of the different sources of oxaloacetate. The amount of recycling of tracer via this route is proportional to the percentage of the molecules in the oxaloacetate pool that condenses with acetyl-CoA and enters tricarboxylic acid cycle. Thus, under steady state conditions, the amount of tracer recycling is also proportional to the percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle.

When labelled propionate is infused, the radioactivity on the carbons of oxaloacetate is proportional to

1. The specific radioactivity of the propionate that labels the oxaloacetate pool,

2. The percentage of the molecules in the oxaloacetate pool arising directly from propionate, and

3. The percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle

as shown by the equations in Section 4.4.

It is possible to derive equations for the specific radioactivity of any product synthesised from oxaloacetate using the equations that describe the specific radioactivities of the individual carbons of oxaloacetate. In some ratios of these derived equations (for the specific radioactivities of the products of oxaloacetate) 1 and 2 cancel out and leave the ratio defined in terms of 3. By measuring the specific radioactivities of these products of oxaloacetate (during the appropriate infusions) and calculating the value of the ratio it is possible to solve for 3. For example in Section 4.4.4, a ratio of glucose specific radioactivities is described in terms of the equations of the specific radioactivities of the carbons of oxaloacetate.

In this experiment specifically labelled propionates were infused directly into one jugular vein and the specific radioactivity of glucose determined on mixed venous blood taken through a cathether in the other jugular vein. The glucose specific radioactivities were used to solve the glucose ratio defined in Section 4.4.4.

Because of the difficulties in estimating the specific radioactivity of the propionate presented to the gluconeogenic tissues, it was assumed that both labelled species of propionate would be affected by the same dilutions. Therefore, both species of labelled propionate should have the same specific radioactivity at the site of gluconeogenesis if infused at the same rate, thus, allowing the specific radioactivities of product pools to be directly compared.

The infusions were administered intravenously to avoid the possible complication of differential loss of tracer from [1-^{14}C] or [2-^{14}C]propionate due to metabolism of propionate by rumen microorganisms or by metabolism in the rumen wall.

When propionate is infused into peripheral blood there is oxidation of the propionate in peripheral tissues. In this case the ratio of the specific radioactivities of blood bicarbonate during infusions of [1-^{14}C] and [2-^{14}C]propionate may be different to the ratio of the specific radioactivities of bicarbonate produced in the liver. If blood CO_2 specific radioactivities are used to solve the CO_2 ratio (defined in Section 4.4.4) the value calculated from $^1/_{(1+N1)}$ will be the average percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle in the tissues that oxidize the propionate. This is not the value needed to correct the propionate to glucose transfer quotient for the effects of metabolic crossover. As gluconeogenesis only occurs in gluconeogenic tissues, peripheral metabolism of propionate should not affect the glucose ratio.

6.2.2 Materials And Methods

Most of the materials and methods are given in Chapter 5.

Four mature Merino sheep fed 800g/d (air dry) lucerne chaff were used. Two animals were infused intraveneously with [1-^{14}C]propionate (0.2μCi/min) and the remaining two animals with [2-^{14}C]propionate (0.1μCi/min) for 12h (2400h to 1200h).

Samples for the assay of blood glucose specific radioactivity were taken at 50min intervals over the last 5h of the infusions.

6.2.3 Results

The rates of infusion of labelled propionate and the blood bicarbonate and glucose specific radioactivities corrected to the same infusion rate (0.25μCi/min) are shown in Table 6-1.

6.2.4 Calculations

The glucose ratio[1]

$$= \frac{\text{tracer incorporated into glucose from [2-}^{14}\text{C]propionate}}{\text{tracer incorporated into glucose from [1-}^{14}\text{C]propionate}}$$

$$= \frac{\text{specific radioactivity of glucose when [2-}^{14}\text{C]propionate infused}}{\text{specific radioactivity of glucose when [1-}^{14}\text{C]propionate infused}}$$

If all glucose is synthesised from oxaloacetate, the specific radioactivities of the carbons of oxaloacetate directly determine the specific radioactivities of the carbons of glucose.

Therefore the glucose ratio

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[1] values corrected to the same infusion rates

Table 6-1

The infusion rates, HCO_3^- and glucose specific radioactivities (SR) during intravenous infusions of [1-^{14}C] and [2-^{14}C]propionate

		Infusion rate (ηCi/min)	HCO_3^- SR[1] (μCi/gC)	Glucose SR[1] (μCi/gC)
[1-^{14}C]propionate	Sheep J	171 (3.4)[2]	1.95 (0.39)	1.18 (.057)
	Sheep K	205 (4.1)	2.04 (.028)	1.15 (.089)
[2-^{14}C]propionate	Sheep L	93 (1.9)	1.09 (.019)	3.25 (.239)
	Sheep M	110 (2.2)	1.09 (.033)	3.02 (.191)

[1] Corrected to an infusion rate of 250ηCi/min

[2] The values in ( ) are the standard errors

$$= \frac{2 \times \left[\begin{array}{c}\text{specific radioactivity of the methyl carbon of oxaloacetate} \\ (\text{[2-}^{14}\text{C]propionate infused})\end{array}\right] + \left[\begin{array}{c}\text{specific radioactivity of the carboxyl carbon of oxaloacetate} \\ (\text{[2-}^{14}\text{C]propionate infused})\end{array}\right]}{\begin{array}{c}\text{specific radioactivity of the carboxyl carbon of oxaloacetate} \\ (\text{[1-}^{14}\text{C]propionate infused})\end{array}}$$

$$= \frac{5+4NI}{1+2NI}$$

(From Section 4.2.2, NI (net influx) = the rate of influx of 4 and 5 carbon compounds relative to the rate of condensation of acetyl-CoA with oxaloacetate, which is taken as unity.

Therefore, using the mean values of glucose specific radioactivities from Table 1,

$$\frac{3.135\mu C/g \text{ glucose carbon}}{1.165\mu C/g \text{ glucose carbon}} = \frac{5+4NI}{1+2NI}$$

$$NI = 1.67 \qquad (S.E. = .42)$$

The percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle in gluconeogenic tissue

$$= \frac{1}{1+NI} \times \frac{100}{1}$$

$$= 37\%$$

6.2.5 Discussion

Metabolic crossover causes the transfer quotient to underestimate the percentage of the glucose pool being provided by propionate. The extent of the underestimation depends on the percentage of the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle. In sheep fed 800g/d lucerne chaff it is estimated in this experiment that 37% of the oxaloacetate pool arises from cycling of

the tricarboxylic acid cycle. The effects of this on the distribution of tracer in glucose and some key intermediates are illustrated in the following examples.

If the oxaloacetate pool formed from net inputs had a carboxyl carbon specific radioactivity of 100 and cycling of the tricarboxylic acid cycle provided 37% of the total oxaloacetate pool, then the specific radioactivity of the carboxyl carbon in the total oxaloacetate pool would be 63. Glucose synthesised from this pool would have radioactivity only on carbons 3 and 4 (the specific radioactivity of carbons 3 and 4 would each be 63 and the whole molecule specific radioactivity would be 21). This is illustrated in Figure 6-1.

If the oxaloacetate pool formed from net inputs had a specific radioactivity of 100 on each of the middle carbons before interaction with the tricarboxylic acid cycle derived oxaloacetate, then the initial metabolic crossover dilution would cause the middle carbon specific radioactivity to fall to 63. However, recycling of tracer in the tricarboxylic acid cycle cancels out part of the dilution and causes the specific radioactivity of the middle carbons to increase - in this example by a factor of 1.23 ($1.23 = {}^{(2+2NI)}/_{(1+2NI)}$, Section 4.4.3). Therefore, the specific radioactivity of the middle carbons after equilibration of tracer in the tricarboxylic acid cycle would be 77.5.

Tracer cycling in the tricarboxylic acid cycle causes the carboxyl carbons of oxaloacetate to become labelled. At steady state the specific radioactivity of the carboxyl carbons is 14.5 ($77.5 \times {}^{1}/_{(2+2NI)}$, Section 4.4.3). Glucose synthesised from this oxaloacetate pool would have a specific radioactivity on carbons 1,2,5 and 6 of

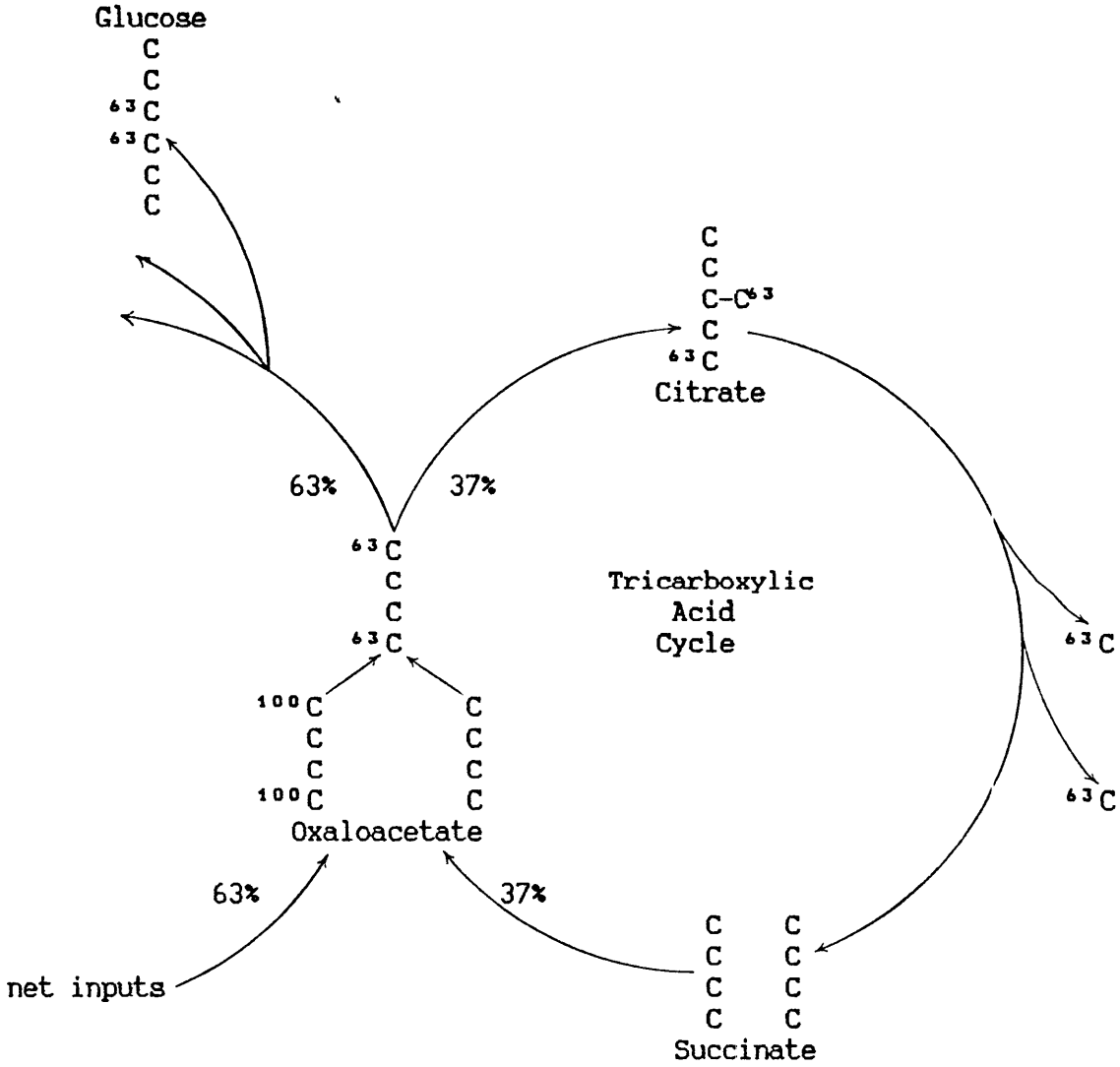


Figure 6-1

The labelling pattern in some intermediates when

1. The specific radioactivity on the carboxyl carbons of the net inputs oxaloacetate pool = 100

2. 37% of the oxaloacetate pool arises from cycling of the tricarboxylic cycle

77.5 and a specific radioactivity on carbons 3 and 4 of 14.5 . The whole molecule specific radioactivity would be 56.5 . This is illustrated in Figure 6-2.

As illustrated in the above examples, metabolic crossover not only decreases the tracer incorporation due to dilution but can change the whole pattern of tracer flow. This makes interpretation of the transfer quotient more difficult. To interpret the transfer quotient correctly, the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle must be known.

If it was assumed that all the net inputs to the oxaloacetate pool were being provided by propionate, then the specific radioactivity of the labelled atom in the propionate pool would have to be 200 in order to give an specific radioactivity of 100 on the appropriate carbons of the nets inputs oxaloacetate pool. This would give a whole molecule propionate to glucose transfer quotient of 31.5% ($^{21}/_{67}$) for [1-^{14}C]propionate. The propionate to glucose transfer quotient using [2-^{14}C]propionate would be 85% ($^{56.5}/_{67}$). These values are considerably higher than values reported in the literature (e.g. Leng, Steel and Luick 1967).

To obtain values similar to those obtained by Leng et al. (1967) propionate would have to be supplying only half the net inputs oxaloacetate pool. The specific radioactivity of the labelled atom in the propionate pool would have to be 400 to give a specific radioactivity of 100 on the appropriate carbons in the net inputs oxaloacetate pool. In this case the whole molecule propionate carbon to glucose carbon transfer quotient would be 16 ($^{21}/_{133}$) when [1-^{14}C]propionate was infused. When [2-^{14}C]propionate was infused the whole molecule propionate carbon to glucose carbon transfer quotient

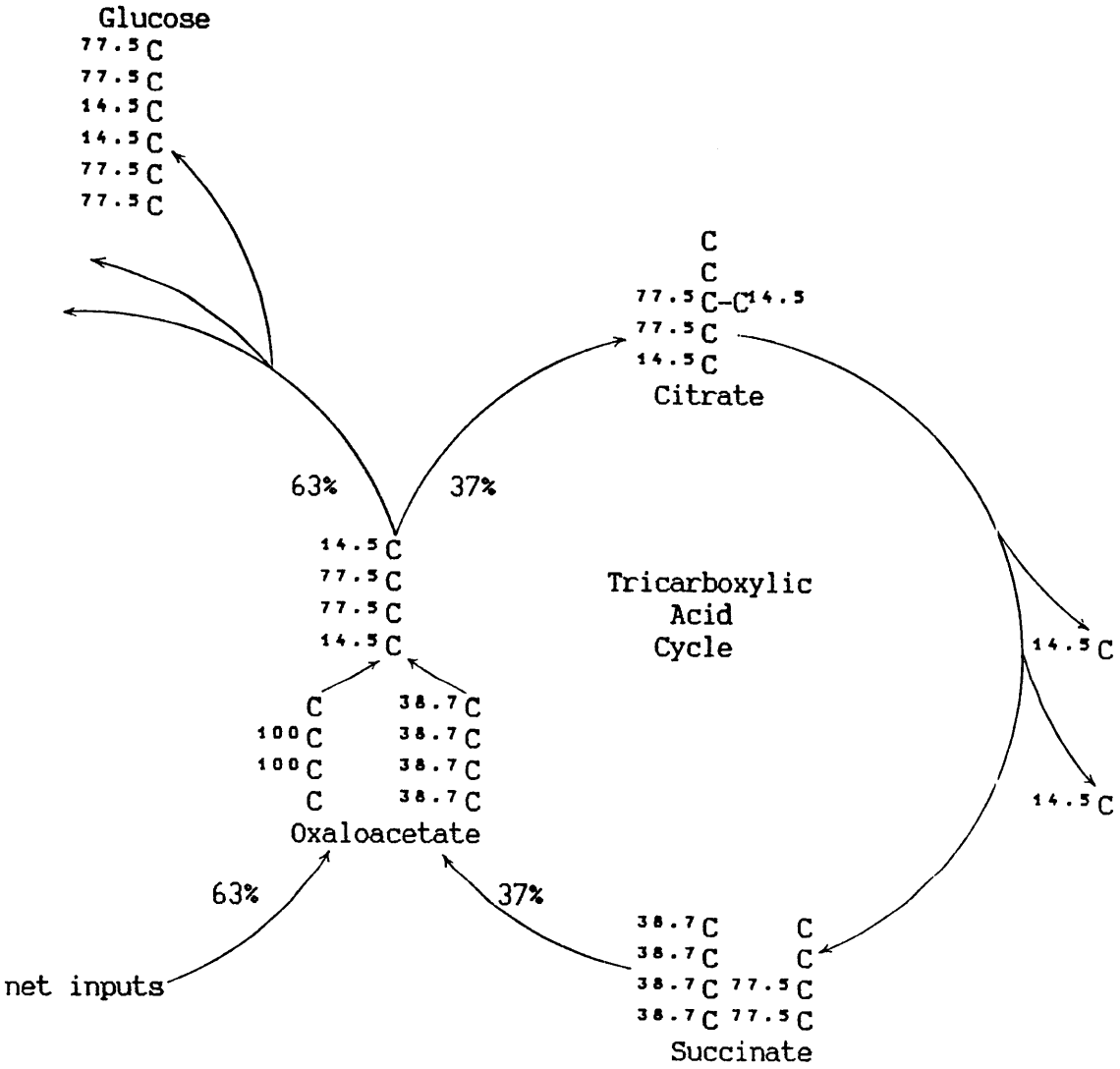


Figure 6-2
The labelling pattern in some intermediates when

1. The specific radioactivity on the methyl carbons of the net inputs oxaloacetate pool = 100

2. 37% of the oxaloacetate pool arises from cycling of the tricarboxylic cycle

would be 42% ($^{56.5}/_{133}$). These values are similar to those obtained by Leng et al. (1967) and suggest that propionate is providing about half the net inputs to the oxaloacetate pool or about 32% of the total oxaloacetate pool. To correct the propionate to glucose transfer quotient for the effects of metabolic crossover the transfer quotient would have to be multiplied by 1.49 ($^{100}/_{100-37}$).

As will be discussed in later sections, there is a significant flow of tracer from propionate to glucose via the bicarbonate pool. During intraruminal infusions of [2-^{14}C]propionate, approximately 2% (Section 8.2) of the tracer in glucose passed through the bicarbonate pool. When [1-^{14}C]propionate was infused intraruminally, about 20% (Section 8.3) of the tracer in glucose passed through the bicarbonate pool. The theory used in developing the glucose equation assumed direct flow of tracer to glucose. Therefore, the transfer quotient has to be adjusted for this indirect flow to obtain an appropriate value to use in the glucose ratio.

If it is assumed that the flows of tracer to glucose via the bicarbonate pools are the same here as calculated for sections 8.2 and 8.3, then the resultant glucose ratio is as follows

$$\frac{3.135\text{uCi/g glucose carbon} \times .98}{1.165\text{uCi/g glucose carbon} \times .80} = \frac{5+4NI}{1+2NI}$$

$$NI = 0.657$$

From this, the percentage of the molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle ($^{1}/_{(1+NI)}$) is 60%. The effects of 60% of the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle are illustrated in the following examples.

If the oxaloacetate pool formed from net input sources had a carboxyl carbon specific radioactivity of 100 and cycling of the tricarboxylic acid cycle provided 60% of the total oxaloacetate pool, then the specific radioactivity of each carboxyl carbon in the total oxaloacetate pool would be 40. Glucose synthesised from this pool would have radioactivity on carbons 3 and 4 (the specific radioactivity of carbons 3 and 4 would each be 40 and the whole molecule specific radioactivity would be 13). This is illustrated in Figure 6-3.

If the oxaloacetate pool formed from net inputs had a specific radioactivity of 100 on each of the middle carbons before interaction with the tricarboxylic acid cycle derived oxaloacetate, then the initial metabolic crossover dilution would cause the middle carbon specific radioactivity to fall to 40. However, recycling of tracer in the tricarboxylic acid cycle cancels out part of the dilution and causes the specific radioactivity of the middle carbons to increase - in this example by a factor of 1.43 ($1.43 = {}^{(2+2NI)}/_{(1+2NI)}$, Section 4.4.3). Therefore, the specific radioactivity of the middle carbons after equilibration of tracer in the tricarboxylic acid cycle would be 57.3 . Tracer cycling in the tricarboxylic acid cycle causes the carboxyl carbons to become labelled. At steady state the specific radioactivity of the carboxyl carbons in the oxaloacetate pool is 17.2 ($57.3 \times {}^{1}/_{(2+2NI)}$, Section 4.4.3). Glucose synthesised from this oxaloacetate pool would have a specific radioactivity on carbons 1,2,5 and 6 of 57.3 and a specific radioactivity on carbons 3 and 4 of 17.2 . The whole molecule specific radioactivity would be 43.9 . This is illustrated in Figure 6-4.

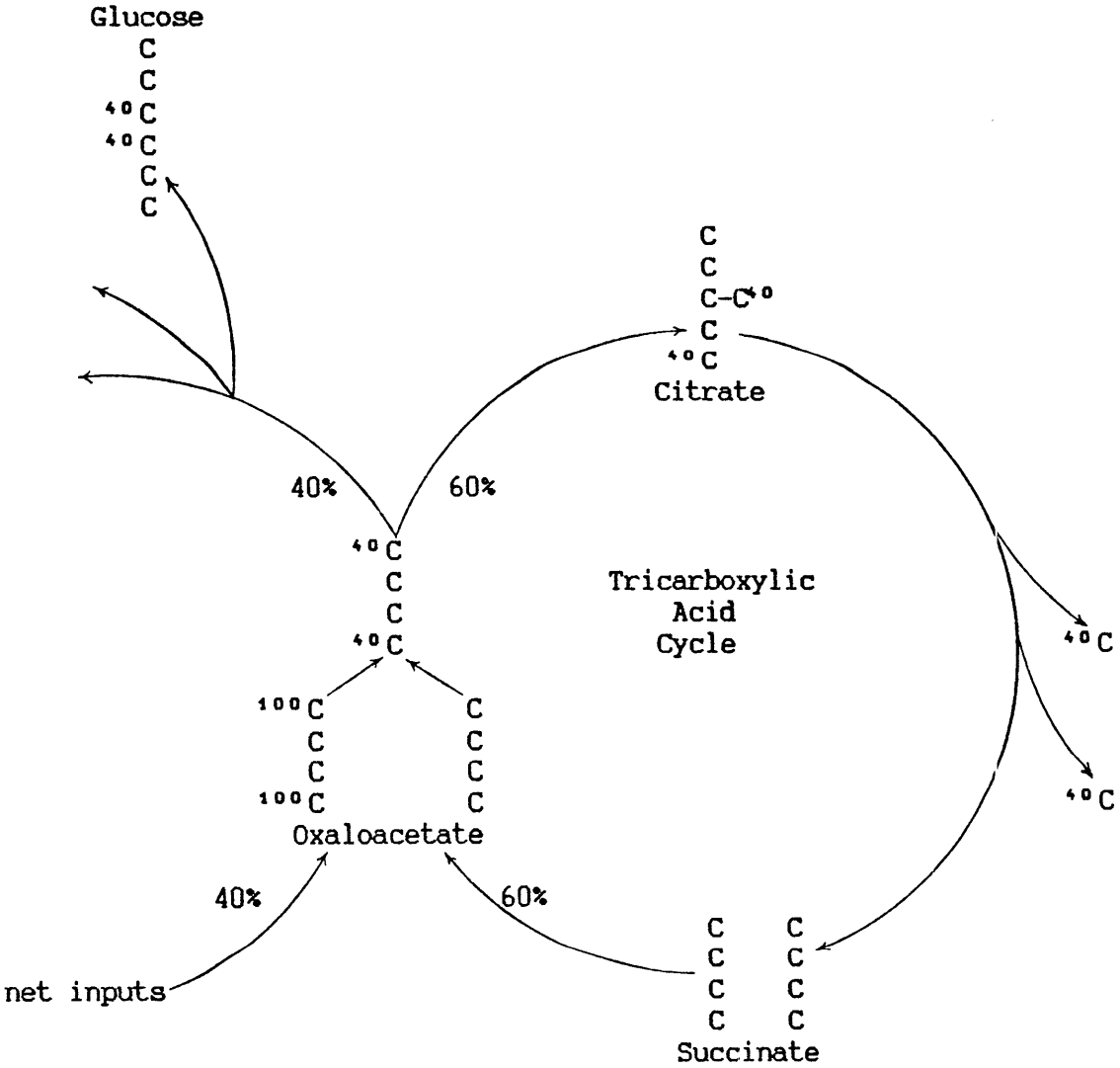


Figure 6-3
The labelling pattern in some intermediates when

1. The specific radioactivity on the carboxyl carbons of the net inputs oxaloacetate pool = 100

2. 60% of the oxaloacetate pool arises from cycling of the tricarboxylic cycle

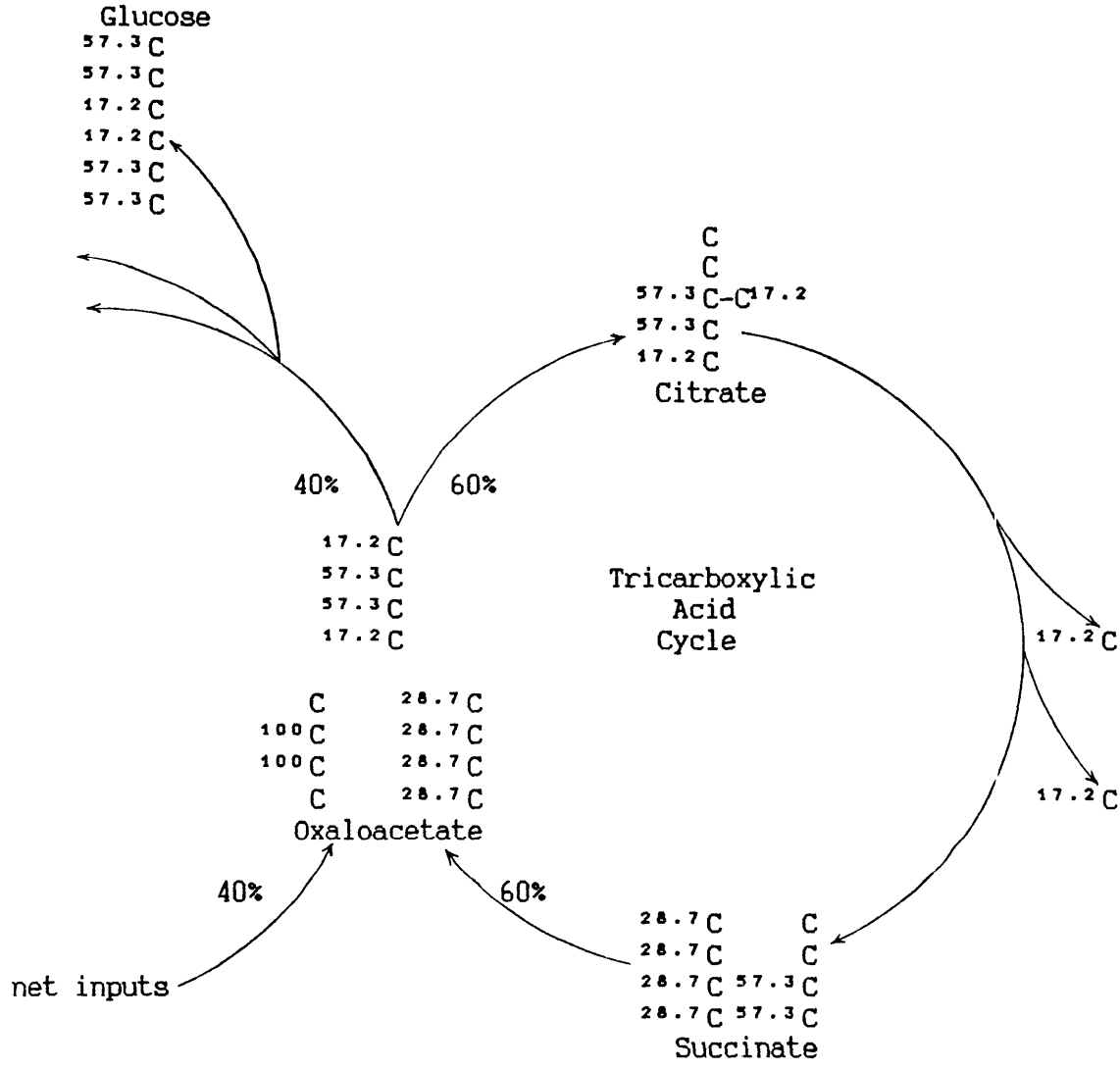


Figure 6-4
The labelling pattern in some intermediates when

1. The specific radioactivity on the methyl carbons of the net inputs oxaloacetate pool = 100

2. 60% of the oxaloacetate pool arises from cycling of the tricarboxylic cycle

Again, if it is assumed that all the net inputs were being provided by propionate, then the specific radioactivity of the labelled atom in the propionate pool would be 200. This would give a whole molecule propionate to glucose transfer quotient of 20% ($^{13}/_{67}$) for [1-^{14}C]propionate. The propionate to glucose transfer quotient for [2-^{14}C]propionate would be 65% ($^{44}/_{67}$). These values are slightly higher than values reported in the literature (e.g. Leng et al. 1967).

If less than 100% of the net inputs oxaloacetate pool was provided by propionate, then the specific radioactivity of propionate needed to make this pool have a specific radioactivity of 100 will increase. This, in turn, will lower the calculated transfer quotient from propionate to glucose. If propionate was providing 80% of the net inputs oxaloacetate pool, then the specific radioactivity on the labelled atom of propionate would have to be 250 to give a specific radioactivity of 100 on the appropriate atoms in the net inputs oxaloacetate pool. This would give whole molecule transfer quotients of propionate to glucose for [1-^{14}C] and [2-^{14}C]propionate of 16% and 53% respectively. These are very similar to the values obtained by Leng et al. (1967). Therefore, this interpretation suggests that propionate provides approximately 80% of the net inputs oxaloacetate pool and thus about 80% of the glucose pool. The propionate to glucose transfer quotient has to be multiplied by 2.5 ($^{100}/_{100-60}$) to correct for the effects of metabolic crossover. However, this value is very dependent on the correction for the indirect flow of tracer to the glucose pool via the bicarbonate pools.

6.3 ESTIMATE OF OXALOACETATE IN GLUCONEOGENIC TISSUES ARISING FROM CYCLING OF THE TRICARBOXYLIC ACID CYCLE, USING [1-^{14}C] AND [2-^{14}C]ACETATE

6.3.1 Introduction

On every turn of the tricarboxylic acid cycle an acetyl-CoA molecule enters the cycle and two carbons leave it as CO_2. However, it is not the acetyl-CoA carbons that are converted to CO_2, but the carboxyl carbons of the oxaloacetate that condensed with the acetyl-CoA. Therefore, oxaloacetate molecules that are formed by the tricarboxylic acid cycle will become labelled when acetyl-CoA is labelled. The specific radioactivity of the oxaloacetate molecules that arose from cycling of the tricarboxylic acid cycle is diluted by oxaloacetate from other sources. However, tracer on the middle carbons of oxaloacetate remains in the intermediates of the tricarboxylic acid cycle for several turns of the cycle. This recycled tracer contributes to the radioactivity in the oxaloacetate pool and partially cancels the effect of the dilution caused by the mixing of the different sources of oxaloacetate.

The amount of tracer cycling, and thus the increase in specific radioactivity of the oxaloacetate pool when [2-^{14}C]acetate is infused over that obtained when [1-^{14}C]acetate is infused, is proportional to the percentage of the molecules in the oxaloacetate pool that leaves via reactions of the tricarboxylic acid cycle. At steady state this percentage is the percentage of the molecules in the oxaloacetate pool that arose from cycling of the tricarboxylic acid cycle.

The specific radioactivities of the carbons of oxaloacetate when labelled species of acetate are infused are proportional to

1. The specific radioactivity of the acetyl-CoA entering the tricarboxylic acid cycle, and

2. The percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle

as indicated by the equations developed in Chapter 4.

It is possible to derive equations for the specific radicactivity of any product synthesised from oxaloacetate using the equations that decribe the specific radioactivities of the individual carbons of oxaloacetate. In some ratios of these derived equations (for the specific radioactivities of the products of oxaloacetate) the specific radioactivity of the acetyl-CoA entering the tricarboxylic acid cycle cancels out and leaves the ratio defined in terms of the percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle. By measuring the specific radioactivities of these products of oxaloacetate (during the appropriate infusions) and calculating the value of the ratio it is possible to solve the ratio for the percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle. For example in Section 4.3.6, a ratio of glucose specific radioactivities is described in terms of the equations of the specific radioactivities of the carbons of oxaloacetate.

In this experiment [1-^{14}C] and [2-^{14}C]acetate were infused intraruminally and blood and rumen samples taken. The acetate to glucose transfer quotients were to be calculated and used to solve the glucose ratio (from Section 4.3.6).

6.3.2 Materials And Methods

The general materials and methods are given in Chapter 5.

Two mature Merino sheep given 800g/d (air dry) lucerne chaff were used. This is the same feeding regime as used in the last experiment.

The animals were infused intraruminally with [2-^{14}C]acetate (approximately 0.5μCi/min) and [1-^{14}C]acetate (approximately 1μCi/min) for 12h (700h to 1900h) on consecutive days.

Samples of blood and rumen fluid were taken at hourly intervals over the last 6h of each infusion. On the second day a pre-infusion sample was also taken. Blood samples were assayed for glucose specific radioactivity. Rumen fluid samples were assayed for the specific radioactivity of acetate.

6.3.3 Results

Figure 6-5 is an example of the rumen acetate specific radioactivity over the two days of the experiment (sheep A). Figure 6-6 is an example of the glucose specific radioactivity over the two days of the experiment (sheep A). The plateau specific radioactivities of the carboxyl carbon of acetate and the specific radioactivities of glucose in the pre-infuson and last sample taken during the [1-^{14}C]acetate infusion are presented in Table 6-2.

6.3.4 Discussion

The glucose ratio (using acetate) requires an estimate of the incorporation of tracer from acetate into glucose at steady state for both [1-^{14}C] and [2-^{14}C]acetate. It is apparent from Figure 6-6, that

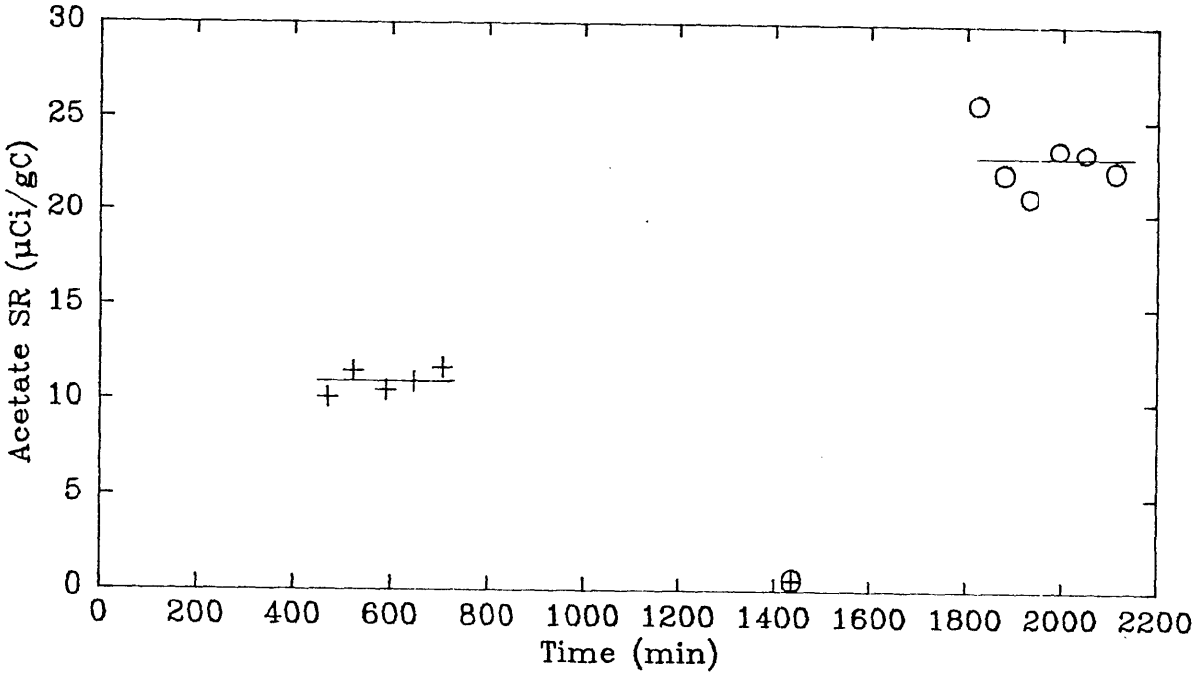


Figure 6-5

An example (sheep A) of the specific radioactivity of rumen acetate when the [2-^{14}C]acetate (+) and [1-^{14}C]acetate (○) were infused intraruminally; (⊕) is the specific radioactivity of acetate when the pre-infusion sample was taken on the second day.

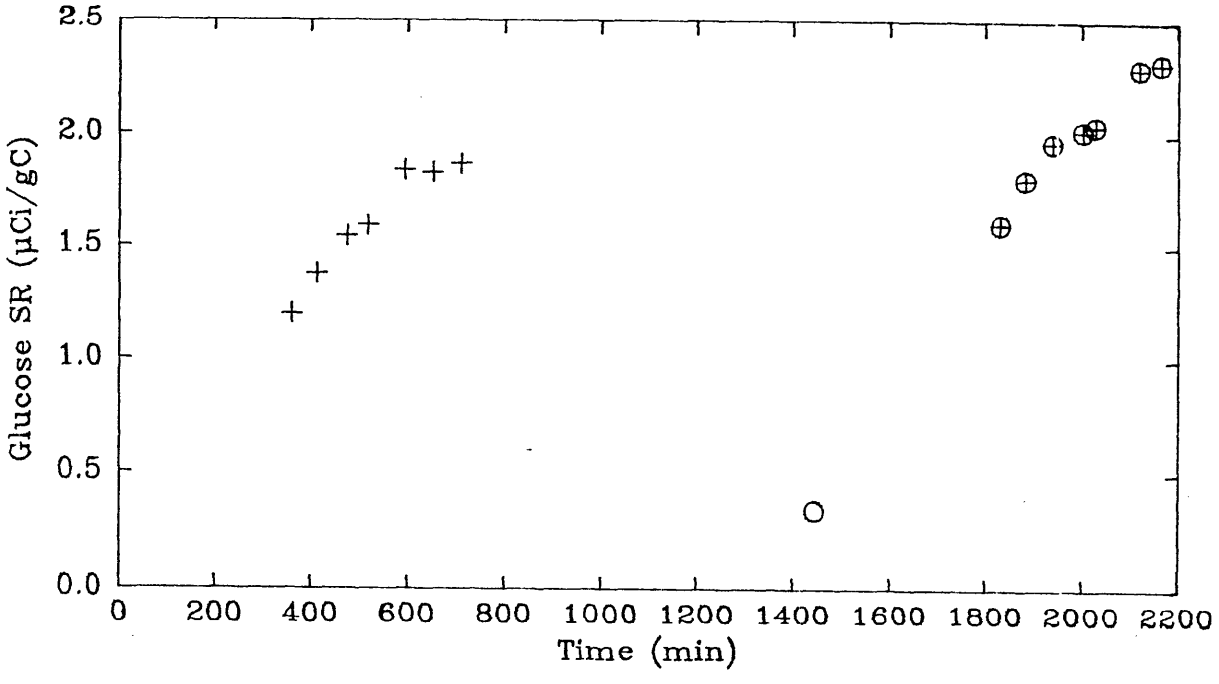


Figure 6-6

An example (sheep A) of the specific radioactivity of glucose when labelled acetate was infused into the rumen: (+) [2-^{14}C]acetate infused, (○) the specific radioactivity of glucose in the pre-infusion sample on the second day, (⊗) [1-^{14}C]acetate infused.

SR = specific radioactivity

Table 6-2

The specific radioactivities of the carboxyl carbon of acetate and glucose during an intraruminal infusion of [1-^{14}C]acetate

	Sheep A	Sheep B
Acetate carboxyl carbon specific radioactivity (μCi/gC)	46 (1.4)[1]	58 (3.6)
Glucose specific radioactivity pre-infusion sample (μCi/gC)	0.34	0.35
Glucose specific radioactivity end of infusion sample (μCi/gC)	2.32	2.26

[1] Values in ( ) are the standard errors

the glucose specific radioactivities are still increasing with time. Because the carbons of oxaloacetate are metabolised differently, their respective glucose specific radioactivities may take different times to reach plateau. At any time before plateau the glucose specific radioactivities may be at different percentages of their respective plateau specific radioactivities. Therefore, the glucose ratio can not be solved. To solve the glucose ratio using acetate, the acetate infusions must be longer than 12h to allow the glucose pools to reach plateau.

Another problem with the experiment is that 12h is not long enough to allow the residual activity in the glucose pool to dissipate. As shown in Figure 6-6, there was a significant amount of activity still in the glucose pool when the pre-infusion sample was taken on Day 2. The glucose specific radioactivity during the sampling period would, therefore, be elevated due to this residual activity. However, 12h is long enough to allow the activity in the acetate pools to fall to negligible values. In later experiments more than 12h will be left between infusions.

Even though the glucose specific radioactivities did not reach a plateau value, a minimum estimate of the percentage of the molecules in the oxaloacetate pool being provided by cycling of the tricarboxylic acid cycle can be obtained from the specific radioactivity of glucose in the last sample taken. After substraction of the specific radioactivity of glucose in the pre-infusion sample, the carboxyl carbon of acetate to glucose transfer quotient is 4.30% (sheep A) and 3.29% (sheep B). Thus, 12.9% (sheep A) and 9.9% (sheep B) of glucose carbons 3 or 4 are being provided by rumen acetate. To estimate the percentage of the molecules in the oxaloacetate pool that

condensed with acetyl-CoA provided by rumen acetate, these values have to be multiplied by 2 to account for the loss of tracer caused by equilibration about the carboxyls of succinate and subsequent loss of one carboxyl in the decarboxylation reaction. Therefore, it is estimated that at least 26% (sheep A) and 20% (sheep B) of the molecules in the oxaloacetate pool arose from cycling of the tricarboxylic acid cycle. These values are minimum values due to the specific radioactivities of the glucose pools not having reached plateau and, the elevation of glucose specific radioactivity due to residual activity would have decreased over the time of the experiment.

Pethick et al. (1981), using sheep given grass hay and a concentrate supplement, found that even if all acetate taken up by the liver was oxidized, acetate could only account for about 30% of the oxygen consumption of the liver. Therefore, it appears that other substrates are contributing the bulk of the acetyl-CoA for oxidation. As acetyl-CoA molecules derived from rumen acetate are only a proportion of the acetyl-CoA molecules that enter the tricarboxylic acid cycle, labelled rumen acetate will only label a proportion of the oxaloacetate molecules that arise from cycling of the tricarboxylic acid cycle. Using the estimate of maximal acetyl-CoA from rumen acetate in the liver (30%) obtained by Pethick et al. (1981), an estimate of the percentage of the molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle can be obtained by adjusting the measured percentage of the molecules in the oxaloacetate pool from rumen acetate for this dilution (i.e. multiply by 100/30). This indicates that the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle is about 80% (sheep A) and 66% (sheep B). The figure of 30% for the

percentage of acetyl-CoA provided by rumen acetate is an overestimate (therefore underestimating the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle) because not all acetate would be used for oxidation.

Considering that several factors are known to be causing the above calculation to underestimate the percentage of the molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle, the calculated estimates seem too high. The reason for this is probably incorporation of tracer from acetate into glucose by an indirect route via CO_2. Later work in this thesis (Section 6.5) found that 57% of the tracer in glucose when [1-^{14}C]acetate was infused intraruminally, was tracer that had passed through the CO_2 pool. Adjusting the above values for this indirect flow of tracer gives values of 34% (sheep A) and 28% (sheep B) of the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle.

Therefore, the arguments above suggest that the propionate to glucose transfer quotient would underestimate the contribution of propionate to glucose by at least 34% for sheep A and 28% for sheep B.

6.4 ESTIMATE OF OXALOACETATE IN GLUCONEOGENIC TISSUES ARISING FROM THE TRICARBOXYLIC ACID CYCLE VIA PATHWAYS NOT INVOLVING CARBOXYLATION REACTIONS

6.4.1 Introduction

Under the assumption that the contribution to the net inputs oxaloacetate pool from pathways not involving carboxylation reactions is negligible relative to the contribution from pathways involving carboxylation reactions, there are only the two sources of

oxaloacetate - cycling of the tricarboxylic acid cycle and pathways involving carboxylation reactions. If the CO_2 pool is labelled, oxaloacetate molecules formed from pathways involving carboxylation reactions will also become labelled. By comparing the specific radioactivity of the carboxyl carbons of oxaloacetate to the specific radioactivity of the CO_2 pool, an estimate of the percentage of the molecules in the oxaloacetate pool provided by pathways involving carboxylation reactions can be obtained. Then, by difference, an estimate of the percentage of the molecules in the oxaloacetate pool provided by cycling of the tricarboxylic acid cycle can be obtained.

In this experiment glucose specific radioactivity was used to obtain an estimate of the oxaloacetate specific radioactivity. As explained in Section 4.5, some glucose precursors, e.g. lactate, do not have a symmetrical compound in their direct pathway to glucose. For these precursors of glucose, CO_2-carbon is incorporated into glucose only if there is equilibration of the dicarboxylic acids and thus passage through a symmetrical compound which distributes the tracer about both the carboxyl carbons. As only one carboxyl is removed in the decarboxylation step, CO_2-carbon is incorporated into glucose.

It was assumed in developing the equation (Section 4.5.4) relating the specific radioactivity of glucose to the specific radioactivity of oxaloacetate that complete equilibration of tracer occurred in the dicarboxylic acid pools. Non-complete adherence to the assumptions would cause the percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle to be overestimated.

The transfer quotient of propionate to glucose was also measured in this experiment. The above estimates of percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle were used to correct this value for the effects of metabolic crossover.

6.4.2 Materials And Methods

Most of the materials and methods are given in Chapter 5.

The four mature Merino sheep used for this experiment were on the same diet (800g/d air dry lucerne chaff) and feeding regime as used in the last experiment.

The animals were infused intravenously with $NaH^{14}CO_3$ (approximately 0.5μCi/min) for 10h (600 - 1600h) on day 1. On day 8, the sheep were infused intraruminally with [1-^{14}C]propionate (approximately 0.25μCi/min) for 24h (1600 - 1600h).

Blood samples were taken at 45min intervals over the last 4.5h of the ^{14}C-bicarbonate infusion. Samples of blood and rumen fluid were taken at hourly intervals over the last 8h of the [1-^{14}C]propionate infusion. A pre-infusion sample of blood and rumen fluid was taken on day 8. Blood samples were analysed for glucose and bicarbonate specific radioactivities. Rumen fluid samples were analysed for propionate specific radioactivities.

6.4.3 Results

Analysis of the pre-infusion samples revealed that there was negligible residual radioactivity in the studied pools.

The infusions were of a sufficient length of time to allow the glucose pools to reach plateau. Examples (sheep F) of the relationships between glucose specific radioactivities and time are shown in Figures 6-7 and 6-8.

The blood bicarbonate specific radioactivities, glucose specific radioactivities and bicarbonate to glucose transfer quotients are shown in Table 6-3. The propionate, blood bicarbonate and glucose specific radioactivities during the [1-^{14}C]propionate infusion are shown in Table 6-4. The rates of propionate irreversible loss, propionate to blood bicarbonate transfer quotients and propionate to glucose transfer quotients are shown in Table 6-5.

6.4.4 Calculations

The specific radioactivity of the carboxyl carbons of oxaloacetate (OCSR) was estimated via the equation,

OCSR = specific radioactivity of glucose carbons 3 or 4

= 3 x glucose specific radioactivity

The following equation was used to calculate NHCO3 (the proportion of the oxaloacetate pool formed from all other sources relative to the proportion formed from carboxylation of 3 carbon compounds, which is defined as unity),

$$NHCO3 = \frac{HCO3SR - 2 \times OCSR}{2 \times OCSR}$$

The percentage of the molecules in the oxaloacetate pool coming from carboxylation of 3 carbon compounds

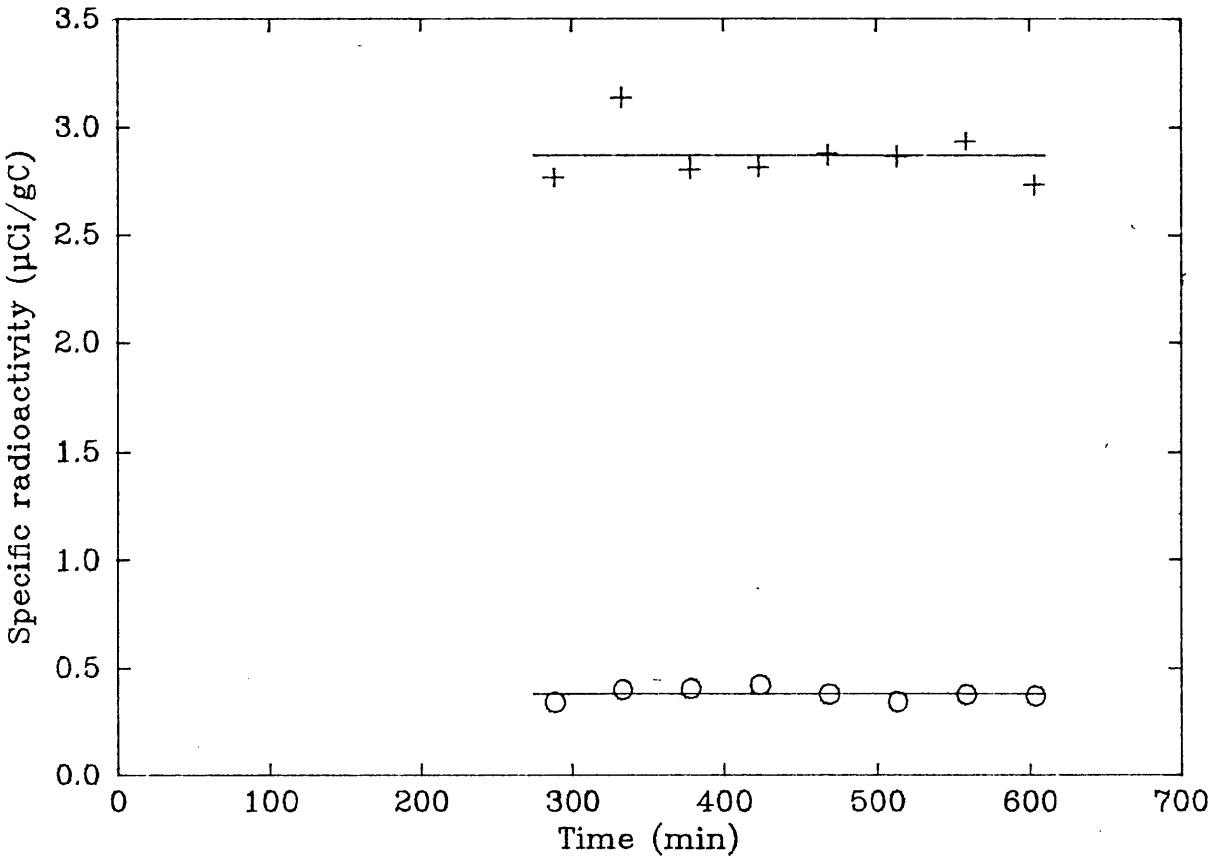


Figure 6-7

An example (sheep F) of the specific radioactivities of blood bicarbonate (+) and glucose (O) during an intravenous infusion of ^{14}C-bicarbonate.

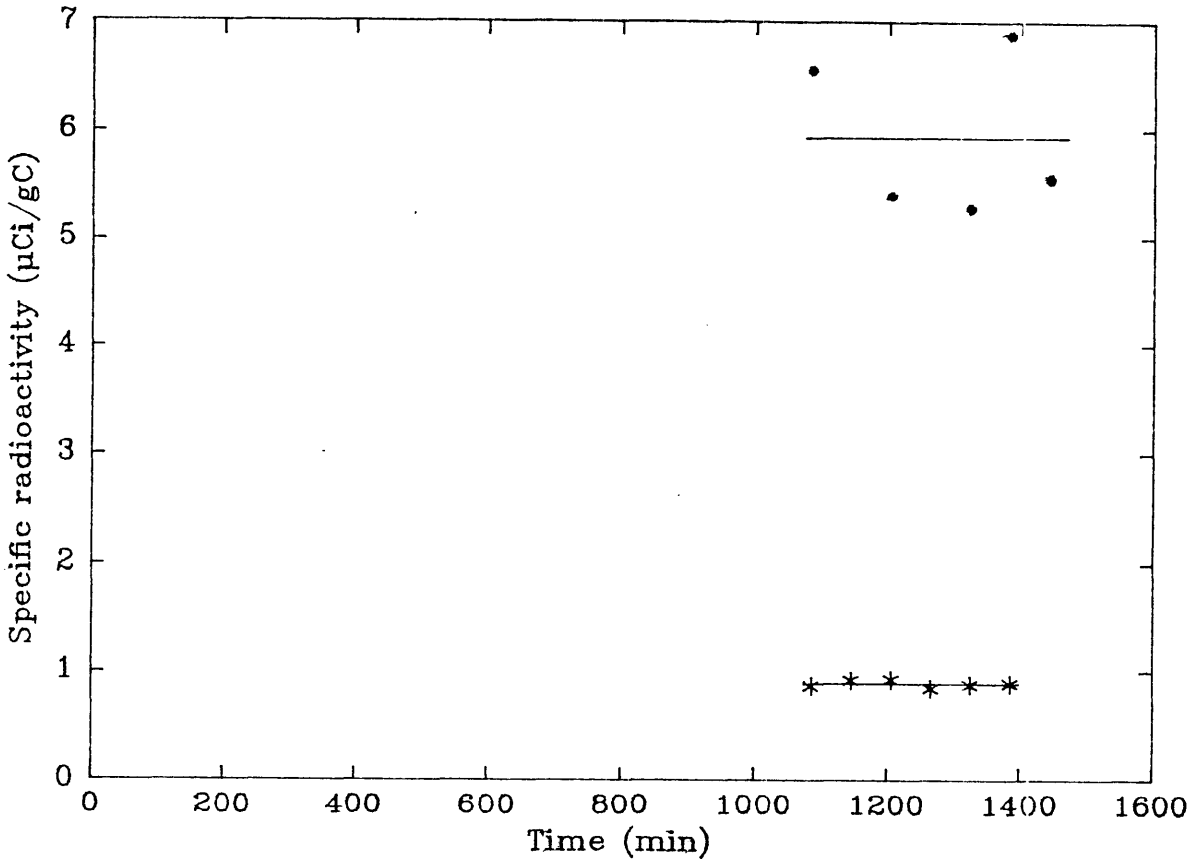


Figure 6-8

An example (sheep F) of the specific radioactivities of rumen propionate (•) and glucose (*) during an intraruminal infusion of [1-^{14}C]propionate

Table 6-3

The blood bicarbonate and glucose specific radioactivities and the bicarbonate to glucose transfer quotient during an intravenous infusion of $H^{14}CO_3$

	blood HCO_3^- SR (μCi/gC)	glucose SR (μCi/gC)	transfer Quotient (%)
Sheep D	5.0 (.24)[1]	0.55 (.005)	11 (1.5)
Sheep E	2.8 (.08)	0.34 (.006)	12 (1.2)
Sheep F	2.9 (.05)	0.38 (.010)	13 (1.2)
Sheep G	2.8 (.07)	0.43 (.010)	15 (1.3)

[1] Values in ( ) are the standard errors

Table 6-4

The specific radioactivities of propionate, blood bicarbonate and glucose during an intraruminal infusion of [1-^{14}C]propionate

	Propionate SR (μCi/gC)	Blood HCO_3^- SR (μCi/gC)	Glucose SR (μCi/gC)
Sheep D	5.1	1.25	0.89
	(.16)[1]	(.020)	(.009)
Sheep E	8.3	1.25	1.02
	(.14)	(.024)	(.013)
Sheep F	5.9	1.25	0.88
	(.33)	(.039)	(.012)
Sheep G	3.3	1.17	0.84
	(.05)	(.032)	(.022)

[1] Values in ( ) are the standard errors

SR = specific radioactivity

Table 6-5

The rates of propionate irreversible loss (IL), propionate to blood bicarbonate and propionate to glucose transfer quotients (TQ) during an intraruminal infusion of [1-^{14}C]propionate

	Propionate IL (gC/d)	Propionate HCO_3^- TQ (%)	Propionate Glucose TQ (%)
Sheep D	57 (3.8)[1]	24 (1.9)	17 (1.2)
Sheep E	38 (1.4)	15 (0.9)	12 (0.6)
Sheep F	53 (6.6)	21 (3.1)	15 (1.9)
Sheep G	90 (3.3)	36 (3.0)	26 (2.1)

[1] Values in ( ) are the standard errors

$$= {}^{1}/_{(1+NHCO3)}$$

Combining the above 2 equations, the percentage of the molecules in the oxaloacetate pool coming from pathways involving the carboxylation of 3 carbon compounds

$$= {}^{2\times OC3R}/_{HCO33R}$$

$$= {}^{2\times(3\times \text{glucose specific radioactivity})}/_{HCO3SR}$$

= 6 x the transfer quotient of blood bicarbonate to glucose

Under the assumptions discussed in Section 4.9, by difference from 100%, the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycling can be estimated.

To correct the propionate to glucose transfer quotient for the effects of metabolic crossover, the transfer quotient has to be divided by 1 minus the proportion of the oxaloacetate pool provided by cycling of the tricarboxylic acid cycle.

The values of the percentage of the molecules in the oxaloacetate pool from pathways involving carboxylation reactions and the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle (according to the above interpretation) are shown in Table 6-6. The propionate to glucose transfer quotients, with and without correction for the effects of the decarboxylation reaction and correction for the effects of metabolic crossover are presented in Table 6-7.

Table 6-6

The percentage of oxaloacetate provided by pathways that involve a CO_2 fixation reaction and, by difference from 100%, the percentage of oxaloacetate provided by cycling of the tricarboxylic cycle (calculated as described in the text)

	from CO_2 fixation pathways (%)	from the tricarboxylic cycle (%)
Sheep D	66 (9.0)	34
Sheep E	72 (7.2)	28
Sheep F	78 (7.2)	22
Sheep G	90 (7.8)	10

[1] Values in ( ) are the standard errors

Table 6-7

Estimates of the percentage of glucose provided by propionate;

1. the propionate to glucose transfer quotient
2. the above value corrected for the loss of tracer due to the decarboxylation reaction (i.e. multiply by 2)
3. the result obtained in (2) corrected for the metabolic crossover dilution (using the estimates of per cent of oxaloacetate arising from cycling of the tricarboxylic acid cycle given in Table 6-6).

	1	2	3
Sheep D	17	34	52
Sheep E	12	24	33
Sheep F	15	30	38
Sheep G	26	52	58

6.4.5 Discussion

The percentage of the molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle differed considerably between animals. The variation was greater than expected considering the animals were fed the same diet and kept under the same conditions. The values of the percentage of oxaloacetate from pathways involving a CO_2 fixation reaction were also higher than expected considering that non-complete adherence to the assumptions causes the values to be underestimated, thus causing the percentage of the molecules in the oxaloacetate pool arising from the tricarboxylic acid cycle to be overestimated. The bicarbonate-glucose transfer quotient that would give 100% of the oxaloacetate coming from carboxylation of 3 carbon compounds is 16.7. Values higher than this have been reported in the literature (e.g., Judson and Leng, 1973).

Recycling of molecules via the pathway; oxaloacetate, phosphoenolpyruvate, pyruvate then back to oxaloacetate (or oxaloacetate/HCO_3^- exchange reactions) would cause the incorporation of CO_2 in glucose to be higher than accounted for by the theory used to develop the equation for NHCO3. However, this need not be the only explanation compatible with the results.

Veneziale, Gabrielli and Lardy (1970) reported that in isolated, perfused, fasted rat liver the phosphoenolpyruvate carboxykinase inhibitor, quinolinate, only decreased the utilization of pyruvate carbon for glucose synthesis by 40%. They suggested the possibility of the existence of a pathway for the conversion of pyruvate to glucose that did not require phosphoenolpyruvate carboxykinase. Veneziale (1971), using perfused liver from fasted rats, found that when pyruvate and ^{14}C-bicarbonate were added to the perfusate the

specific radioactivity of the 3-phosphoglycerate exceeded that of one of its supposed precursors, malate, after 4min. In a later experiment Veneziale (1972) found that when [1-^{14}C]pyruvate was used the mean ratios of 3-phosphoglycerate to phosphoenolpyruvate was 2.86 (SD=0.70;n=6). He concluded that his results failed to support the current concept of gluconeogenesis from pyruvate and the results were consistent with the suggestion that undiscovered enzyme reactions or compartmentation phenomena, or both are operative.

Mullhofer et al. (1977b) also found that the current theory could not account for all their results. The observed labelling patterns of metabolites when [1-^{14}C] or [2-^{14}C]lactate were infused were not too different from expected. However, the ^{14}C incorporation into glucose was higher than expected when ^{14}C-bicarbonate was infused and much lower than expected when [1-^{14}C]octanoate was infused. Their results could not be explained by invoking HCO_3^- exchange reactions between oxaloacetate and phosphoenolpyruvate or between malate and pyruvate because, although this could explain the ^{14}C-bicarbonate and [1-^{14}C]octanoate results, it would also postulate low levels of [1-^{14}C]pyruvate and [1-^{14}C]lactate incorporation into glucose - results which were not observed. Mullhofer et al. (1977a) repeated the work using isolated rat liver parenchymal cells to remove the problem of heterogeneity of cell types that would have been present in the liver perfusion. Although this did change some things, major discrepancies still existed. There appears to be some mechanism operative that increases the incorporation of ^{14}C into glucose from ^{14}C-bicarbonate, while having considerably lesser effects on the incorporation from [1-^{14}C]lactate and [1-^{14}C]pyruvate. Mullhofer et al. (1977a,b) also suggested the possibility of unknown intracellular interactions although the possibility of hepatocytes having differing

metabolic functions could not be ruled out.

It is possible that these isolated reports (Vezeziale, 1971,1972; Mullhofer et al., 1977a,b) are merely artifacts of the particular system used by the researchers. However, if the results cannot be explained with or without the known forms of tracer recycling, then unknown intracellular interactions may have to be postulated.

6.5 ESTIMATE OF OXALOACETATE FROM THE TRICARBOXYLIC ACID CYCLE VIA THE ACETATE GLUCOSE RATIO AND PATHWAYS NOT INVOLVING CARBOXYLATION REACTIONS

6.5.1 Introduction

In this study an attempt was made to estimate, by two different methods, the percentage of the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle in the same animals under conditions kept as constant as possible. The methods used were those of the preceding two sections, i.e. utilizing $^{14}CO_2$ to measure the percentage of the molecules in the oxaloacetate pool coming from carboxylation of 3 carbon compounds and solving the glucose ratio using results obtained during infusions of [1-^{14}C] and [2-^{14}C]acetate.

In this experiment the transfer quotient of propionate carboxyl carbon to glucose was also estimated. The estimates of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle given by the above methods were used to correct this transfer quotient for the effects of metabolic crossover.

The acetate infusions were for 24h to allow the specific radioactivities of the glucose pools to attain a plateau value. One week was left between infusions to allow the residual radioactivity in the pools to return to negligible values. To avoid the possible problem of intraruminal metabolism of acetate, the labelled acetates were infused intravenously.

6.5.2 Materials And Methods

Most of the materials and methods are given in Chapter 5.

The two mature Merino sheep used in this experiment were on the same diet (800g/d lucerne chaff) and feeding regime as used in the last experiment.

On day 1, the animals were infused intravenously with [1-^{14}C]acetate (0.5μCi/min), day 8 with [2-^{14}C]acetate (0.25μCi/min), on day 15 with [2-^{3}H]glucose (0.02μCi/min) and $NaHCO_3^-$ (0.6μCi/min) and on day 22 with [1-^{14}C]propionate (0.5μCi/min).

The [2-^{3}H]glucose and $NaHCO_3^-$ infusions were for 10h (700h to 1700h). The [1-^{14}C], [2-^{14}C]acetate and [1-^{14}C]propionate infusions were for 24h (1700h to 1700h).

Blood samples were taken at hourly intervals over the last 8h of the [1-^{14}C], [2-^{14}C]acetate and [1-^{14}C]propionate infusions and at 45min intervals over the last 5.5h of the [2-^{3}H]glucose and ^{14}C-bicarbonate infusion. The blood was analysed for bicarbonate and glucose specific radioactivities. Rumen fluid samples were taken at hourly intervals over the last 8h of the [1-^{14}C]propionate infusion and assayed for propionate specific radioactivity. A pre-infusion sample was taken from each of the sampled pools before all infusions.

6.5.3 Results

Unfortunately, one animal would not consume its daily ration during the course of the infusions. Therefore, the samples from this animal were discarded.

The interval of one week between infusions was sufficient to allow the residual radioactivity in the sampled pools to fall to negligible values. 24h was sufficient time for the glucose specific radioactivities to plateau during the acetate infusions (as illustrated in Figures 6-9 and 6-10). The specific radioactivities of the pools that were sampled are presented in Table 6-8. The infusion rates, rates of irreversible loss and the percentage of tracer (therefore tracee) entering the sampled secondary pools are presented in Table 6-9.

6.5.4 Discussion

6.5.4.1 Percentage Of Oxaloacetate From Cycling Of The Tricarboxylic Acid Cycle Estimated From Pathways Not Containing A Carboxylation Reaction -

From Section 6.4.4, the proportion of the oxaloacetate pool from pathways involving a carboxylation reaction

= 6 x the transfer quotient of blood bicarbonate to glucose

$$= 6 \times {}^{0.77}/_{5.72}$$

$$= .81$$

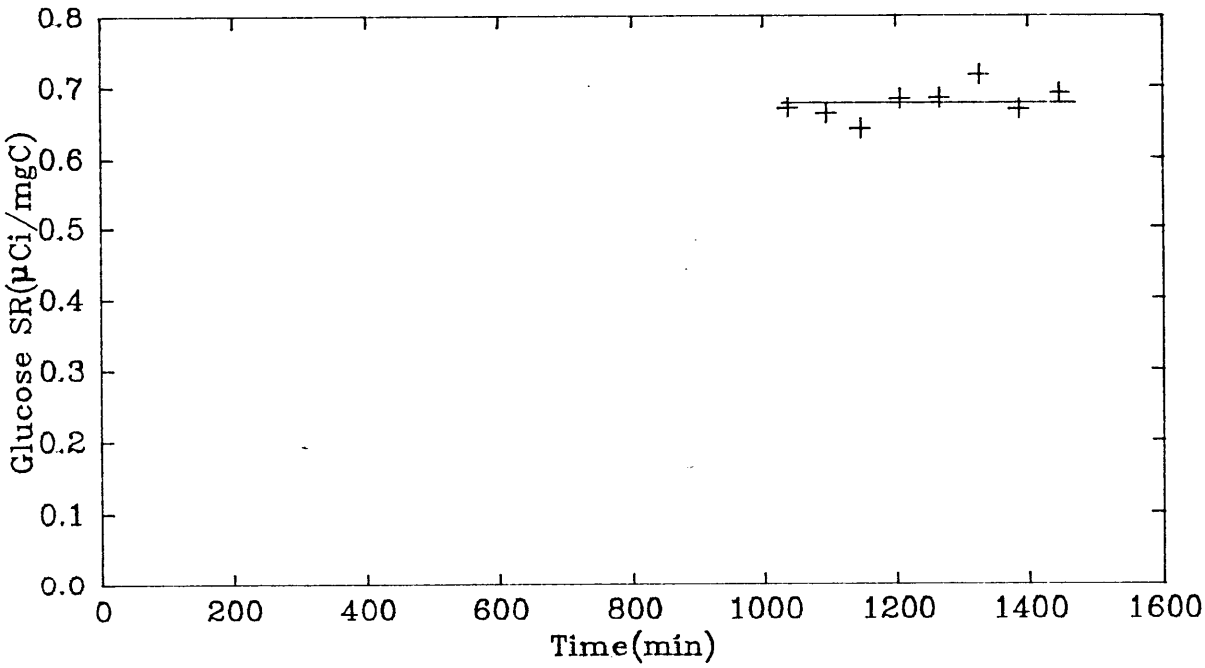


Figure 6-9

The specific radioactivity of glucose in sheep C during an intravenous infusion of [1-^{14}C]acetate

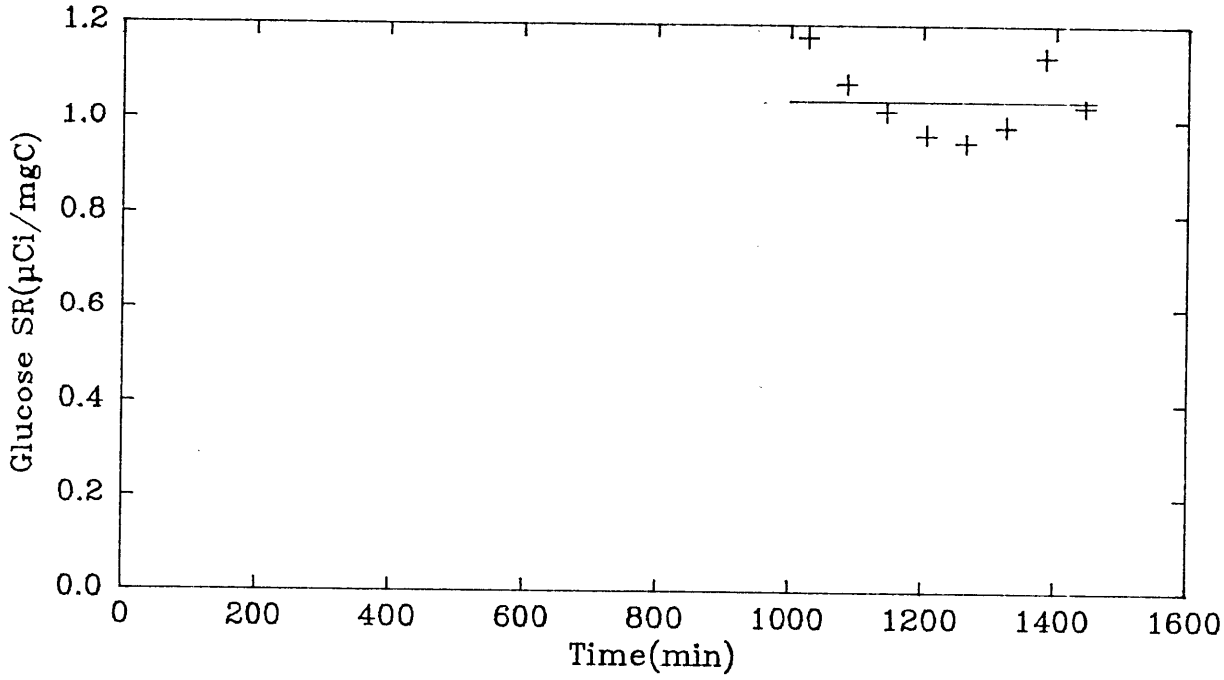


Figure 6-10

The specific radioactivity of glucose in sheep C during an intravenous infusion of [2-^{14}C]acetate

Table 6-8

The specific radioactivities of the pools measured in this experiment.

compound infused	secondary pool	SR (μCi/gC)	standard error
[1-^{14}C]acetate	- Glucose	0.68	0.008
	- Blood bicarbonate	2.85	0.074
[2-^{14}C]acetate	- Glucose	1.04	0.028
	- Blood bicarbonate	1.49	0.032
[2-^{3}H]glucose	- Glucose	1.33	0.024
$H^{14}CO_3^-$	- Glucose	0.77	0.029
	- Blood bicarbonate	5.72	0.242
[1-^{14}C]propionate	- Glucose	1.52	0.027
	- Blood bicarbonate	5.91	0.391
	- Propionate	13.34	0.669

Table 6-9

The infusion rates, irreversible losses and percentage tracer flows from this experiment

		Standard error
[1-^{14}C]acetate infusion rate (ηCi/min)	375	7.5
[2-^{14}C]acetate infusion rate (ηCi/min)	234	4.7
[2-^{3}H]glucose infusion rate (ηCi/min)	20.9	0.43
[^{14}C]bicarbonate infusion rate (ηCi/min)	581	11.6
[1-^{14}C]propionate infusion rate (ηCi/min)	343	6.8
Glucose irreversible loss (gC/d)	22.6	1.11
Bicarbonate irreversible loss (gC/d)	146	17.7
Propionate irreversible loss (gC/d)	37.0	3.78
[1-^{14}C]acetate to glucose (%)[1]	2.8	0.15
[2-^{14}C]acetate to glucose (%)	7.0	0.39
[^{14}C]bicarbonate to glucose (%)	2.1	0.11
[1-^{14}C]propionate to glucose (%)	7.0	0.39
[1-^{14}C]acetate to bicarbonate (%)	77	11.1
[2-^{14}C]acetate to bicarbonate (%)	65	8.9
[1-^{14}C]propionate to bicarbonate (%)	83	14.7

[1] $\%\ X \text{ to } Y = \frac{Y \text{ irreversible loss} \times Y \text{ specific radioactivity (X infused)}}{X \text{ infusion rate}}$

By difference from 100%, the percentage of the molecules in the oxaloacetate pool coming from pathways that do not involve a CO_2 fixation reaction is 19%. The effect of non-adherence to the assumptions is to decrease the incorporation of HCO_3^- into glucose and thus to underestimate the percentage of oxaloacetate from CO_2 fixation reactions. Therefore, 19% is theoretically a maximum value for oxaloacetate coming from cycling of the tricarboxylic acid cycle (according to this method of estimation).

6.5.4.2 Percentage Of The Molecules In The Oxaloacetate Pool From Cycling Of The Tricarboxylic Acid Cycle Estimated From The Glucose Ratio Using Acetate -

The glucose ratio requires values for the incorporation of tracer into glucose from [1-^{14}C] and [2-^{14}C]acetate. From the values in Table 6-9, 2.84% of [1-^{14}C]acetate tracer infused and 7.02% of the [2-^{14}C]acetate tracer infused was incorporated into glucose.

Using these values in the glucose ratio:

$$\frac{5+4NI}{1+2NI} = \frac{7.02}{2.84}$$

$$NI = 2.68$$

The percentage of the molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle:

$$\frac{1}{1+NI} \times \frac{100}{1} = 27\%$$

This value is higher than the estimate obtained by the first method, even though the first method should overestimate the percentage of the molecules in the oxaloacetate pool coming from

cycling of the tricarboxylic acid cycle.

6.5.4.3 Indirect Flows Via The Bicarbonate Pools -

The pathway by which acetate carbon is incorporated into glucose used in developing the theory is as follows: acetate tracer entered the tricarboxylic acid cycle by condensation of acetyl-CoA with oxaloacetate, left the cycle via decarboxylation of oxaloacetate to phosphoenolpyruvate, then was incorporated into glucose via reversal of the Embden-Meyerhof pathway. If tracer from acetate was incorporated into glucose via other pathways, the transfer quotient would overestimate the flow along the pathway assumed in the theory and thus, if used to solve the glucose ratio, result in a spurious answer.

The blood bicarbonate pool becomes labelled during infusions of both species of acetate, indicating a flow of tracer from acetate to bicarbonate. As there is a flow from bicarbonate to glucose, it is possible that the flow of tracer from acetate through the bicarbonate pool to glucose is having a significant effect on the value of the glucose ratio. Kleiber, Black, Brown and Tolbert (1953) reported that 32% of the tracer incorporated into lactose after a single injection of [1-^{14}C]propionate had passed through the CO_2 pool. When [2-^{14}C]propionate was injected only 6% of the tracer incorporated into lactose had passed through the CO_2 pools. Therefore, the values needed to solve the glucose ratio probably have to be adjusted for this flow.

6.5.4.3.1 Effect On The Incorporation Of Tracer From [1-^{14}C]acetate Into Glucose -

From Table 6-9, 2.84% of the [1-^{14}C]acetate tracer was incorporated into glucose, 77.24% of the [1-^{14}C]acetate tracer entered the blood HCO_3^- pool and 2.09% of the $H^{14}CO_3^-$ tracer was incorporated into glucose. Therefore, the amount of tracer from [1-^{14}C]acetate that was incorporated into glucose via the bicarbonate pool was 2.09% of the 77.24% that went to bicarbonate, i.e. 1.61%. Of the 2.84% of [1-^{14}C] acetate tracer that was incorporated into glucose only 1.23% (43% of the tracer in glucose) went via pathways that did not involve the CO_2 pools. Therefore, the maximum percentage of acetate incorporated into glucose via the pathways assumed in the theory was 1.23%. A significant proportion of the carbon atoms in the glucose pool that originated in the acetate pool (i.e. the transfer quotient) was incorporated due to indirect flows via the bicarbonate pool.

6.5.4.3.2 Effect On The Incorporation Of Tracer From [2-^{14}C]acetate Into Glucose -

When [2-^{14}C]acetate was infused

7.02% of acetate tracer was incorporated into glucose

64.87% of acetate tracer went to blood bicarbonate

2.09% of bicarbonate tracer went to glucose.

The percentage of tracer from [2-^{14}C]acetate that was incorporated into glucose via bicarbonate was 2.09% of the 64.87% that went to bicarbonate from [2-^{14}C]acetate] i.e. 1.35%. Therefore, only 5.67% (80.7% of the tracer that is in glucose) of the tracer from the methyl carbon of acetate was incorporated into glucose via pathways other than via CO_2. Again, if the indirect flow via bicarbonate was

not accounted for, the transfer quotient would overestimate the flow from the methyl carbon of acetate to glucose along the direct route.

6.5.4.3.3 Effect On The Estimate Of Oxaloacetate From Cycling Of The Tricarboxylic Acid Cycle Calculated From The Glucose Ratio -

These corrected values were used in the glucose ratio

$$\frac{5+4NI}{1+2NI} = \frac{5.67}{1.23}$$

$$NI = 0.075$$

The percentage oxaloacetate from cycling of the tricarboxylic acid cycle

$$= \frac{1}{1+NI} \times \frac{100}{1}$$

$$= 93\%$$

This value is considerably different from that using the uncorrected flows. The flows of tracer via bicarbonate are having a significant effect on the glucose ratio and hence the estimate of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle.

6.5.4.3.4 Conclusion -

The estimates of the percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle are 93% from the glucose ratio using acetate and 19% from the incorporation of CO_2 into glucose. The values are not compatible and therefore indicate that the theory used to develop the equations is not adequate to explain the data. It appears that the glucose ratio overestimates the percentage of the molecules in the oxaloacetate pool

from cycling of the tricarboxylic acid cycle and the value from the incorporation of CO_2 underestimates it. Recycling of tracer via the pathway; oxaloacetate, phosphoenolpyruvate , pyruvate, oxaloacetate and/or exchange reactions between CO_2 and oxaloacetate would result in effects consistent with the above observations.

6.5.4.4 Tracer Recycling Back To Oxaloacetate Via Phosphoenolpyruvate And Pyruvate And/or Exchange Reactions Between CO_2 And Oxaloacetate -

6.5.4.4.1 Effect On The Specific Radioactivities Of The Carbons Of Oxaloacetate -

The effects of this type of tracer recycling on the specific radioactivities of the carbons of oxaloacetate are illustrated in the following examples. Figures 6-11 to 6-14 illustrate the effects of recycling via phosphoenolpyruvate and pyruvate or a form of cycling that has the same effects on the tracer flows. The following assumptions are incorporated into the illustrations:

1. 3 units of oxaloacetate are provided by propionate,
2. 2 units of oxaloacetate are provided from other sources,
3. the system is in steady state,
4. for the purposes of clarity, the effects of interaction with the tricarboxylic acid cycle are not included,
5. complete equilibrium of the tracer about the carboxyl or middle carbons of oxaloacetate occurs due to complete equilibration of the dicarboxylic acid pools.

Figure 6-11 illustrates the situation where the middle carbon of propionate has a specific radioactivity of 200 and no recycling is occurring.

Figure 6-12 illustrates the same situation as Figure 6-11 except that the carboxyl carbon, and not the middle carbon, is labelled. In both the above illustrations the specific radioactivity on the labelled carbons of oxaloacetate is 60.

Figure 6-13 is the same as Figure 6-11 except that 5 units of oxaloacetate are recycled from phosphoenolpyruvate to oxaloacetate via pyruvate (or a form of recycling that has the same effect on the tracer flows). For this to occur the flow from oxaloacetate to phosphoenolpyruvate must double. The molecules returning to the oxaloacetate pool have the same specific radioactivity as the molecules in the pool, thus the specific radioactivity of the pool is unchanged. This is as expected because no tracer is lost in the cycling process.

Figure 6-14 is the same as Figure 6-13 except that the carboxyl carbon of propionate is labelling the oxaloacetate pool. In this case half the tracer is removed in the decarboxylation reaction; oxaloacetate to phosphoenolpyruvate. The carbon that is incorporated in the reaction, pyruvate to oxaloacetate, will be from the CO_2 pool and assumed to have the specific radioactivity of the CO_2 pool (zero in the illustration). Equilibration with the symmetrical dicarboxylic acids causes the tracer to again distribute about both carboxyl positions. The specific radioactivity of the carboxyl carbons on the recycled molecules is lower than the specific radioactivity of the carboxyl carbon pool without recycling. Therefore, recycling must cause the specific radioactivity of the pool to decrease.

Figure 6-11

The specific radioactivities of the carbons of oxaloacetate, phosphoenolpyruvate and glucose when propionate is converted to glucose under the assumptions described in the text.

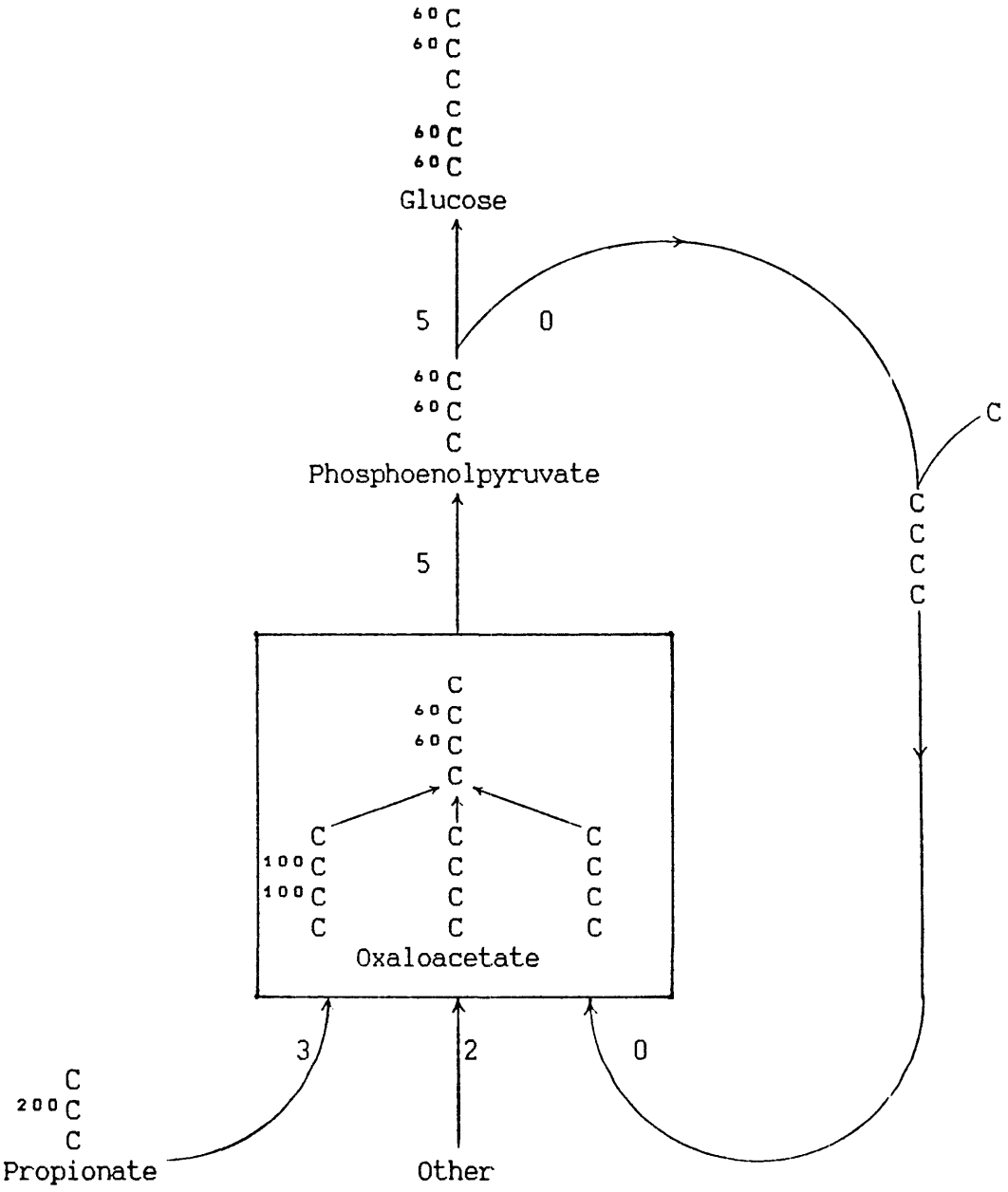


Figure 6-12

The specific radioactivities of the carbons of oxaloacetate, phosphoenolpyruvate and glucose when propionate is converted to glucose under the assumptions described in the text.

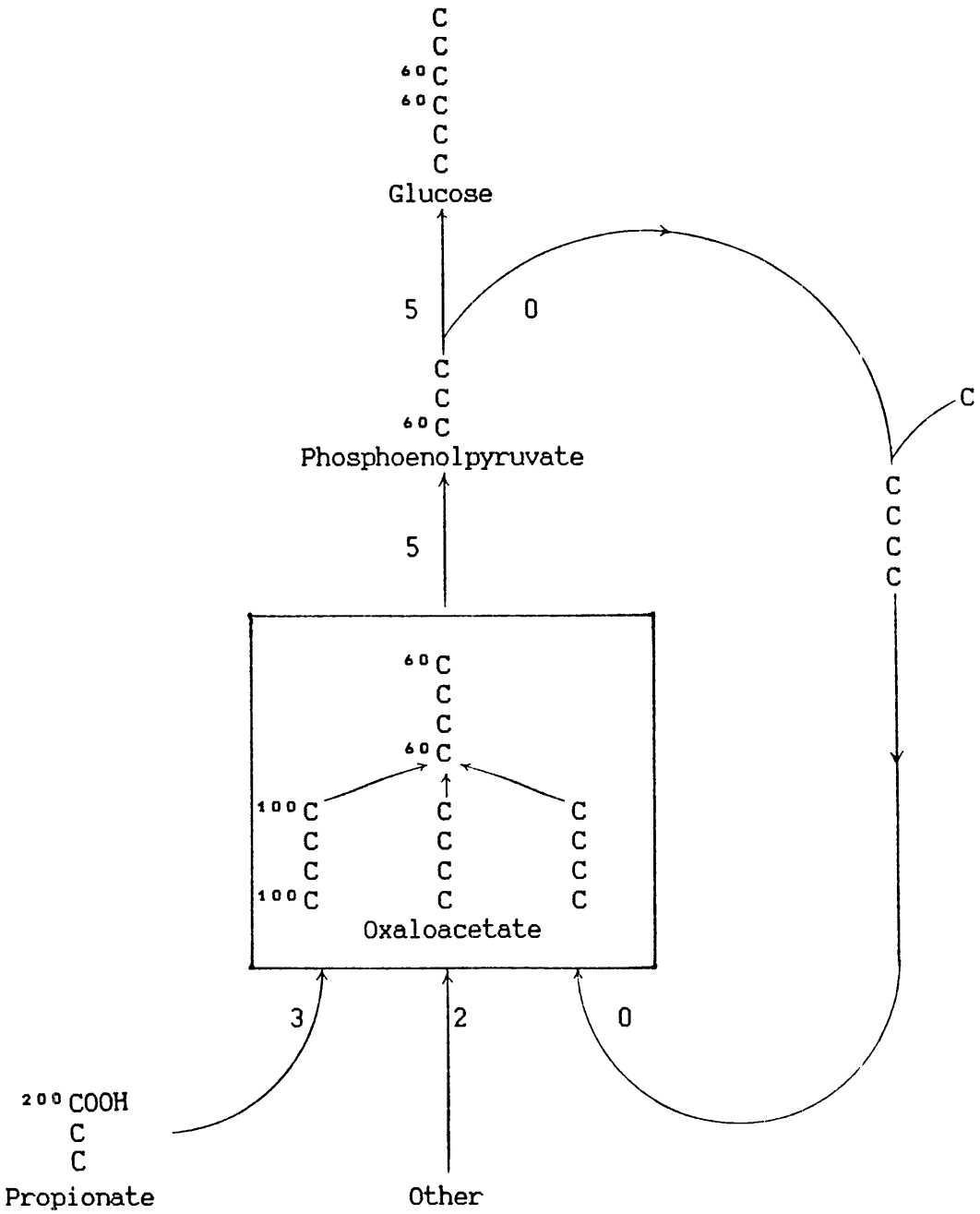


Figure 6-13

The effects of recycling via the pathway; oxaloacetate, phosphoenolpyruvate, pyruvate, oxaloacetate, on the specific radioactivities of the carbons of oxaloacetate, phosphoenolpyruvate and glucose when propionate is converted to glucose under the assumptions described in the text.

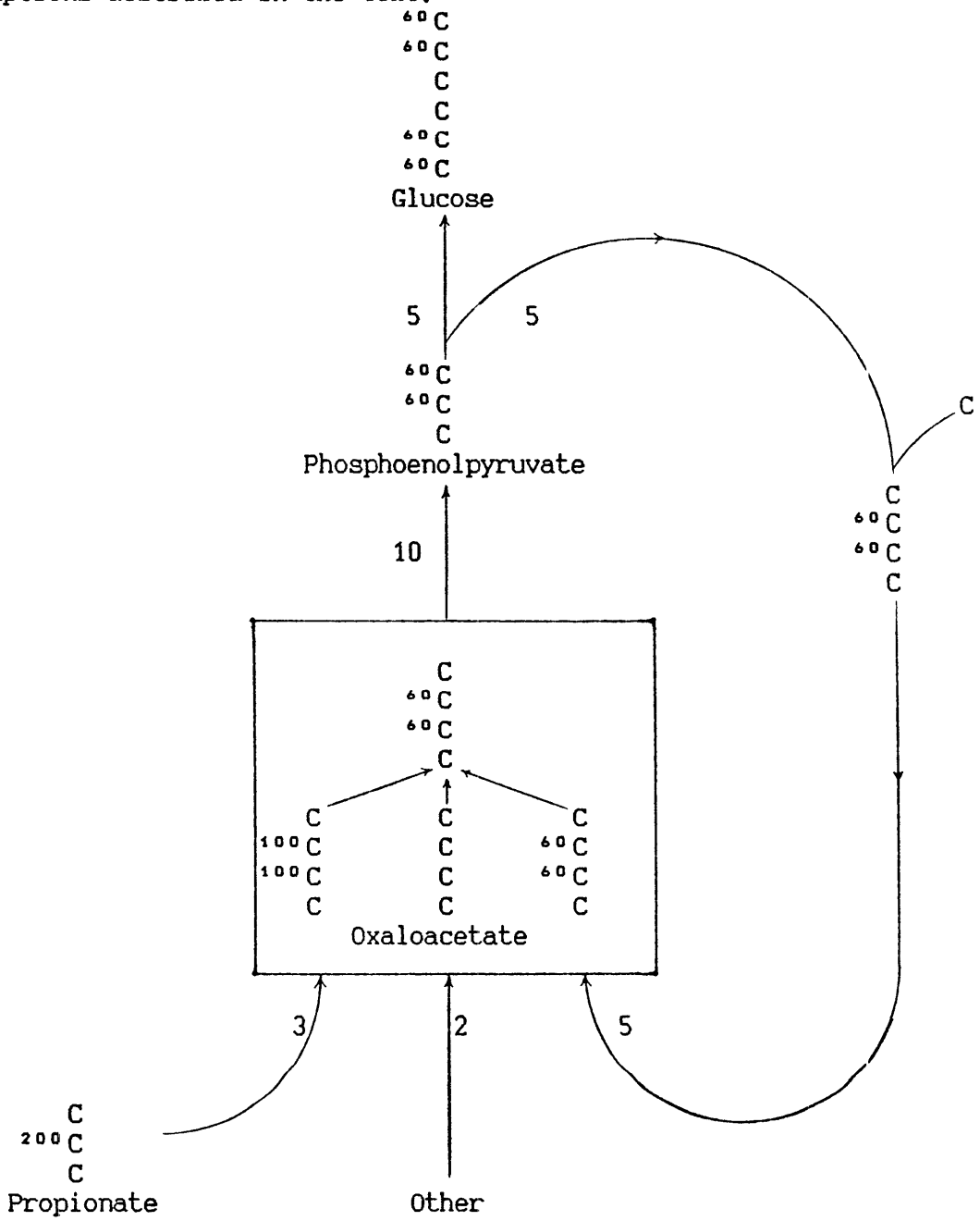
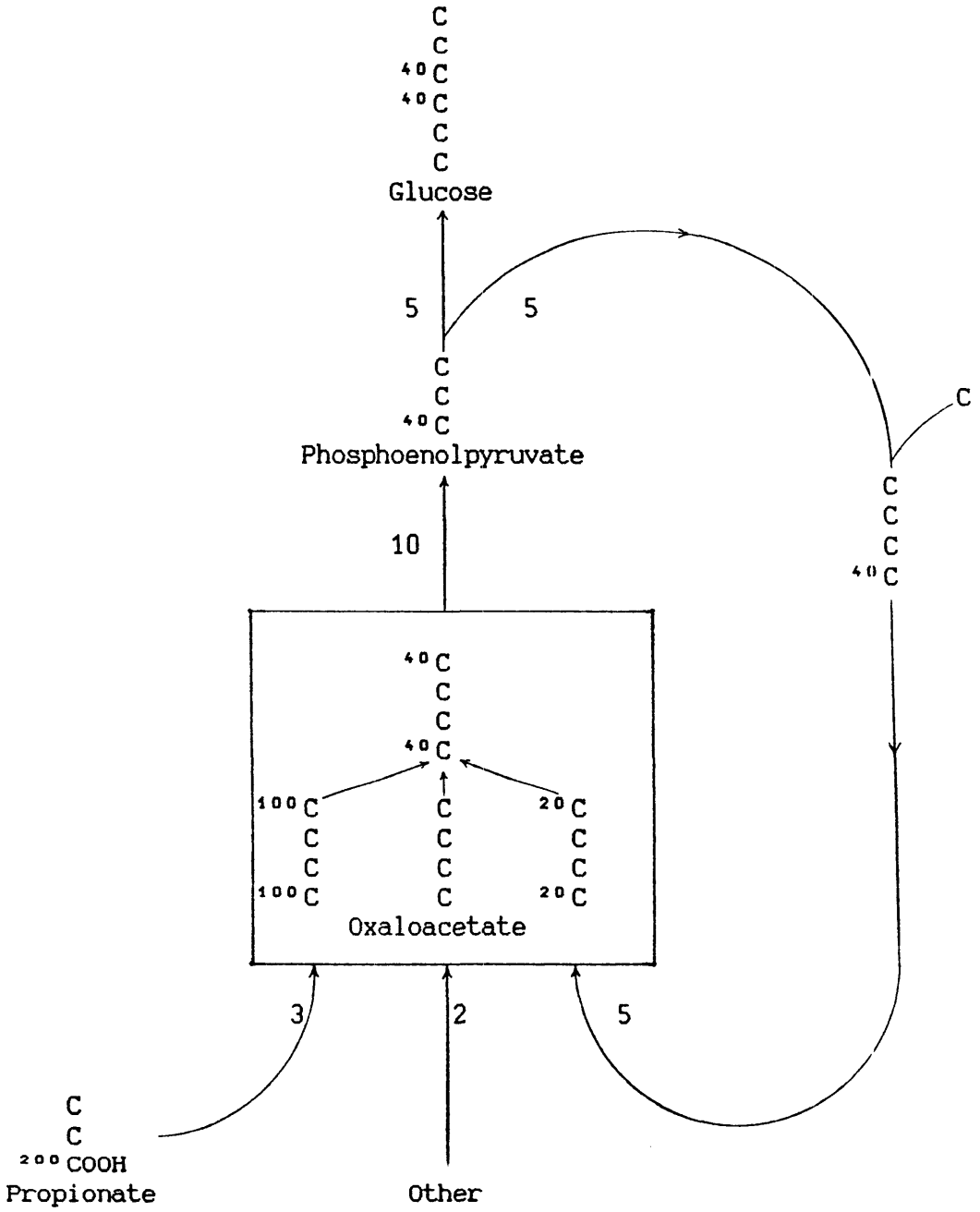


Figure 6-14

The effects of recycling via the pathway; oxaloacetate, phosphoenolpyruvate, pyruvate, oxaloacetate, on the specific radioactivities of the carbons of oxaloacetate, phosphoenolpyruvate and glucose when propionate is converted to glucose under the assumptions described in the text.



The extent of equilibration with the dicarboxylic pools is critical because if it does not occur, the carbon that is incorporated in the carboxylation reaction is the carbon that is removed in the decarboxylation reaction and thus will not affect tracer flows. If there is any reincorporation of CO_2 formed in the oxaloacetate to phosphoenolpyruvate reaction then the effect of the recycling on the tracer flows will be partially negated.

6.5.4.4.2 Effect On The Glucose Ratio -

In terms of the specific radioactivity of oxaloacetate carbons, the glucose ratio equals

$$\frac{2 \times \left[\begin{array}{c}\text{specific radioactivity of}\\ \text{the middle carbons of}\\ \text{oxaloacetate}\\ \text{([2-}^{14}\text{C]acetate infused)}\end{array}\right] + \left[\begin{array}{c}\text{specific radioactivity of}\\ \text{the carboxyl carbons of}\\ \text{oxaloacetate}\\ \text{([2-}^{14}\text{C]acetate infused)}\end{array}\right]}{\begin{array}{c}\text{specific radioactivity of carboxyl carbons of oxaloacetate}\\ \text{when [1-}^{14}\text{C]acetate infused}\end{array}}$$

$$= [2({}^{ASR}/_{(1+2NI)}) + ({}^{ASR}/_{(2+2NI)(1+2NI)})] \; / \; [{}^{ASR}/_{(2+2NI)}]$$

$$= {}^{3+4NI}/_{1+2NI} \qquad \text{(from Section 4.4.4)}$$

Recycling of tracer via phosphoenolpyruvate and pyruvate and/or exchange reactions between oxaloacetate and CO_2 only affects the incorporation of the carboxyl carbons of oxaloacetate into glucose. It will decrease the denominator and part of the numerator of the glucose ratio. Because only part of the numerator of the glucose ratio is decreased, the effect on the denominator will be proportionally greater. Therefore, recycling causes the value of the glucose ratio to increase, NI to decrease, and the estimate of the percentage of the molecules in the oxaloacetate pool that arises from cycling of the tricarboxylic acid cycle to increase. Correcting for this form of recycling will decrease the estimate of the percentage of

the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle.

Molecules that recycle through lactate or alanine have to pass through the pyruvate pool and thus the effects on tracer flows will be as described above.

In order to estimate the effects of the recycling let X be the proportional decrease in the specific radioactivity of the carboxyl carbon of oxaloacetate that is incorporated into glucose due to recycling of molecules via pyruvate and/or exchange reactions with CO_2. Therefore, $(^1/_{(1-X)})$ is the proportional increase in the incorporation of CO_2 carbon into glucose due to this type of recycling.

To adjust the glucose ratio for the effects of the above form of recycling the specific radioactivity of the carboxyl carbons of oxaloacetate has to be multiplied by (1-X). Let B equal the value of the glucose ratio. Therefore, B

$$=[2(^{ASR}/_{(1+2NI)})+(^{ASR(1-X)}/_{(2+2NI)(1+2NI)})] \;/\; [^{ASR(1-X)}/_{(2+2NI)}]$$

$$= {}^{(5+4NI-X)}/_{(1+2NI)(1-X)}$$

6.5.4.4.3 Effect On The Incorporation Of CO_2 Into Glucose -

From Section 6.4.4, the percentage of the molecules in the oxaloacetate pool from pathways that involve a carboxylation reaction equals 6 times the transfer quotient of blood bicarbonate to glucose.

Let the apparent percentage of the molecules in the oxaloacetate pool from pathways that contain a carboxylation reaction = A (i.e. A = 6 times the blood bicarbonate to glucose transfer quotient).

Under the assumptions stated earlier (Section 4.5), the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle equals 1-A. Therefore,

$$^{1}/_{(1+NI)} = 1-A$$

To correct this equation for the effects of recycling via pyruvate and/or exchange reactions between oxaloacetate and CO_2, A (the apparent percentage of the molecules in the oxaloacetate pool from pathways that involve a carboxylation reaction) has to be multiplied by (1-X). Therefore, the equation becomes

$$^{1}/_{(1+NI)} = 1 - A(1-X)$$

6.5.4.4.4 Effect On The Percentage Of The Molecules In The Oxaloacetate Pool From Cycling Of The Tricarboxylic Acid Cycle -

The value of the glucose ratio and the incorporation of CO_2 can be measured experimentally. This leaves 2 unknowns (NI and X) in the above two equations. Therefore, it is possible to solve for the unknowns. Substituting the equation for the CO_2 incorporated into glucose into the glucose ratio results in the following quadratic equation,

$$NI^2(4A-2B) + NI(8A+1-B) + 4A = 0$$

Using the value of the glucose ratio corrected for the indirect flows via the bicarbonate pools (4.61) and the value of the percentage of the molecules in the oxaloacetate pool apparently coming from pathways that involve a CO_2 fixation reaction (0.81) the following equation is obtained,

$$0 = -5.98NI^2 + 2.87NI + 3.24$$

The roots of this equation are 1.01 and -0.53. The negative root is biologically meaningless. Therefore, the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle ($^1/_{(1+NI)}$)

$$= 50\%$$

The value of X calculated using this value of NI is 0.38. This means that recycling of tracer via pyruvate and/or exchange reactions between oxaloacetate and CO_2 decreases the specific radioactivity of the carboxyl carbon of oxaloacetate that is incorporated into glucose by 38% when [1-^{14}C]acetate or [1-^{14}C]propionate are infused and increases the specific radioactivity by 61% when ^{14}-bicarbonate is infused.

Although X has been ascribed to the forms of cycling mentioned above, it actually represents the combined effects of all forms of cycling that affect the flows of tracer in the same way as described above. For example, the effects of loss of tracer from the carboxyl position of propionate owing to metabolism of propionate to lactate in the rumen wall would also be included in X.

6.5.4.4.5 Effect On The Percentage Of Glucose Being Provided By Propionate -

As there is a flow of tracer from [1-^{14}C]propionate to blood bicarbonate, the transfer quotient of propionate carboxyl carbon to glucose will overestimate the tracer incorporated into glucose via the direct route.

From the data given in Table 6-9,

7.0% of [1-^{14}C]propionate tracer was incorporated into glucose

82.5% of [1-^{14}C]propionate tracer entered the blood bicarbonate pool

2.1% of ^{14}C bicarbonate was incorporated into glucose

The percentage of tracer from [1-^{14}C]propionate that was incorporated into glucose via bicarbonate was 2.1% of the 82.5% that went to bicarbonate, i.e. 1.7%. Therefore, 5.3% (75% of the tracer in glucose) of the propionate tracer went to glucose by pathways other than via the CO_2 pool. Using the data in Table 6-8 the transfer quotient of propionate to glucose is 11.4. Correcting the value for the flow via CO_2 gives,

adjusted transfer quotient = 11.4 x 0.75

= 8.6%

To obtain the percentage of the molecules in the oxaloacetate pool being provided by propionate this transfer quotient has to be multiplied by 2 to account for the loss of tracer at the decarboxylation step.

percentage oxaloacetate from propionate = 8.6 x 2

$$= 17.2\%$$

Correcting this value for the loss of tracer due to the possible recycling via phosphoenolpyruvate and pyruvate and/or exchange reactions between oxaloacetate and CO_2, i.e. divide by (1-X) gives

$$^{17.2}/_{0.62} = 27.7\%$$

of the oxaloacetate pool was provided by propionate. As 50% of the oxaloacetate pool was provided by cycling of the tricarboxylic acid cycle, only 50% was provided by net input sources and therefore only 50% is available for anabolic purposes. Propionate provided 27.7% of the 50% of the oxaloacetate pool from net inputs to the tricarboxylic acid cycle. Therefore, propionate must be providing 55.4% of the molecules used for anabolic purposes, and thus 55.4% of the molecules used to synthesise glucose.

6.5.4.4.6 The Pattern Of Tracer Flow -

The pattern of tracer flow indicated by the above interpretation is illustrated in Figure 6-15. As shown in this figure, 70% of the flow from oxaloacetate to phosphoenolpyruvate returns to the oxaloacetate pool via pyruvate (or via a pathway that has similar effects on the tracer flows). If there is less than 100% equilibration of the carboxyls the actual recycling will be higher. The proportion of the recycled molecules that do not equilibrate does not affect the tracer flows.

The predicted pattern of tracer flow for [2-^{14}C]propionate if it had been infused is shown in Figure 6-16. Unfortunately [2-^{14}C]propionate was not infused in this experiment and the predicted specific radioactivity cannot be verified with a measured specific

Figure 6-15

The pattern of molecule flow indicated by the interpretation of the results obtained in Section 6.5

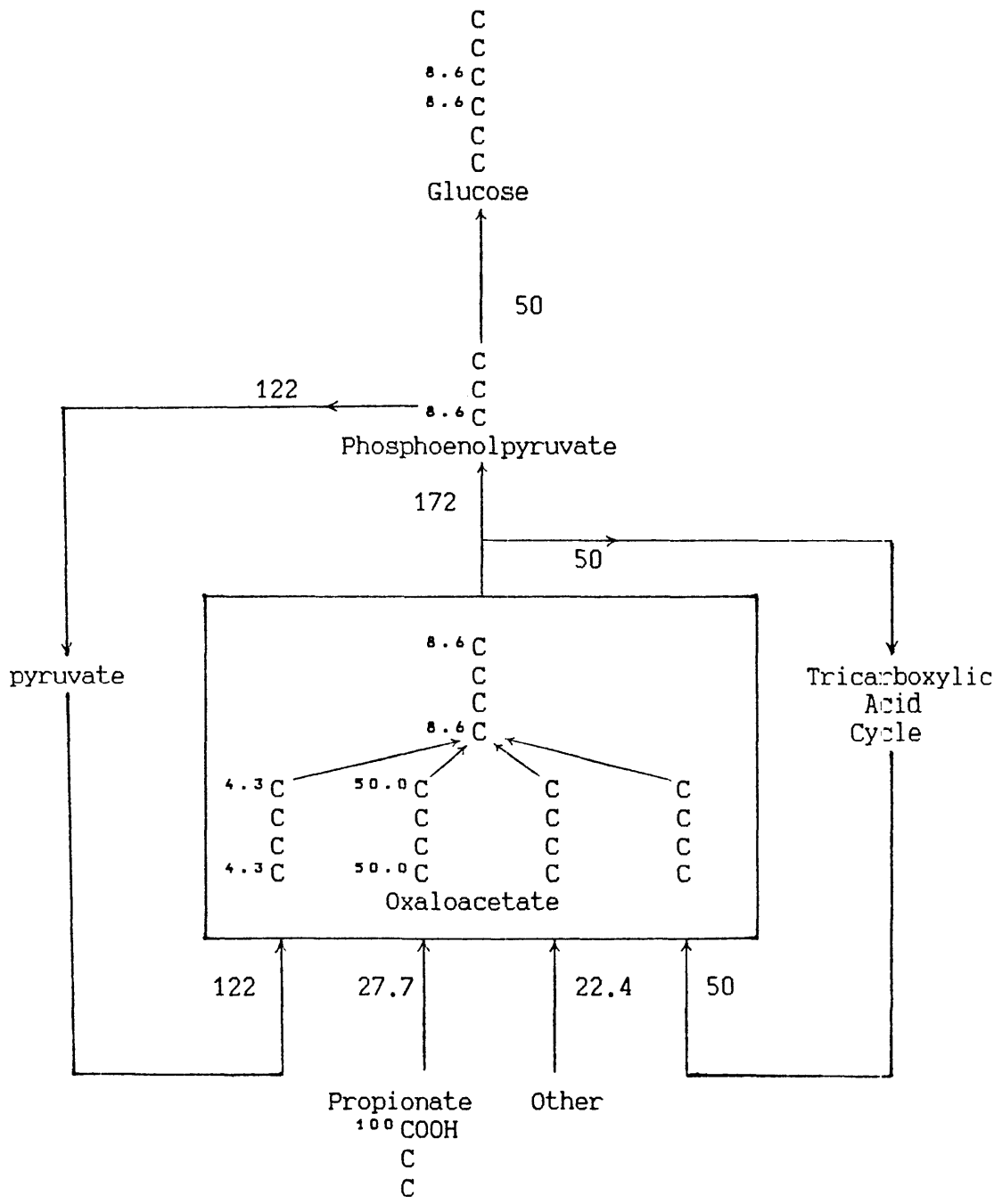
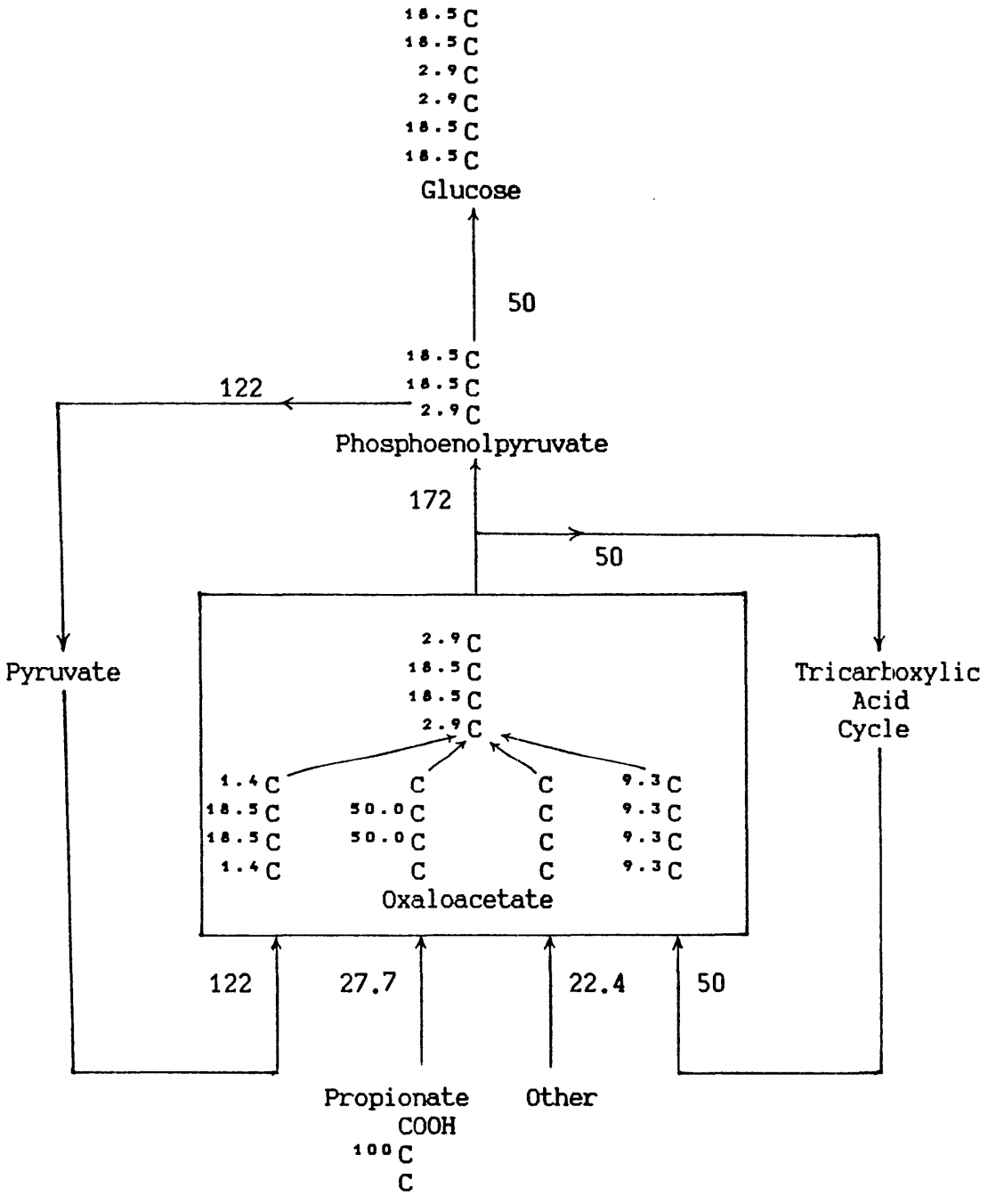


Figure 6-16

The predicted pattern of tracer flow if [2-^{14}C]propionate had been infused into the rumen of sheep in the experiment reported in Section 6.5.



radioactivity. Annison, Leng, Lindsay and White (1963) presented results for distribution of tracer in the individual carbons of glucose during an infusion of [2-^{14}C]propionate into the portal vein of anaesthetized sheep. These animals were given the same diet (800g/d lucerne chaff), but unlike the experiment reported here, their sheep were on once a day feeding and were not fed on the day of the experiment. They found that 8% of the tracer in glucose was in positions 3 and 4. The above interpretation predicts that 7% of the tracer would be in positions 3 and 4 of glucose if [2-^{14}C]propionate had been infused in the experiment reported here. Although these figures are in good agreement, the percentages of tracer from [2-^{14}C]propionate in positions 3 or 4 of glucose will always be small and therefore subject to large percentage errors. This makes real differences difficult to measure.

As will be discussed in Section 7.3.6, the CO_2 ratio is not affected by recycling of tracer via phosphoenolpyruvate and pyruvate (or a form of recycling that has similar effects on the tracer flows). Therefore, the CO_2 ratio can be used as an independent check of the above interpretation. If the CO_2 ratio gives estimates of the percentage of molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle similar to estimates from the simultaneous solving of the glucose ratio and CO_2 incorporation into glucose equation, then there will be evidence that recycling via phosphoenolpyruvate and pyruvate (or a form of recycling that has similar effects on the tracer flows) is affecting the results and has to be accounted for in any interpretation of the data. In the next experiment the CO_2 ratio is measured along with the glucose ratio and the incorporation of CO_2 into glucose to test if the above interpretation is valid.

CHAPTER 7

QUANTIFYING METABOLIC CROSSOVER USING POOL MODELLING TECHNIQUES

7.1 INTRODUCTION

One of the problems with comparing the estimates reported in the last Chapter, of the percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle using the different methods was that not all methods of estimation were used on the same animals. Therefore, in the experiment described here all the parameters necessary to estimate the percentage of the molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle by each of the four methods (i.e., the CO_2 and glucose ratios using propionate, the glucose ratio using acetate and the percentage of molecules in the oxaloacetate pool not coming from pathways involving carboxylation of three carbon compounds) were estimated in individual animals. This should remove between animal variation from the calculations and increase the accuracy of the comparison of methods.

It was decided to perform this experiment on sheep fed a diet that was low in gluconeogenic precursors in order to ensure that a large percentage of the main gluconeogenic precursors were being used for gluconeogenesis. The diet used was 800g/d oaten straw chaff (all

grain completely removed).

7.2 EXPERIMENTAL

7.2.1 Materials And Methods

The general materials and methods are presented in Chapter 5.

Two mature Merino sheep were used in this experiment. The animals were well accustomed to being handled and appeared not to be stressed by the experimental procedures.

The daily ration was 800g (air dry) oaten straw chaff. This chaff was produced by putting oaten chaff (straw chaff plus grain) through a grain cleaner and collecting the straw pieces. The straw chaff contained some grain husk but was completely free of all grain and grain chips. The order, site, duration and appropimate tracer infusion rates of the infusions are shown in Table 7-1. All infusions were timed to finish at 1800h. At least 1 week was allowed to elapse between infusions to ensure that residual radioactivity in the animals had dissipated.

Samples of blood and rumen fluid were taken at 45min intervals over the last 6h of each infusion. Blood samples were assayed for glucose and bicarbonate specific radioactivities. Rumen fluid samples were assayed for the specific radioactivity of bicarbonate, propionate and acetate.

Table 7-1

The order, site, duration and approximate infusion rates of the infusions carried out as part of experiment 7.

Order	Isotope infused	Site of infusion	Duration of infusion (h)	Approximate infusion rate (μCi/min)
1	[1-^{14}C]propionate	IR	12	0.2
2	[2-^{14}C]propionate	IR	12	0.1
3	$H^{14}CO_3^-$	IV	10	0.2
4	$H^{14}CO_3^-$	IR	12	0.4
5	[U-^{14}C]glucose	IV	10	0.4
6	[2-^{14}C]acetate	IR	24	0.4
7	[1-^{14}C]acetate	IR	24	0.4

IR = infusion into the rumen

IV = infusion into the jugular vein

7.2.2 Results

The dry matter, organic matter and total N contents of the oaten straw chaff are presented in Table 7-2.

The specific radioactivities of the primary and secondary pools during each infusion are presented in Tables 7-3 to 7-9. In the cases where specific carbon atoms of the infused compound are labelled, the specific radioactivity for the primary pool is expressed as the specific radioactivity of the tracee atom. The transfer quotients represent the proportion of the total carbon of the secondary pool arising from the tracee atom. The rates of irreversible loss are presented in Table 7-10. In the cases where specific atoms of the infused compound are labelled, the rates of irreversible loss presented are the rates of irreversible loss of the tracee atoms.

7.3 DISCUSSION

Because direct flows have been assumed in the theory used to develop the equations and ratios, the transfer quotients have to be adjusted for indirect flows of tracer before they are appropriate to use in solving the ratios. The mathematical equations used to solve the open system models of Mann and Gurpide (1966) and Nolan et al. (1976) make use of the labelling of secondary pools and indirect tracer flows so as to calculate the tracee flows. The values of the flows calculated in these models are corrected for the indirect flows of tracer if an intermediate of the indirect pathway is included in the model. Therefore, the values calculated in these models are suitable for using to solve the ratios developed in Chapter 4.

Table 7-2

The dry matter (DM), organic matter and total nitrogen content of the oaten chaff straw

Dry matter content (g/kg)	886
Organic matter content (g/kg DM)	934
Total nitrogen content (g/kg DM)	12

Table 7-3

The specific radioactivity (SR) of the carboxyl carbon of propionate and the specific radioactivities of the secondary pools during an intraruminal [1-^{14}C]propionate infusion. The percentages of the carbon atoms in the secondary pools that arose from the pool into which the tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep H		Sheep I	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Propionate carboxyl	35.4 (0.82)	-	17.6 (1.47)	-
Rumen HCO_3^-	2.41 (0.030)	6.8 (0.42)	1.86 (0.034)	10.6 (2.05)
Blood HCO_3^-	1.45 (0.070)	4.1 (0.56)	1.43 (0.038)	8.1 (1.63)
Glucose	1.16 (0.042)	3.3 (0.36)	1.23 (0.036)	7.0 (1.42)

The values in ( ) are the standard errors

Table 7-4

The specific radioactivity (SR) of the middle carbon of propionate and specific radioactivities of the secondary pools during an intraruminal [2-^{14}C]propionate infusion. The percentages of the carbon atoms in the secondary pools that arise from the pool into which tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep H		Sheep I	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Propionate middle carbon	24.0 (2.54)	-	12.9 (0.82)	-
Rumen HCO_3^-	0.25 (0.014)	1.0 (0.26)	0.21 (0.027)	1.6 (0.55)
Blood HCO_3^-	0.48 (0.031)	2.0 (0.55)	0.46 (0.031)	3.6 (0.74)
Glucose	2.71 (0.101)	11.3 (2.63)	2.63 (0.112)	20.4 (3.23)

The values in ( ) are the standard errors

Table 7-5

The specific radioactivity (SR) of the blood HCO_3^- pool and the specific radioactivities of the secondary pools during an intravenous infusion of $H^{14}CO_3^-$. The percentages of the carbon atoms in the secondary pools that arose from the pool into which tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep H		Sheep I	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Blood HCO_3^-	1.77 (0.048)	-	1.80 (0.061)	-
Propionate	0.22 (0.015)	12.5 (1.90)	0.21 (0.015)	11.6 (1.96)
Acetate	0.04 (0.004)	2.4 (0.48)	0.03 (0.003)	1.6 (0.26)
Rumen HCO_3^-	1.12 (0.028)	63.4 (5.93)	0.64 (0.022)	35.4 (4.34)
Glucose	0.33 (0.007)	18.4 (1.66)	0.42 (0.017)	23.2 (3.31)

The values in ( ) are the standard errors

Table 7-6

The specific radioactivity (SR) of the rumen HCO_3^- pool and the specific radioactivities of the secondary pools during an intraruminal infusion of $H^{14}CO_3^-$. The percentages of the secondary pools arising from the pool into which the tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep H		Sheep I	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Rumen HCO_3^-	5.51 (0.157)	-	5.91 (0.288)	-
Propionate	1.45 (0.02)	26.4 (2.24)	1.53 (0.013)	25.9 (3.40)
Acetate	0.20 (0.028)	3.7 (0.76)	0.17 (0.006)	2.9 (0.41)
Blood HCO_3^-	2.16 (0.067)	39.3 (4.20)	2.65 (0.115)	44.8 (7.76)
Glucose	0.78 (0.050)	14.2 (2.61)	0.78 (0.042)	13.3 (2.54)

The values in ( ) are the standard errors

Table 7-7

The specific radioactivity (SR) of the glucose pool and the specific radioactivities of the secondary pools during an intravenous infusion of [U-^{14}C]glucose. The percentages of the secondary pools arising from the pool into which the tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep H		Sheep I	
	SR µCi/gC	TQ %	SR µCi/gC	TQ %
GLucose	23.44 (0.368)	-	24.51 (1.056)	-
Propionate	0.11 (0.006)	0.5 (0.06)	0.14 (0.019)	0.6 (0.017)
Acetate	0.03 (0.001)	0.1 (0.01)	.03 (0.003)	0.1 (0.03)
Rumen HCO_3^-	1.23 (0.091)	5.3 (1.19)	0.75 (0.028)	3.1 (0.51)
BLood HCO_3^-	1.90 (0.072)	8.1 (1.0)	1.76 (0.127)	7.2 (1.71)

The values in ( ) are the standard errors

Table 7-8

The specific radioactivity (SR) of the methyl carbon of acetate and the specific radioactivities of the secondary pools during an intraruminal [2-^{14}C]acetate infusion. The percentages of the carbon atoms in the secondary pools that arose from the pool into which the tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep H		Sheep I	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Acetate methyl carbon	11.9 (0.37)	-	19.1 (2.47)	-
Rumen HCO_3^-	2.41 (0.062)	20.3 (1.90)	2.67 (0.031)	14.0 (2.04)
Blood HCO_3^-	1.58 (0.039)	13.3 (1.21)	1.73 (0.039)	9.1 (1.42)
Glucose	1.62 (0.065)	22.0 (2.02)	2.93 (0.028)	15.3 (2.21)

The values in ( ) are the standard errors

Table 7-9

The specific radioactivity (SR) of the carboxyl carbon of acetate and the specific radioactivities of the secondary pools during an intraruminal [1-^{14}C]acetate infusion. The percentages of the carbon atoms in the secondary pools that arose from the pool into which the tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep H		Sheep I	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Acetate carboxyl carbon	19.1 (1.56)	-	20.2 (2.84)	-
Rumen HCO_3^-	2.98 (0.012)	15.6 (2.42)	2.98 (0.150)	14.7 (4.22)
Blood HCO_3^-	2.98 (0.044)	15.6 (2.32)	3.26 (0.064)	16.2 (4.04)
Glucose	1.43 (0.019)	7.5 (1.10)	1.44 (0.014)	7.2 (2.64)

The values in ( ) are the standard errors

Table 7-10

The rates of irreversible loss of the carboxyl and middle carbons of propionate, rumen bicarbonate, blood bicarbonate, glucose, and the carboxyl and methyl carbons of acetate.

	Sheep H gC/d	Sheep I gC/d
Propionate carboxyl carbon	8.2 (0.46)	16.6 (3.14)
Propionate middle carbon	6.6 (1.40)	12.2 (1.37)
Blood HCO_3^-	206 (15.3)	204 (18.8)
Rumen HCO_3^-	105 (8.2)	98 (12.8)
Glucose	23.9 (1.17)	23.0 (2.84)
Acetate methyl carbon	52.6 (3.07)	33.1 (8.58)
Acetate carboxyl carbon	28.6 (4.08)	27.3 (6.67)

The values in ( ) are the standard deviations

7.3.1 Development Of Models To Solve The CO_2 Ratio (using Propionate)

The CO_2 ratio is the ratio of the CO_2 produced from [1-^{14}C] and [2-^{14}C]propionate in the compartment of the cells of the tissues that carry out gluconeogenesis. As almost all propionate is removed from the portal blood by the liver and subsequently metabolised there (Annison et al., 1957), most of the $^{14}CO_2$ produced from propionate in the body will be produced in the liver, which is the major gluconeogenic tissue. Dilution with unlabelled HCO_3^- will affect both the denominator and numerator of the CO_2 ratio proportionally and thus not change its value. Therefore, the value of the CO_2 ratio using blood specific radioactivities should be similar to the value of the ratio produced in the liver. However, this will not be true if propionate is metabolized in tissues of differing synthetic activities that release $^{14}CO_2$ of a different ratio into the blood. If there is metabolism of propionate to bicarbonate in the rumen and subsequent exchange of labelled bicarbonate with blood, then the value of the CO_2 ratio using blood bicarbonate specific radioactivities will not reflect the value of the CO_2 ratio produced in the liver.

Mayes, Milne, Lamb and Spence (1980) reported that when [1-^{14}C]propionate was infused intraruminally considerable amounts of propionate carbon were apparently converted to CO_2 in the rumen. However, when [2-^{14}C]propionate was infused intraruminally negligible amounts of ruminal CO_2 were apparently derived from propionate. This indicates that there is metabolism of the carboxyl carbon but not the methyl carbon of propionate in the rumen. In this experiment the transfer quotient of [1-^{14}C]propionate to rumen HCO_3^- is much higher than [2-^{14}C]propionate to rumen HCO_3^-. Therefore, two pool models of the propionate carbons and rumen bicarbonate were set up to quantify

the flows from propionate to bicarbonate in the rumen.

7.3.1.1 Two Pool Models -

7.3.1.1.1 Propionate Carboxyl Carbon - Rumen HCO_3^- -

The models were constructed as described in Section 5.3.2.

The transfer quotient of rumen bicarbonate to propionate indicated that significant amounts of rumen bicarbonate were incorporated into propionate. Rowe, Davies and Broome (1981) reported that all carbon from rumen bicarbonate incorporated into propionate was incorporated into the carboxyl position. Therefore, this was assumed in developing the models (i.e. the transfer quotient of rumen bicarbonate to propionate was multiplied by 3 to obtain the transfer quotient of rumen bicarbonate to the carboxyl carbon of propionate). Figure 7-1 illustrates that there is extensive exchange of carbon between the propionate carboxyl carbon and rumen HCO_3^-.

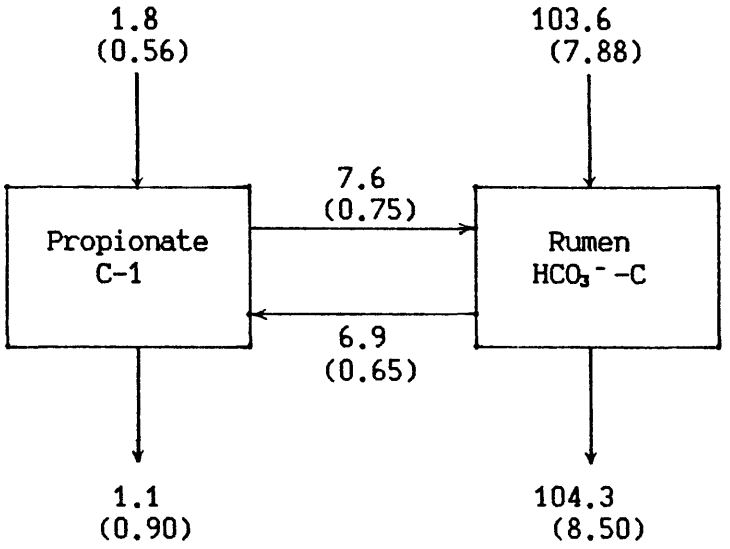
7.3.1.1.2 Propionate Middle Carbon - Rumen HCO_3^- -

When [2-^{14}C]propionate was infused into the rumen the rumen bicarbonate pool became labelled, indicating that some tracer from the middle carbon of propionate enters the rumen HCO_3^- pool. Therefore, it is possible that some metabolism of the middle carbon of propionate occurs within the rumen. The models of the propionate middle carbon and rumen bicarbonate are presented in Figure 7-2. The flow from rumen HCO_3^- to the middle carbon of propionate was deleted from the general model because of the assumption that HCO_3^- is incorporated only into the carboxyl position of propionate.

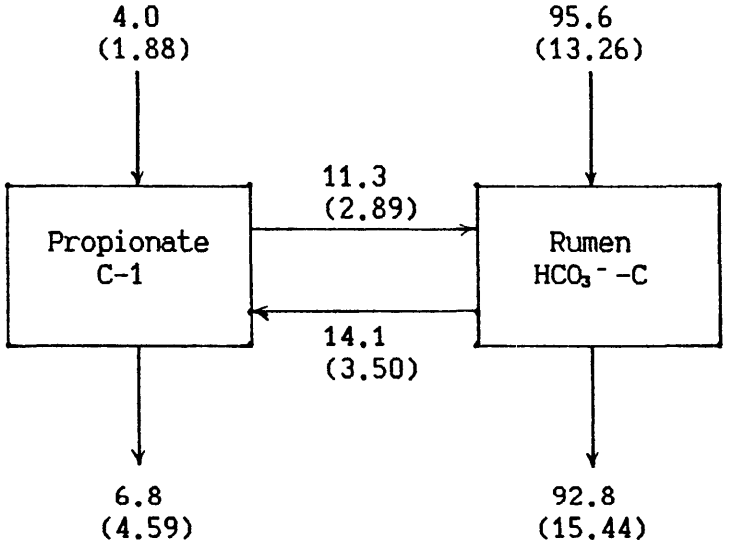
Figure 7-1

The 2 pool models of the carboxyl carbon of propionate and rumen HCO_3^- for sheep H and I (units = gC/d)

Sheep H



Sheep I

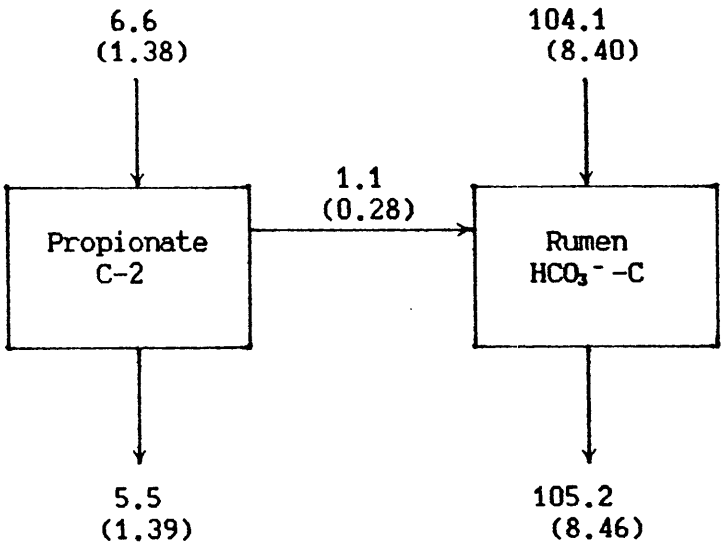


The values in ( ) are estimates of the errors of each flow calculated as described in section 5.3.2

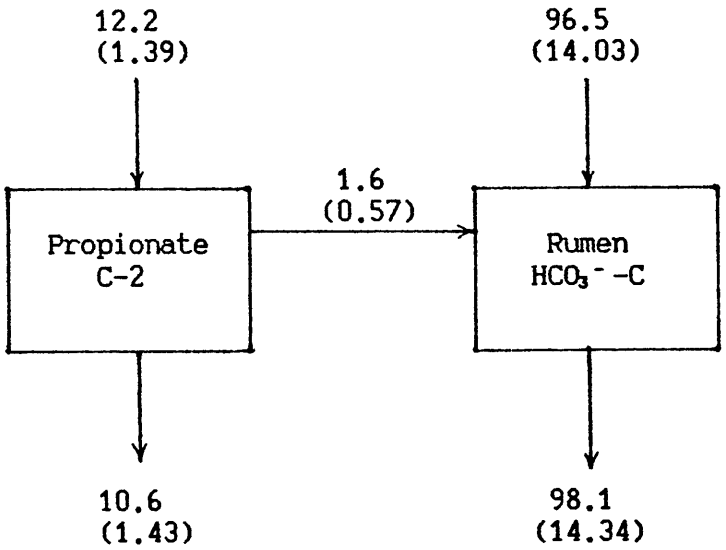
Figure 7-2

The 2 pool models of the middle carbon of propionate and rumen HCO_3^- for sheep H and I (units = gC/d)

Sheep H



Sheep I



The values in ( ) are estimates of the errors of each flow calculated as described in section 5.3.2

7.3.1.1.3 Rumen HCO_3^- - Blood HCO_3^- -

If any of the labelled HCO_3^- from the rumen exchanges with the blood HCO_3^- pool the CO_2 ratio will be affected. The two pool models of rumen and blood bicarbonate are presented in Figure 7-3. These models show that, since there is extensive exchange of carbon between the blood HCO_3^- and rumen HCO_3^- pools, there must be a flow of tracer from the propionate carboxyl carbon to the blood bicarbonate pool via the rumen bicarbonate pool. This means that the specific radioactivity of blood HCO_3^- does not accurately reflect the specific radioactivity of HCO_3^- produced in the body.

7.3.1.2 Three Pool Models -

7.3.1.2.1 Propionate Carboxyl Carbon - Rumen HCO_3^- - Blood HCO_3^- -

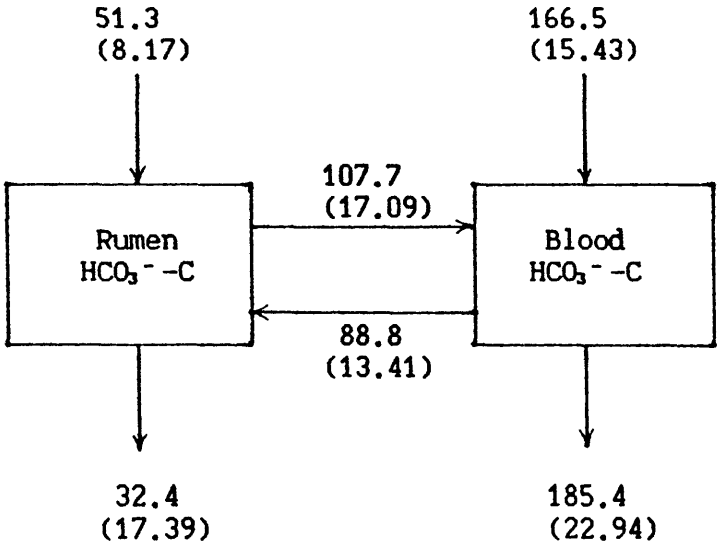
Three pool models of propionate carboxyl carbon, rumen HCO_3^- and blood HCO_3^- (Figure 7-4) were developed to correct the estimate of the flow of tracer from labelled propionate to blood bicarbonate for the flow via the rumen bicarbonate pool. The flow from blood HCO_3^- to propionate carboxyl carbon was deleted from these models because blood HCO_3^- has to pass through the rumen HCO_3^- pool before it can be incorporated into propionate.

In the solution to the model the flow of traced propionate carbon to outside the modelled system was negative. Although this value is mathematically the correct solution to the equations used to develop the model, it is biologically meaningless. Efforts were made to keep the conditions of the experiment the same for each infusion. However, it is unrealistic to think that this was achieved perfectly. Because the HCO_3^- pools are so large in comparison with the propionate

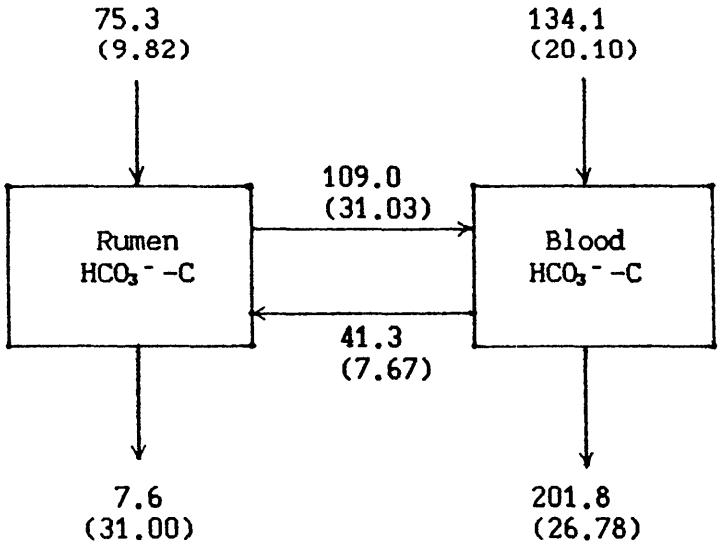
Figure 7-3

The 2 pool models of rumen HCO_3^- and blood HCO_3^- for sheep H and I (units = gC/d)

Sheep H



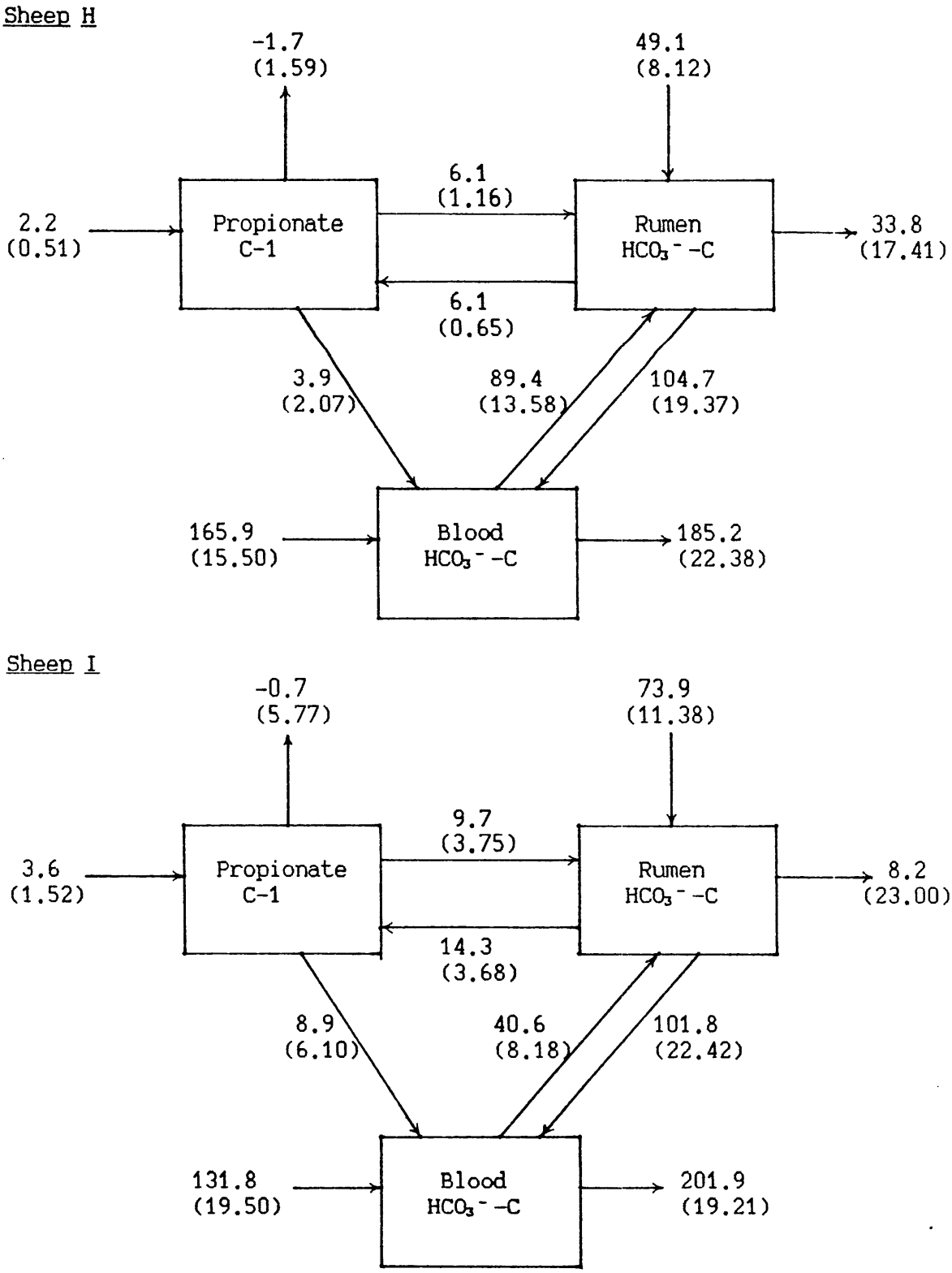
Sheep I



The values in ( ) are estimates of the errors of each flow calculated as described in section 5.3.2

Figure 7-4

The 3 pool models of the carboxyl carbon of propionate, rumen HCO_3^- carbon and blood HCO_3^- carbon. (Units = gC/d)



The values in ( ) are estimates of the standard errors

carboxyl pool, very minor changes in entry rates or transfer quotients can induce proportionally major changes in the calculated flows of the smaller pool.

Figure 7-5 illustrates the 3 pool models of propionate carboxyl, rumen HCO_3^- and blood HCO_3^- incorporating the assumption that all the propionate carboxyl carbon is metabolized through the HCO_3^- pools.

The percentage of the blood bicarbonate pool being provided by the propionate carboxyl carbon, estimated as the transfer quotient, is 4.1% and 8.1% for sheep H and I respectively. However, the 3 pool models indicate that only 1.44% (sheep H) and 3.67% (sheep I) of the blood bicarbonate pool was provided by the carboxyl carbon of propionate by all routes other than via rumen HCO_3^-. Therefore, 65% (sheep H) and 52% (sheep I) of the tracer in blood bicarbonate when [1-^{14}C]propionate was infused into the rumen came from the rumen bicarbonate pool. The values from the models will overestimate the direct flow from the carboxyl carbon of propionate to CO_2 in the body if any tracer enters the blood HCO_3^- pool via any other intermediate compounds.

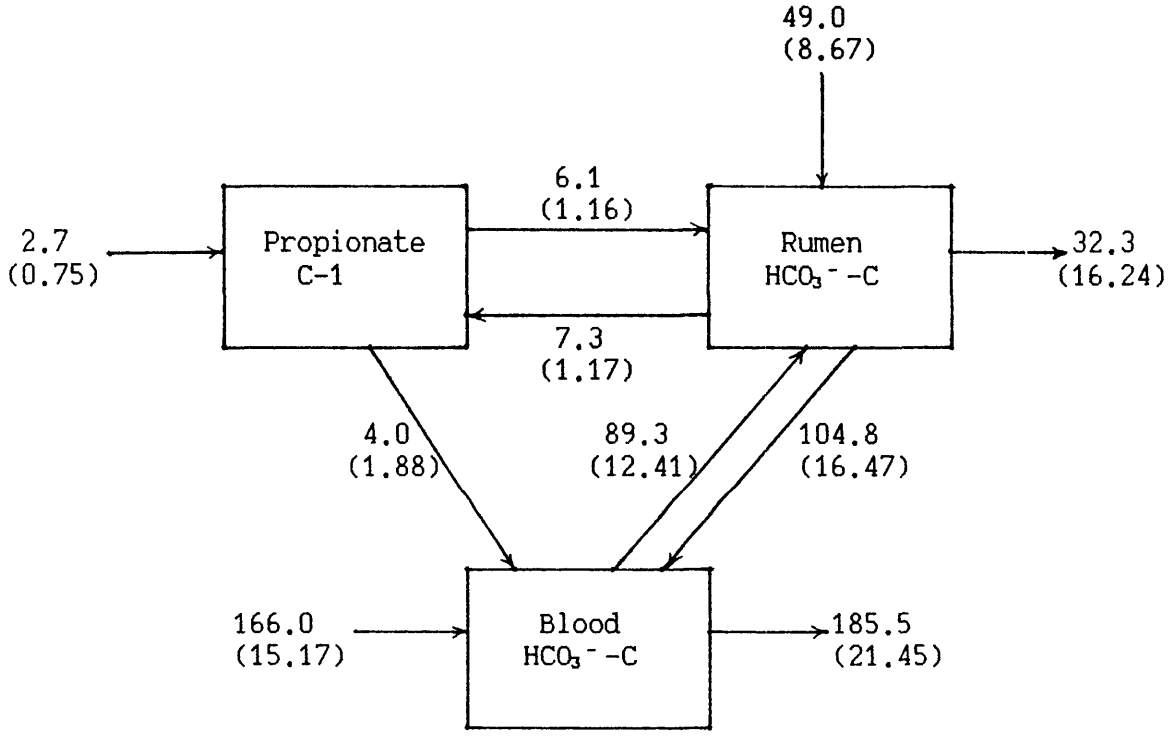
7.3.1.2.2 Propionate Middle Carbon - Rumen HCO_3^- - Blood HCO_3^- -

Figure 7-6 shows the 3 pool models of the middle carbon of propionate, rumen HCO_3^- and blood HCO_3^-. The flow from rumen propionate to rumen HCO_3^- is negative for sheep H and within the estimated error for sheep I. It was therefore concluded that there is no metabolism of the middle carbon of propionate to bicarbonate in the rumen. This conclusion has been incorporated into Figure 7-7.

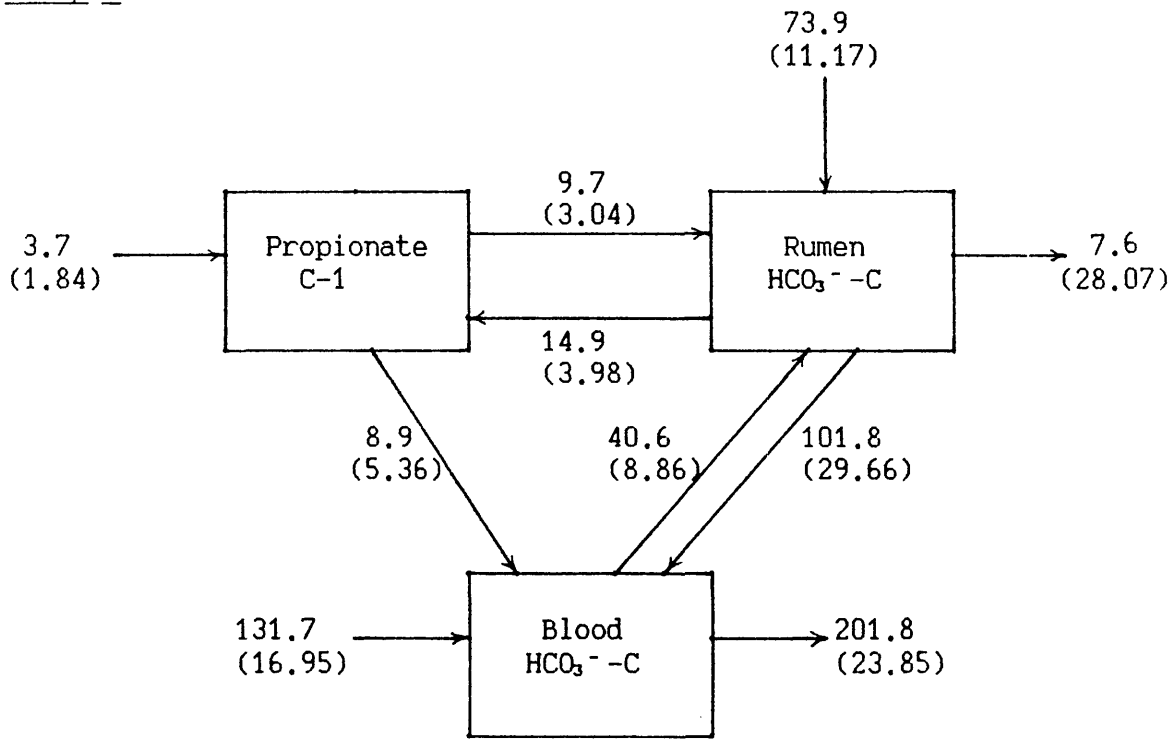
Figure 7-5

The 3 pool models of the carboxyl carbon of propionate, rumen HCO_3^- carbon and blood HCO_3^- carbon (units = gC/d)

Sheep H



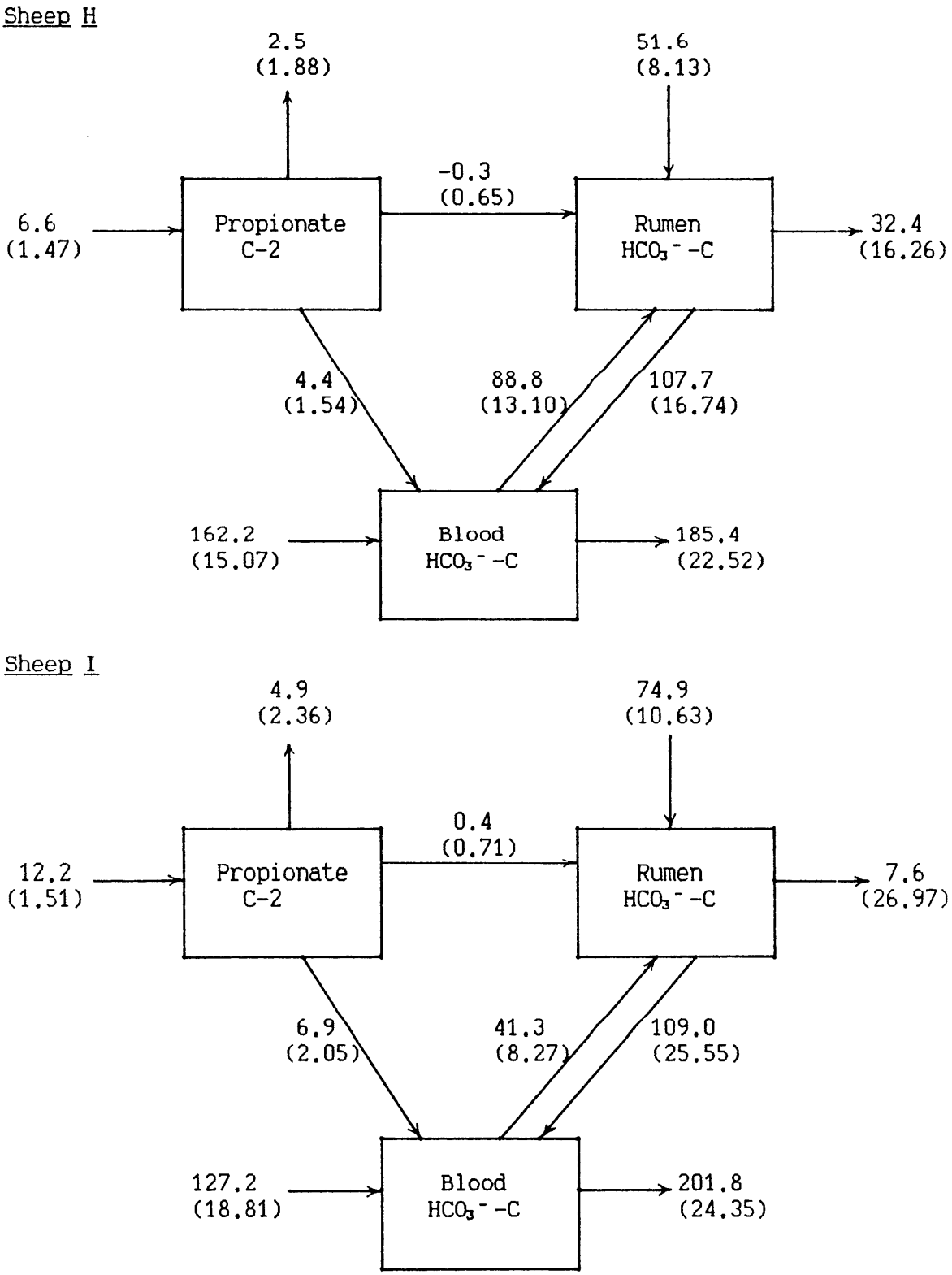
Sheep I



The values in ( ) are estimates of the standard errors

Figure 7-6

The 3 pool models of the methyl carbon of propionate, rumen HCO_3^- carbon and blood HCO_3^- carbon (units = gC/d)

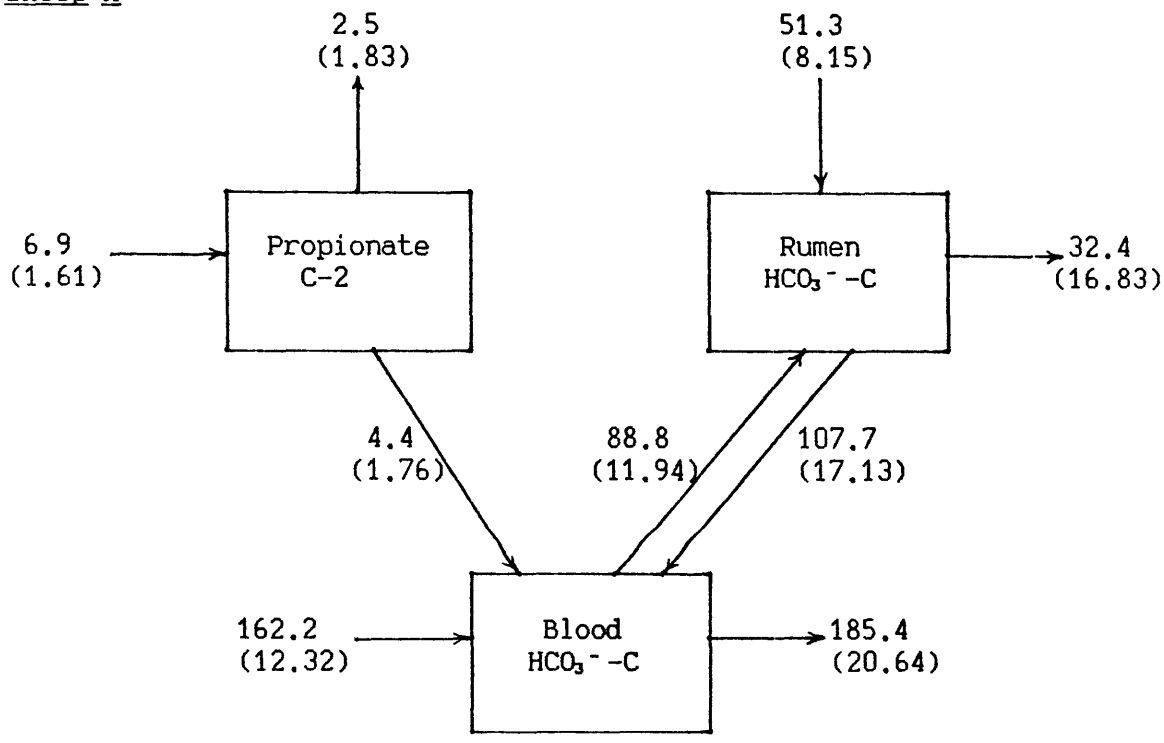


The values in ( ) are estimates of the standard errors

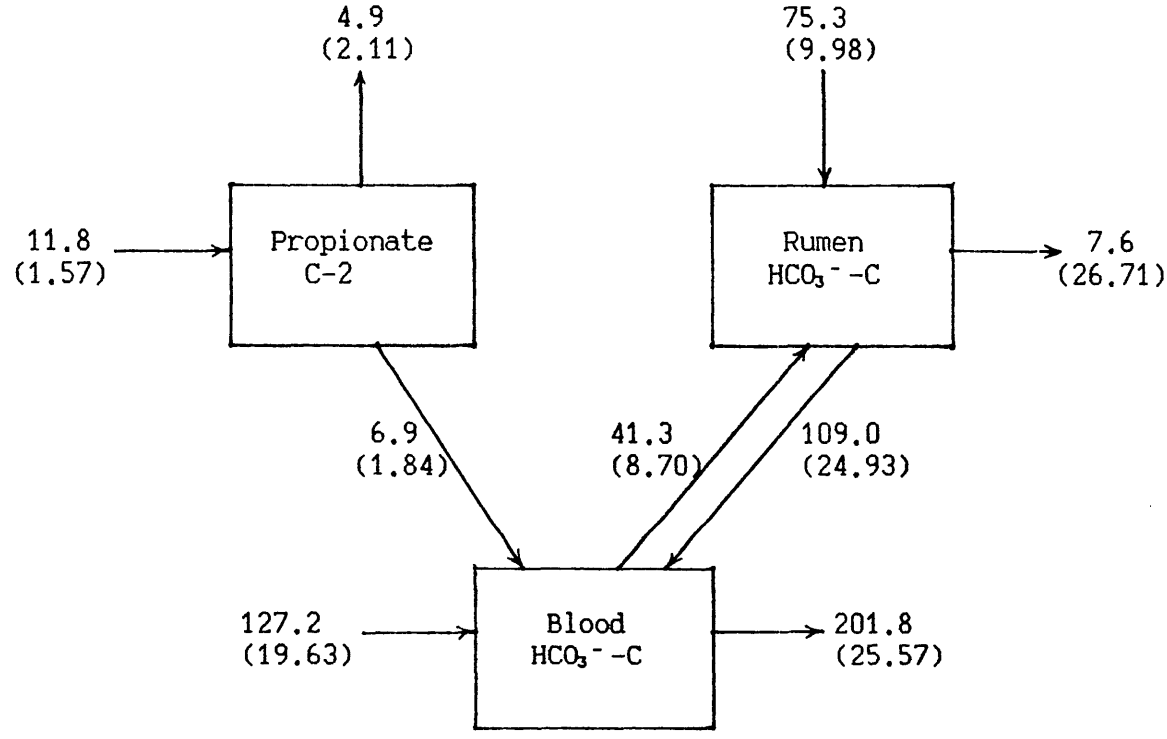
Figure 7-7

The 3 pool models of the methyl carbon of propionate, rumen HCO_3^- carbon and blood HCO_3^- carbon (units = gC/d)

Sheep H



Sheep I



The values in ( ) are estimates of the standard errors

The transfer quotients indicated that 2.0% (sheep H) and 3.6% (sheep I) of the blood bicarbonate pool was provided by the middle carbon of propionate. However, the 3 pool models suggest that only 1.60% (sheep H) and 2.84% (sheep I) was provided directly by the middle carbon of propionate. The difference is due to cycling of tracer back to the blood bicarbonate pool from the rumen bicarbonate pool.

7.3.1.3 Solving The CO_2 Ratio Using Values From The Three Pool Models -

The proportions of the blood HCO_3^- pools coming from propionate after correction for the flow via rumen HCO_3^-, i.e. 1.44% (sheep H) and 3.67% (sheep I) for the carboxyl carbons and 1.60% (sheep H) and 2.84% (sheep I) for the middle carbons, were used to solve the CO_2 ratio (Section 4.4.4.1). The resulting value of NI is negative for sheep H and nonsensically low for sheep I, i.e. the values of NI (the rate of influx to the tricarboxylic acid cycle of 4 and 5 carbon compounds relative to the rate of condensation of acetyl-CoA with oxaloacetate, with the latter being defined as unity) are meaningless. Therefore, it appears that the parameters extracted from the models as developed so far are not the parameters required by the theory used to develop the equations.

7.3.1.4 Four Pool Models -

It is expected that a significant amount of the propionate carbon will be incorporated into glucose carbon. As glucose is needed as an energy source by some ruminant tissues, e.g. brain (Murray, 1974), some propionate tracer may enter the blood bicarbonate pool from the

glucose pool. Figure 7-8 shows that 70% (sheep H) and 64% (sheep I) of the glucose carbon is oxidized to HCO_3^-. Therefore, in the previous models the percentage of HCO_3^- coming from propionate may have been overestimated due to a flow of tracer from propionate to the HCO_3^- pool via the glucose pool.

7.3.1.4.1 Propionate Carboxyl Carbon - Rumen HCO_3^- - Blood HCO_3^- - Glucose -

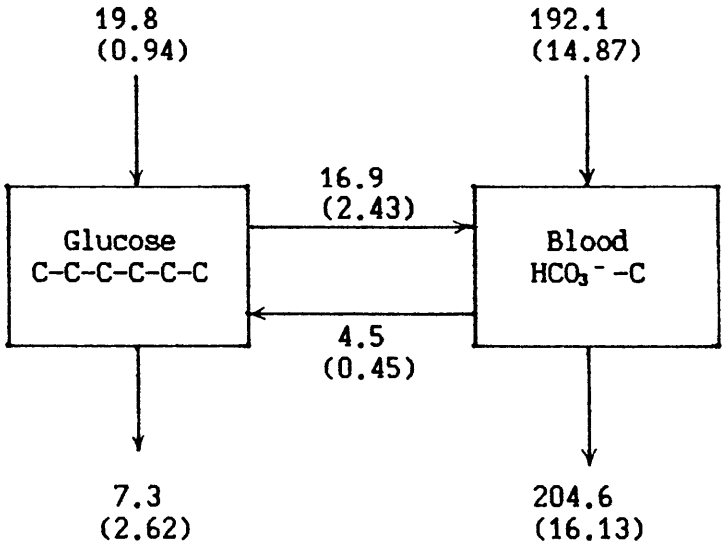
Figures 7-9 and 7-10 are 4 pool models incorporating the glucose pool into the appropriate earlier models. The flow from glucose to rumen bicarbonate was excluded on the assumption that blood glucose does not enter the rumen. The flow from glucose to the carboxyl carbon of propionate was also excluded for the same reason. The flow from rumen HCO_3^- to blood glucose was included because, although unexpected, any flow to glucose via other intermediate compounds would be represented in this flow.

In animal I the flow from rumen bicarbonate to glucose was very small and negative, indicating that no rumen bicarbonate was incorporated into glucose without passing through the blood bicarbonate or propionate carboxyl pool. Deleting this flow from the model has no effect on the other flows. However, in animal H the calculated flow from rumen HCO_3^- to blood glucose was much higher than expected. Possible reasons for this unexpected large flow are discussed in Section 7.3.2.2.

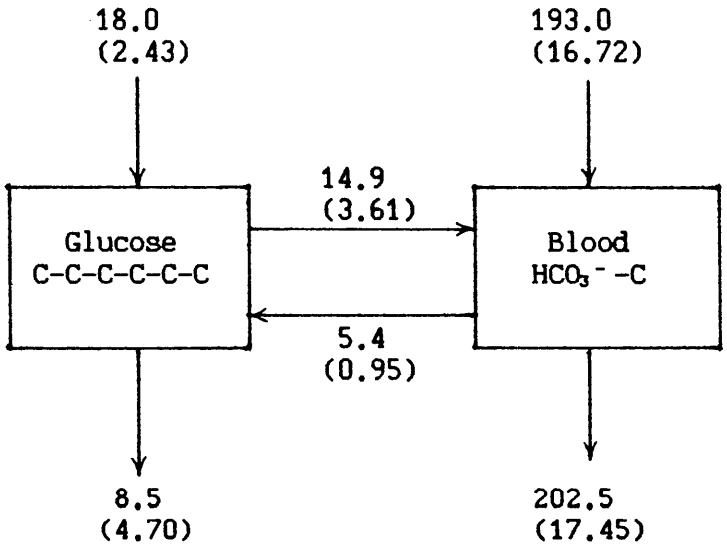
Figure 7-8

The 2 pool models of glucose and blood HCO_3^- for sheep H and I (units = gC/d)

Sheep H



Sheep I



The values in ( ) are estimates of the errors of each flow calculated as described in section 5.3.2

Figure 7-9

A 4 pool model; propionate carboxyl carbon, rumen HCO_3^-, blood HCO_3^- and glucose for sheep H (units = gC/d)

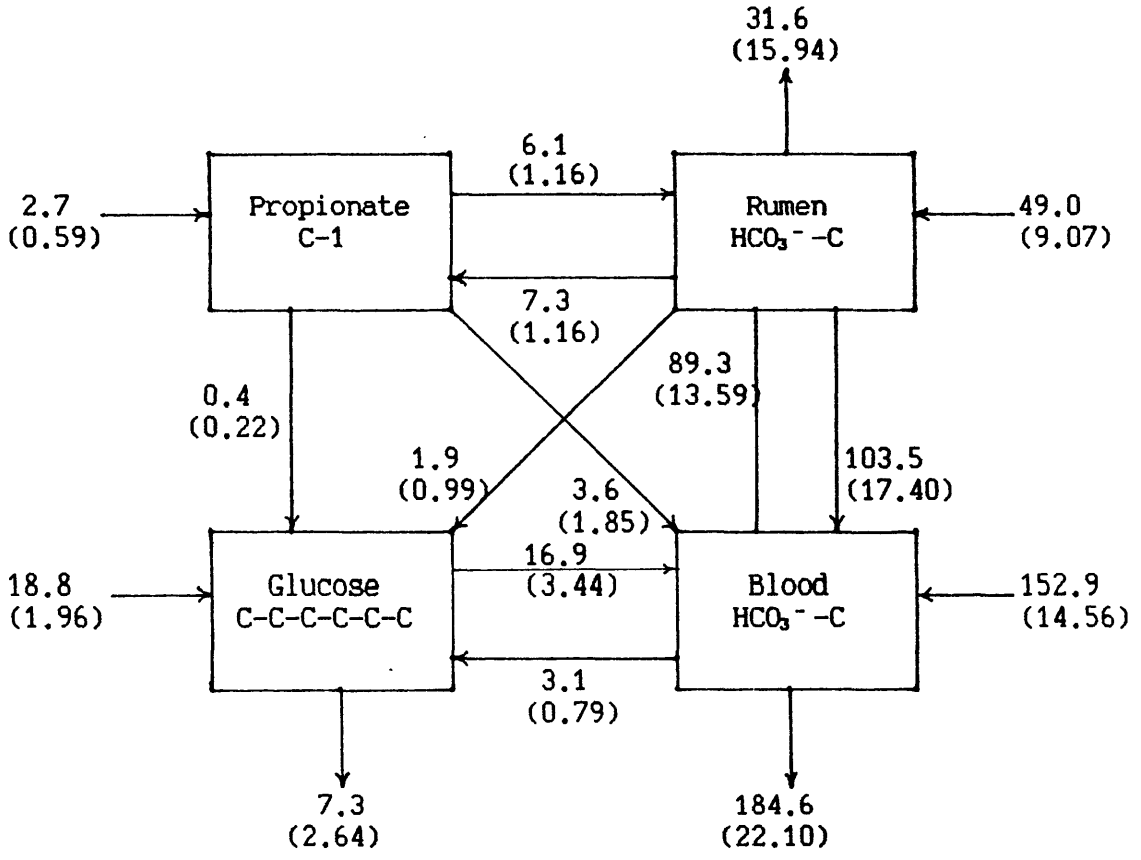
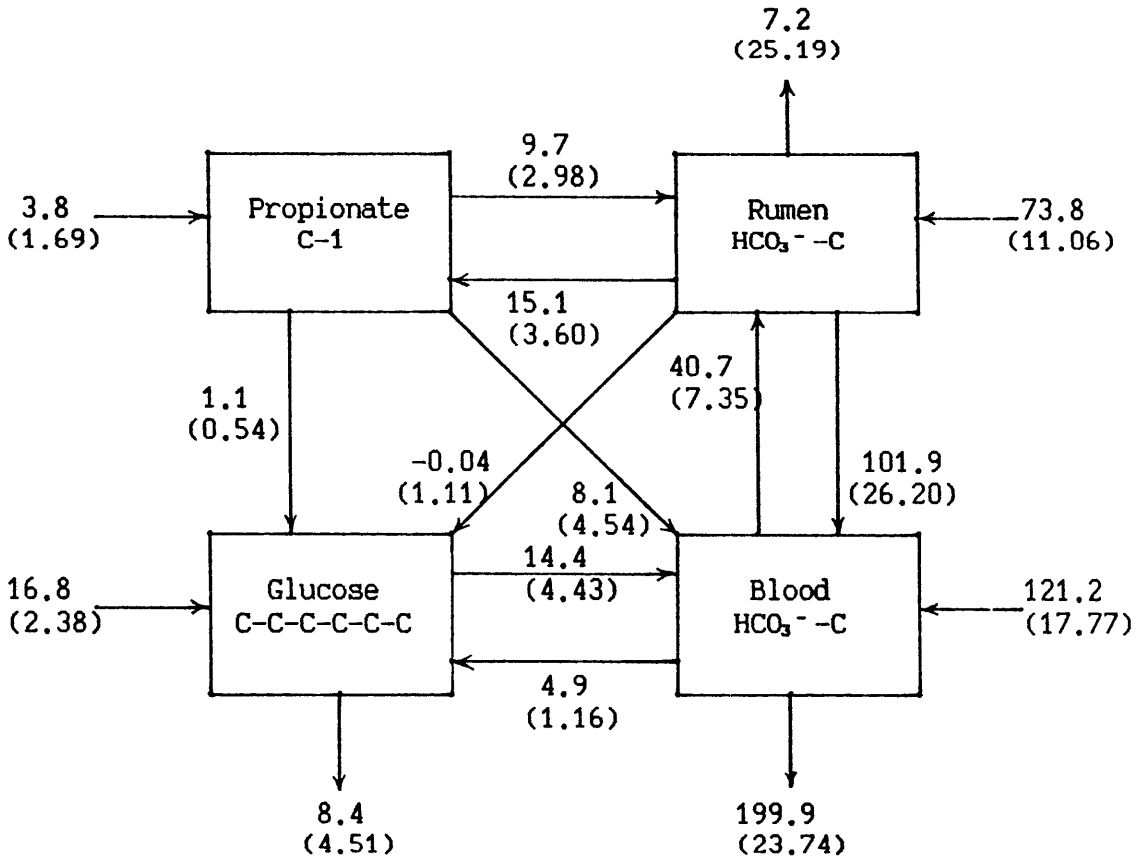


Figure 7-10

A 4 pool model; propionate carboxyl carbon, rumen HCO_3^-, blood HCO_3^- and glucose for sheep I (units = gC/d)



The values in ( ) are estimates of the standard errors

7.3.1.4.2 Propionate Middle Carbon - Rumen HCO_3^- - Blood HCO_3^- - Glucose -

The 4 pool models of the propionate middle carbon, rumen HCO_3^-, blood HCO_3^- and glucose are presented in Figures 7-11 and 7-12. In these models it was expected that there would be flows from rumen bicarbonate to glucose without passing through the blood bicarbonate pools because these represent the flows to glucose via the propionate carboxyl carbon.

7.3.1.5 Five Pool Model: Propionate Middle Carbon - Rumen HCO_3^- - Blood HCO_3^- - Glucose - Propionate Carboxyl Carbon -

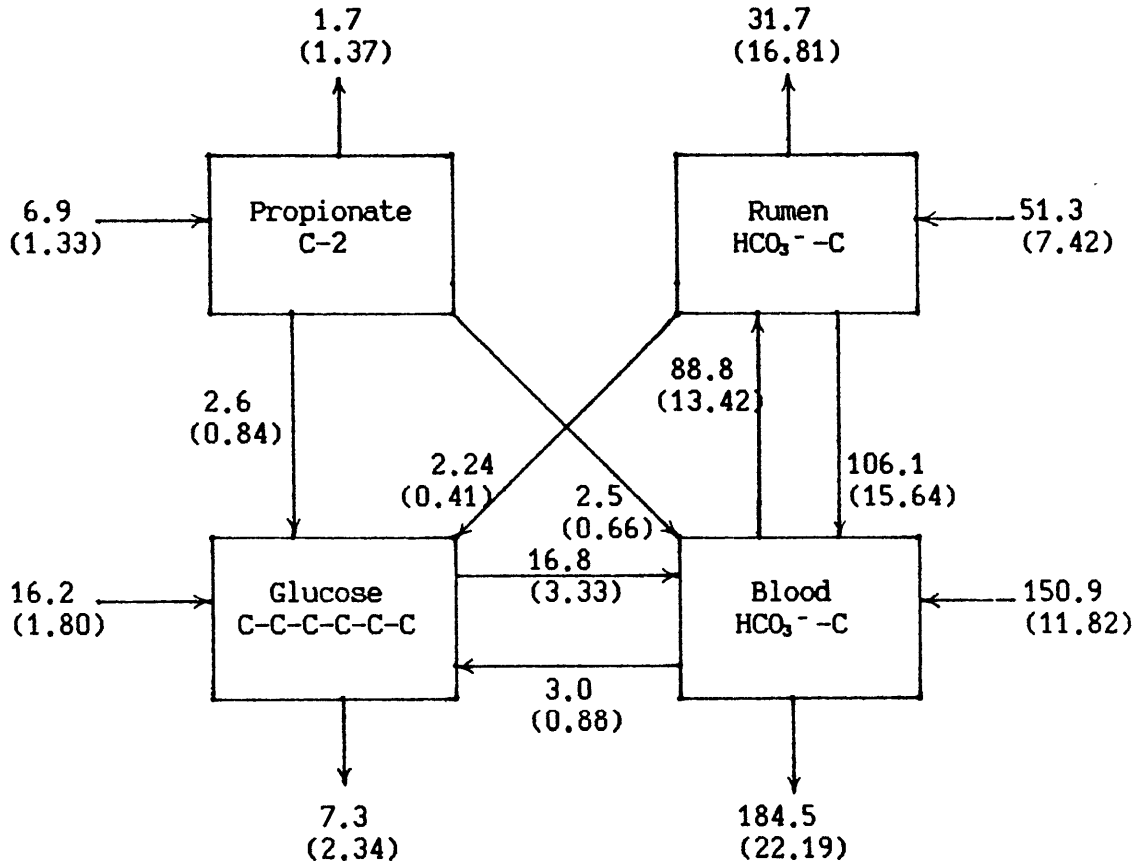
The two four pool models contain different information about the propionate pool and its kinetics. This induces slight differences in all flows because all flows in the models are interrelated to all other flows. To remove these differences and to combine the information from both species of propionate isotope, 5 pool models were constructed.

7.3.1.5.1 Sheep I -

Figure 7-13 is the 5 pool model for sheep I. The flow from rumen bicarbonate to glucose was deleted because this flow was small and negative, therefore indicating that all tracer incorporated into glucose passed through the blood bicarbonate or propionate carboxyl carbon pools. Accordingly, this flow was deleted from the model for this animal.

Figure 7-11

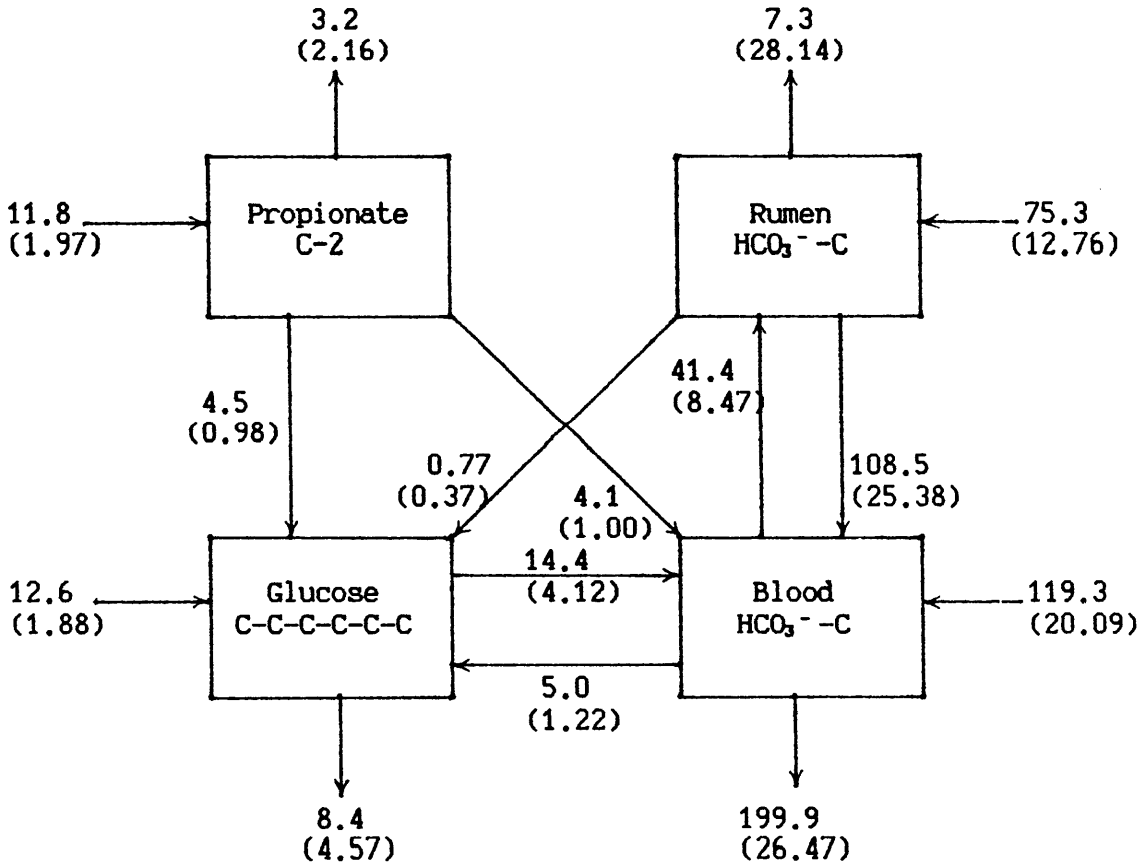
A 4 pool model; propionate middle carbon, rumen HCO_3^-, blood HCO_3^- and glucose for sheep H (units = gC/d)



The values in ( ) are estimates of the errors

Figure 7-12

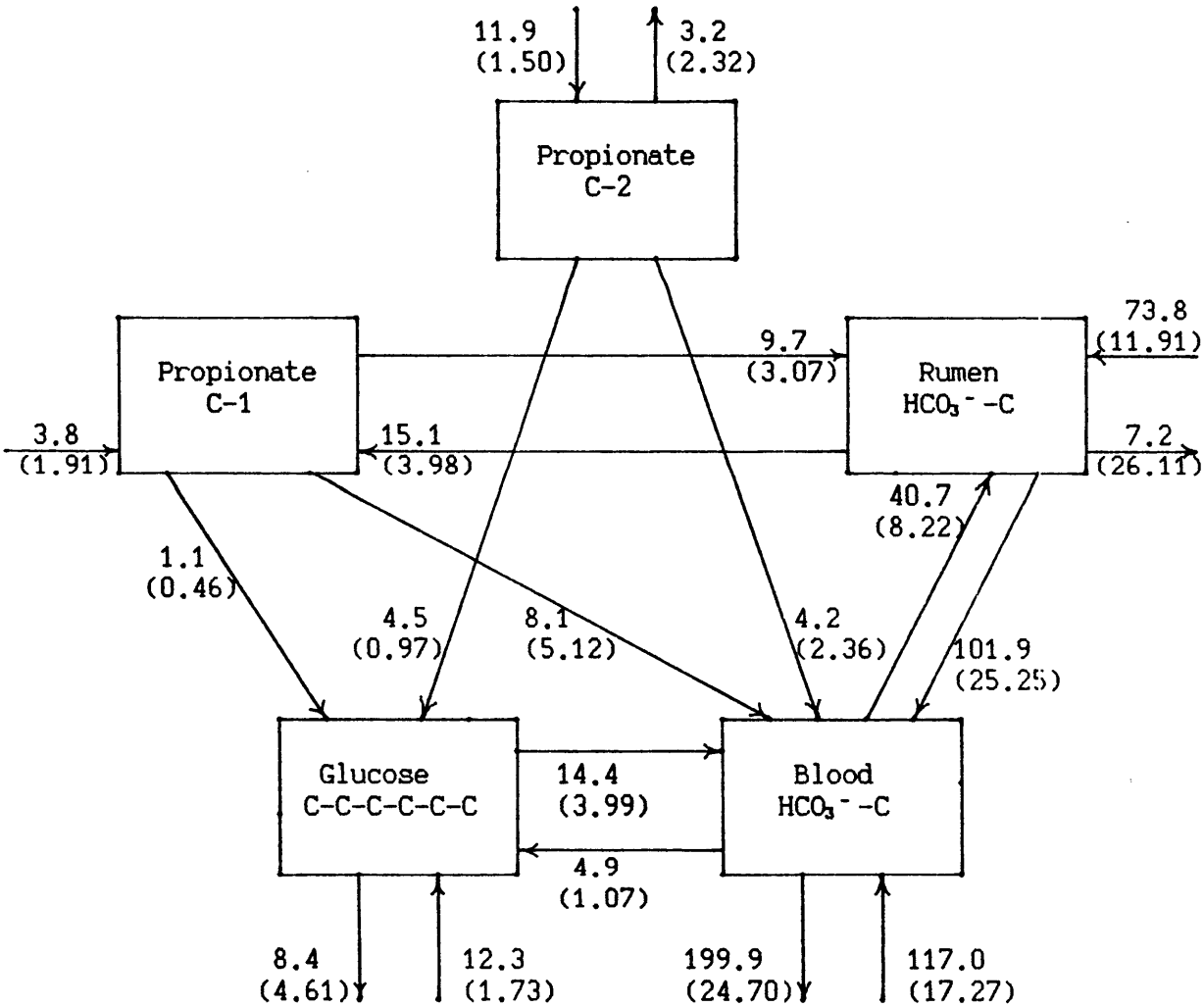
A 4 pool model; propionate middle carbon, rumen HCO_3^-, blood HCO_3^- and glucose for sheep I (units = gC/d)



The values in ( ) are estimates of the errors

Figure 7-13

The 5 pool model (carboxyl and middle carbons of propionate, rumen HCO_3^-, blood HCO_3^- and glucose) for sheep I (units = gC/d)



7.3.1.5.2 Sheep H -

The 5 pool model for sheep H is presented in Figure 7-14. In this animal, the flow from rumen bicarbonate to glucose is positive and larger than the estimate of variance. Possible reasons for this are discussed in Section 7.3.2.2.

7.3.1.6 Solving The CO_2 Ratio Using Values From The Five Pool Model -

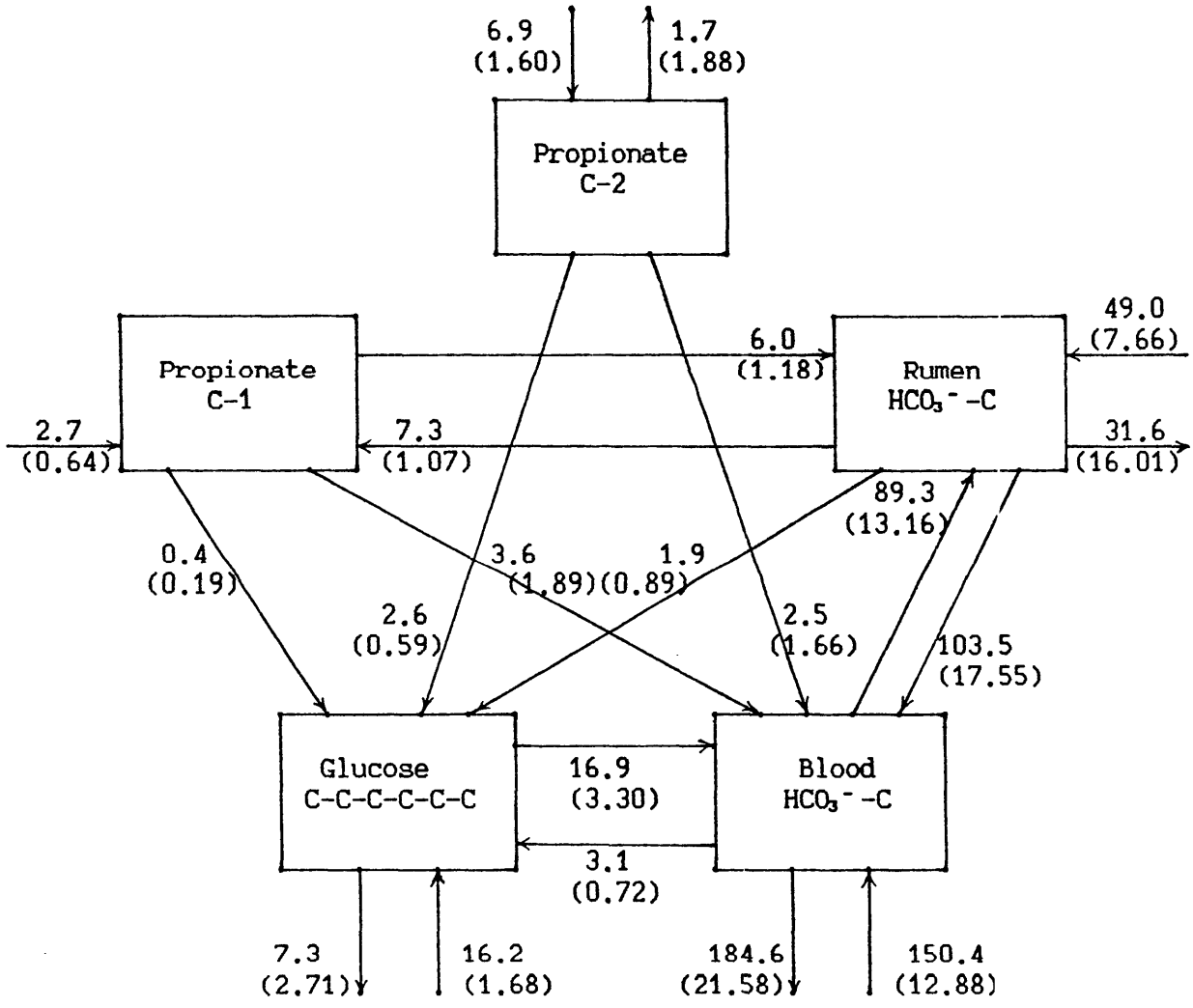
7.3.1.6.1 The Percentage Of The Glucose Pool Provided By Direct Flow From The Carboxyl Carbon Of Propionate -

The percentage of blood bicarbonate carbon atoms originating in the propionate carboxyl carbon pool (i.e. the transfer quotient) is 4.1% (sheep H) and 8.1% (sheep I). However, the 3 pool models (propionate carboxyl, rumen bicarbonate and blood bicarbonate) indicate that 65% (sheep H) and 55% (sheep I) of the carbon atoms in the blood bicarbonate pool passed through the rumen bicarbonate pool and that only 1.44% (sheep H) and 3.67% (sheep I) entered the blood bicarbonate pool via other pathways.

The estimates of the percentage of the blood bicarbonate pool from the carboxyl carbon of propionate in the 5 pool models (Sheep H 1.30% Figure 7-14; Sheep I 3.30% Figure 7-13) are 10% lower than the estimates from the three pool models. These values will overestimate the parameters needed for the CO_2 ratio if there is any significant flow of tracer to blood HCO_3^- via other intermediates.

Figure 7-14

The 5 pool model (carboxyl and middle carbons of propionate, rumen HCO_3^-, blood HCO_3^- and glucose) for sheep H (units = gC/d)



The values in ( ) are estimates of the standard errors

7.3.1.6.2 The Percentage Of The Glucose Pool Provided By Direct Flow From The Middle Carbon Of Propionate -

The percentage of the carbon atoms in the blood bicarbonate pool that originated in the propionate middle carbon pool, estimated as the transfer quotient, is 2.0% (sheep H) and 3.6% (sheep I). The 3 pool models indicate that 20% (sheep H) and 21% (sheep I) of the tracer in the blood bicarbonate pool was there due to turnover and exchange of tracer with the rumen bicarbonate pool and that only 1.6% (sheep H) and 2.8% (sheep I) of the blood bicarbonate pools were provided by the middle carbon of propionate by all other pathways.

The 5 pool models indicate that the percentage of the blood bicarbonate pool provided by the middle carbon of propionate is only 0.94% (Sheep H) and 1.71% (Sheep I). These values are only 45% (Sheep H) and 47% (Sheep I) of the estimates from the respective transfer quotients. They are 56% (Sheep H) and 60% (Sheep I) of the estimates from the 3 pool models. The values indicate that there is a significient flow of tracer from the middle carbon of propionate to the blood bicarbonate pool via the glucose pool. Very serious errors can be introduced into the conclusions if these indirect flows of tracer are not taken into account.

7.3.1.6.3 The CO_2 Ratios -

Assuming that flows through other intermediates are not significant the above values were used to solve the CO_2 ratio. This ratio is defined as ($^{14}CO_2$ derived from [1-^{14}C]propionate) divided by ($^{14}CO_2$ derived from [2-^{14}C]propionate).

Therefore the CO_2 ratio equals

$$\frac{\text{carboxyl carbon of propionate incorporated into blood } HCO_3^-}{\text{middle carbon of propionate incorporated into blood } HCO_3^-}$$

Since the equilibrium specific radioactivity of the carboxyl carbons of oxaloacetate determines directly the amount of $^{14}CO_2$ expired, the above ratio can be written as:

$$\frac{\text{specific radioactivity of the carboxyl carbon of oxaloacetate derived from [1-}^{14}\text{C]propionate}}{\text{specific radioactivity of the carboxyl carbon of oxaloacetate derived from [2-}^{14}\text{C]propionate}}$$

$= 1 + 2NI$ (from Section 4.4.4, NI is defined as the rate of influx to the tricarboxylic acid cycle of 4 and 5 carbon compounds relative to the rate of condensation of acetyl-CoA with oxaloacetate, with the latter defined as unity.)

Therefore, for sheep H

$$1+2NI = \frac{1.30}{0.94}$$

$$NI = 0.19$$

The percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle can be calculated from NI from the formula

$$\frac{1}{(1+NI)} \times \frac{100}{1}$$

Thus, the percentage of the molecules in the oxaloacetate pool being provided by cycling of the tricarboxylic acid cycle in sheep H equals 84%.

The CO_2 ratio for Sheep I is

$$1+2NI = \frac{3.30}{1.71}$$

$$NI = 0.46$$

The percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle for sheep I equals 68%.

7.3.2 Solving The Glucose Ratio Using Propionate

The parameters needed to solve the glucose ratio are the proportions of the carbon in the glucose pool provided by the carboxyl and middle carbons of propionate. As discussed earlier, because the bicarbonate pools become labelled, there is incorporation of tracer into glucose from these pools. Therefore, the transfer quotient will overestimate the direct flow of molecules from propionate to glucose. Thus, the values to be used in solving the glucose ratio have to be corrected for this indirect flow.

The flows in the 5 pool models are corrected for the flow of tracer via the bicarbonate pools and thus should be appropriate values to use in the glucose ratio.

7.3.2.1 Sheep I -

The transfer quotient indicates that 20.4% of the carbon atoms in the glucose pool originated in the propionate middle carbon pool. However, the 5 pool model (Figure 7-13) indicates that only 19.74% of the glucose pool was provided by the middle carbon of propionate. The difference is due to interaction with the blood bicarbonate pool. The 5 pool model also indicates that only 4.82% of the glucose pool was

provided by the propionate carboxyl carbon pool. Therefore, 3.% of the tracer in the glucose pool when [1-^{14}C]propionate is infused into the rumen is there due to interaction with the bicarbonate pools.

The glucose ratio is defined as: (^{14}C incorporated into glucose from [2-^{14}C]propionate) divided by (^{14}C incorporated into glucose from [1-^{14}C]propionate) by way of the pathway: propionate, tricarboxylic acid cycle, oxaloacetate, pyruvate to glucose. Therefore, the glucose ratio equals

$$\frac{\text{glucose derived from the middle carbon of propionate}}{\text{glucose derived from the carboxyl carbon of propionate}}$$

In terms of the specific radioactivity of oxaloacetate carbons, the glucose ratio equals

$$\frac{2 \times \left[\begin{array}{c}\text{specific radioactivity of}\\ \text{the middle carbons of}\\ \text{oxaloacetate}\\ \text{([2-}^{14}\text{C]propionate infused)}\end{array}\right] + \left[\begin{array}{c}\text{specific radioactivity of}\\ \text{the carboxyl carbons of}\\ \text{oxaloacetate}\\ \text{([2-}^{14}\text{C]propionate infused)}\end{array}\right]}{\begin{array}{c}\text{specific radioactivity of carboxyl carbons of oxaloacetate}\\ \text{([1-}^{14}\text{C]propionate infused)}\end{array}}$$

$$= {}^{5+4NI}/_{1+2NI} \qquad \text{(from Section 4.4.4)}$$

Substituting the corrected transfer quotients into the glucose ratio for sheep I gives

$$\frac{5+4NI}{1+2NI} = \frac{19.74}{4.82}$$

$$NI = 0.22$$

Using this value of NI the percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle (${}^{1}/_{(1+NI)}$) equals 82%.

7.3.2.2 Sheep H -

The percent of the glucose pool provided by the propionate middle carbon pool (10.74%) was 5% lower than the estimate from the transfer quotient. Therefore, 5% of the tracer in glucose was there due to interaction with the blood bicarbonate pool.

The propionate carboxyl to glucose transfer quotient is 3.3%. However, according to the 5 pool model (Figure 7-14), the percentage of tracer in glucose that was there due to interaction with the bicarbonate pools was 50%. Therefore, only 1.65% of the glucose pool came from propionate via other pathways (presumably via the direct route).

These values were used to solve the glucose ratio.

$$\frac{5+4NI}{1+2NI} = \frac{10.74}{1.65}$$

$$NI = -0.17$$

A negative value of NI is meaningless and suggests that there is either a problem with the data or the theory used to develop the equations.

The flow from rumen bicarbonate direct to blood glucose in the proposed 5 pool model (Figure 7-14) for this animal is higher than expected. The value of the flow is larger than the estimate of error, indicating that the value should be real according to the data used to solve the model. However, this estimate of error only includes analytical errors and errors due to non-steady state of the animals during the infusions. It does not include the errors introduced due to changes in the animal between days of infusions. This flow would be induced in the calculations if, for example, the incorporation of

tracer from blood bicarbonate to glucose was lower on the day of the blood bicarbonate infusion than it was on the day of the rumen bicarbonate infusion. It is implicit in the equations used to obtain the flows presented in the models that an overestimation of one flow into a pool will force the other flows to be underestimated. Therefore, if the flow from rumen bicarbonate to glucose is overestimated, then this will cause the flows to glucose from blood bicarbonate and the carboxyl carbon of propionate to be underestimated. This could possibly explain the small flow from the carboxyl carbon of propionate to glucose in Figure 7-14 and thus, the negative value obtained in the glucose ratio.

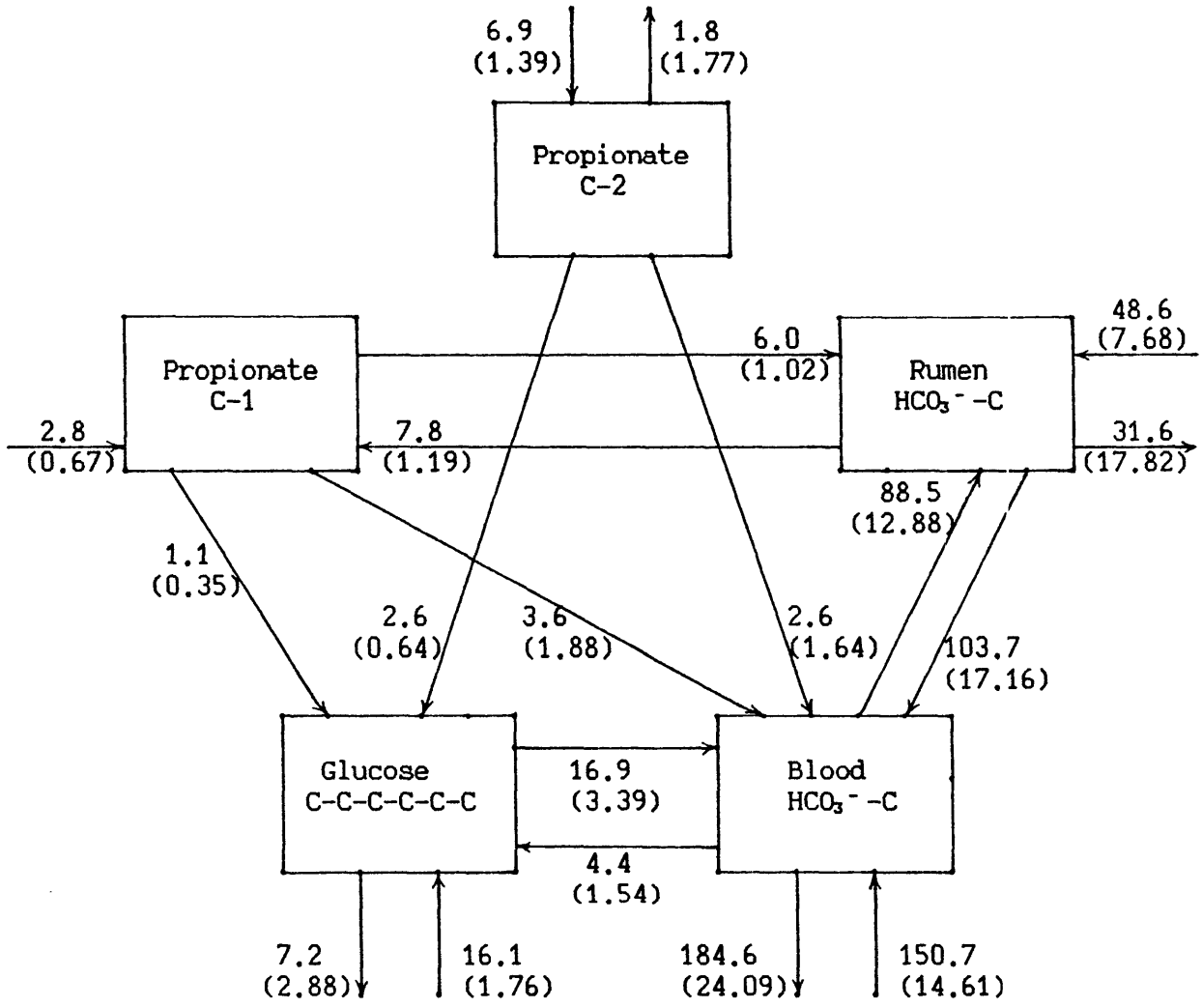
If it is assumed that the model used for sheep I (i.e. that there is no direct flow from rumen bicarbonate to glucose) is also the correct model for sheep H then the model presented in Figure 7-15 results. This interpretation will force an increase in flow along the alternative routes from rumen bicarbonate to glucose (i.e. increase the flow via blood bicarbonate and the flow via the propionate carboxyl carbon pool). On the basis of this interpretation the percentage of glucose apparently provided by the carboxyl carbon of propionate is 4.56%. This value is higher than the transfer quotient. Therefore, this model must be overestimating the flow from the carboxyl carbon of propionate to glucose.

If it is assumed that the percentage of the tracer in glucose due to flow via the bicarbonate pools is the same in this animal as it was for sheep I, i.e. 31%, then the percentage of glucose from the carboxyl carbon of propionate equals 2.31%. Using this value in the glucose ratio gives the following result.

$$\frac{5+4NI}{1+2NI} = \frac{10.74}{2.31}$$

Figure 7-15

The 5 pool model (carboxyl and middle carbons of propionate, rumen HCO_3^-, blood HCO_3^- and glucose) for sheep H incorporating the assumption that there is no flow of carbon from rumen bicarbonate direct to blood glucose. (units = gC/d)



$$NI = 0.06$$

The percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle equals 94%.

7.3.3 Solving The Glucose Ratio Using Acetate

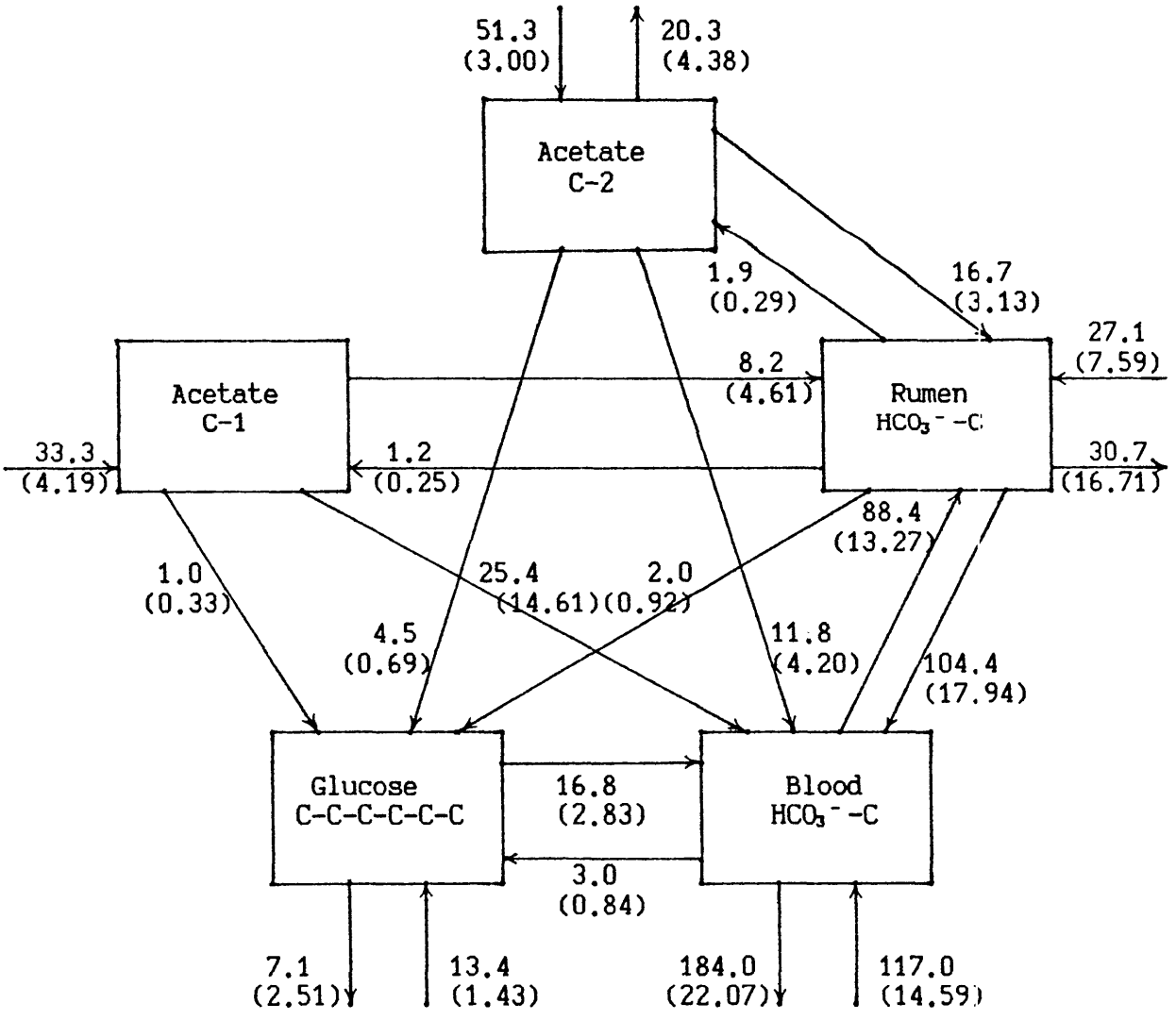
The transfer quotients indicate that tracer from acetate enters the bicarbonate pools. As bicarbonate is incorporated into glucose there must be an indirect flow of tracer from acetate to glucose via these bicarbonate pools. Therefore, the acetate to glucose transfer quotients will overestimate the parameters needed to solve the glucose ratio. Five pool models (methyl carbon of acetate, rumen bicarbonate, blood bicarbonate, glucose and carboxyl carbon of acetate - Figures 7-16 and 7-17) were constructed so as to correct for the indirect flow of tracer via the bicarbonate pools.

The direct flows from glucose and blood bicarbonate to acetate were excluded because all carbon from these compounds would have to pass through the rumen bicarbonate pool before incorporation into acetate. It was assumed that there was no direct exchange of carbon between the methyl and carboxyl positions of acetate. Because it was not known if the tracer incorporated into acetate was incorporated into the carboxyl or methyl positions, it was assumed that tracer was incorporated into both positions equally.

A problem with the solutions to the models was that the flow from the carboxyl carbon of acetate to outside the modelled system was negative. This problem was also encountered with the carboxyl carbon of propionate and possibly for the same reason, i.e. small differences in the entry rates or transfer quotients in the large

Figure 7-16

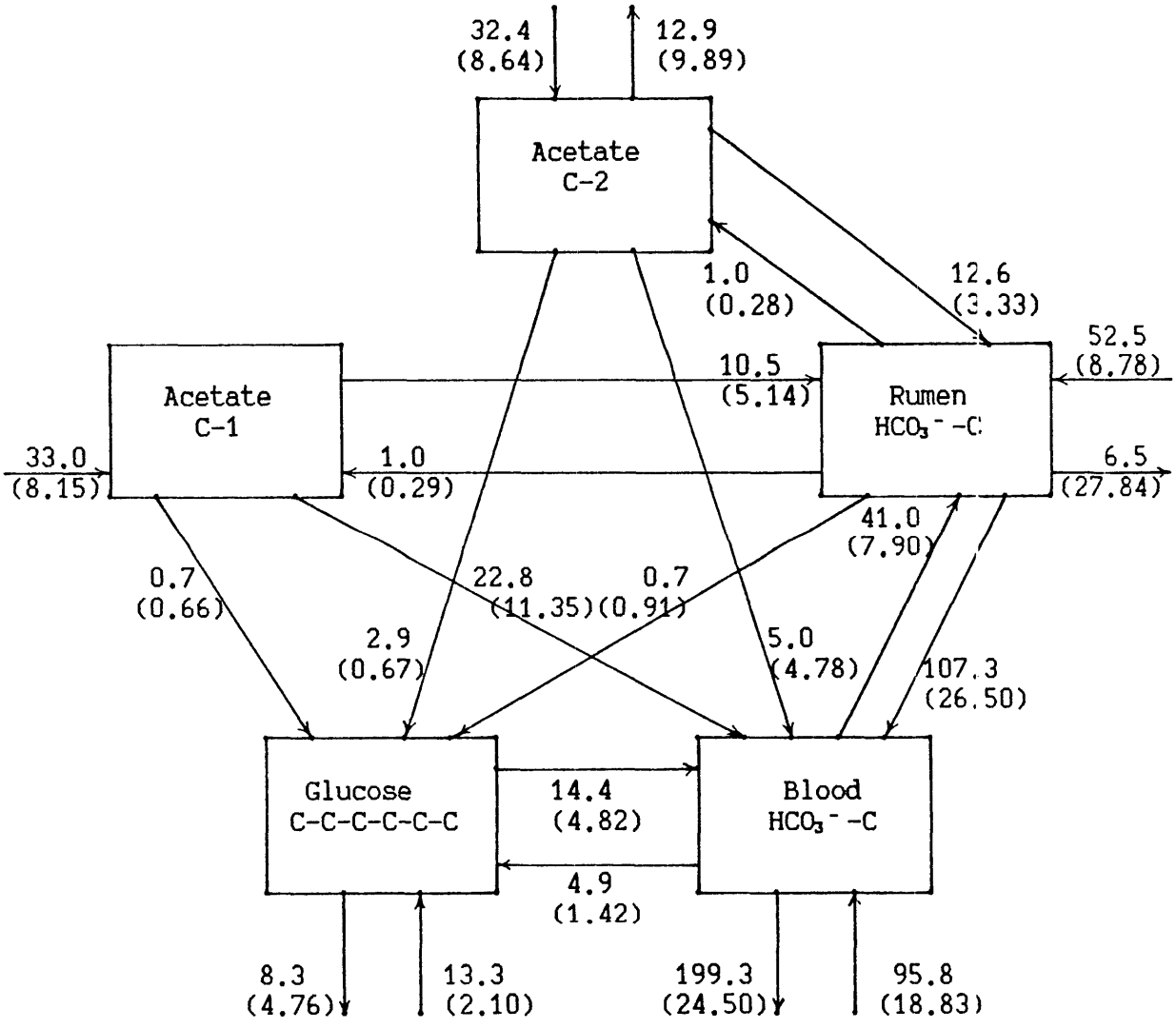
The 5 pool model (carboxyl and methyl carbons of acetate, rumen HCO_3^-, blood HCO_3^- and glucose) for sheep H (units = gC/d)



The values in ( ) are estimates of the errors

Figure 7-17

The 5 pool model (carboxyl and methyl carbons of acetate, rumen HCO_3^-, blood HCO_3^- and glucose) for sheep I (units = gC/d)



The values in ( ) are estimates of the errors

bicarbonate pools can induce proportionally large changes in the smaller pools. Therefore, it was assumed that all the carboxyl carbon was metabolized through the pools incorporated into the model.

The flow from rumen bicarbonate to glucose was included in the acetate models because this flow would include the flow from rumen bicarbonate to glucose via the carboxyl carbon of propionate.

7.3.3.1 Sheep I -

According to the transfer quotient, 15.3% of the carbon atoms in the glucose pool originated in the acetate methyl carbon pool. The 5 pool model (Figure 7-17) indicates that 16% of the tracer in the glucose pool was there due to interaction with the bicarbonate pools and that only 12.9% of the glucose pool was provided by the methyl carbon of acetate via all other pathways. The 5 pool model also indicates that 56% of the acetate carboxyl to glucose transfer quotient (7.2%) was due to interaction with the bicarbonate pools. Therefore, only 3.1% was provided by the carboxyl carbon of acetate via all other pathways (but presumably by the direct route).

These values were used to solve the glucose ratio.

$$\frac{5+4NI}{1+2NI} = \frac{12.9}{3.1}$$

$$NI = 0.19$$

The percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle equals 84%.

7.3.3.2 Sheep H -

The transfer quotient indicates that 22% of the carbon atoms in the glucose pool originated in the acetate methyl carbon pool. However, according to the 5 pool model (figure 7-16), 14% of this was due to interaction with the bicarbonate pools and only 18.8% of the glucose pool was provided by the methyl carbon of acetate by all other pathways. The model also indicates that 44% of the acetate carboxyl carbon to glucose transfer quotient (7.5%) was due to interaction with the bicarbonate pools and only 4.2% of the glucose pool was provided by the carboxyl carbon of acetate by all other pathways.

Therefore, the glucose ratio for this animal is

$$\frac{5+4NI}{1+2NI} = \frac{18.8}{4.2}$$

$$NI = 0.11$$

The percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle equals 90%.

If the flow from rumen bicarbonate to glucose is overestimated in this model as it appears to be in the propionate 5 pool models, then the flow from the carboxyl carbon of acetate will be underestimated. This will cause NI to be underestimated and thus the percentage of the molecules in the oxaloacetate pool being provided by cycling of the tricarboxylic acid cycling to be overestimated.

7.3.4 CO_2 Incorporation Into Glucose

7.3.4.1 Sheep I -

The transfer quotient of blood bicarbonate to glucose for sheep I is 23.2%. The percentage of the glucose pool provided by the blood bicarbonate pool according to the acetate 5 pool model is 21.7 (93% the value of the transfer quotient) which indicates that 7% of the tracer was in the glucose pool due to indirect flows and recycling. As bicarbonate only labels carbons 3 and 4 of glucose, blood bicarbonate provided 65.1% of glucose carbons 3 or 4 (using the value from the 5 pool model). This now has to be multiplied by 2 to account for the loss of tracer due to the decarboxylation reaction (Section 4.5). Thus, according to this reasoning apparently 130% of the oxaloacetate carboxyl carbons are provided by pathways that contain a carboxylation reaction.

Non-adherence to the assumptions (Section 4.5) will cause this method to underestimate the percentage of molecules in the oxaloacetate pool coming from carboxylation of 3 carbon compounds and thus overestimate the percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle. There must, therefore, be pathways by which bicarbonate is incorporated into glucose which are not accounted for in the theory used to develop the equations.

7.3.4.2 Sheep H -

The transfer quotient indicates that 18.4% of the carbon atoms in the glucose pool originated in the blood bicarbonate pool. The 5 pool model indicates that only 12.6% of the carbon atoms of the glucose

pool were provided directly by the blood bicarbonate pool. This value is only 68% the value of the transfer quotient. This is quite different to the situation for sheep I and is due to the apparent flow of tracer from the blood bicarbonate pool to glucose via the rumen bicarbonate pool.

If it is assumed that the difference between the transfer quotient and the value from the 5 pool model is the same for sheep H as it is for sheep I (i.e. 7%) then apparently 17.1% of the glucose pool is provided by the blood bicarbonate pool. This value indicates that 103% of the oxaloacetate pool was provided by compounds that undergo a CO_2 fixation reaction.

These results are similar to that found by other workers. For example, in a 5 pool model (mean of 2 animals) produced by Armentano and Young (1983) for steers given a diet containing 30% cracked corn, 19% of the carbon in the glucose pool entered from the blood bicarbonate pool. Considering that on this diet there would have been absorption of exogenous glucose, the percentage of the endogenously formed glucose coming from blood bicarbonate would be higher than 19%.

7.3.5 Correction Of The Propionate To Glucose Transfer Quotient For The Effects Of Metabolic Crossover

7.3.5.1 Sheep I -

The percentage of the glucose carbon that was provided by the carboxyl carbon of propionate is 4.8% (Figure 7-13). As the carboxyl carbon of propionate can only be incorporated into positions 3 or 4 of the glucose molecule the percentage of these carbons provided by the carboxyl carbon of propionate is 14.4%. This value has to be

multiplied by 2 to account for the loss of tracer in the decarboxylation reaction. Therefore, 28.8% of glucose carbons 3 or 4 are provided by the carboxyl carbon of propionate or the CO_2 molecule fixed to the propionate in the carboxylation step. To obtain the net percentage of glucose carbons 3 or 4 provided by propionate this value (28.8%) has to be corrected for the dilution of tracer caused by mixing with dicarboxylic acids derived from cycling of the tricarboxylic acid cycle, i.e. metabolic crossover.

Table 7-11 presents the estimates of the percentage of molecules in the oxaloacetate pool provided by cycling of the tricarboxylic acid cycle obtained from the present experiment. The estimates from the incorporation of CO_2 into glucose were disregarded.

The glucose ratios should be the most accurate estimators of the percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle because this ratio is not affected by metabolism in non-gluconeogenic tissues. The estimates obtained from the glucose ratio using acetate and propionate are in good agreement and were therefore combined. These glucose ratios indicate that in this animal only 17% of the molecules in the oxaloacetate pool was provided by net inputs to the tricarboxylic acid cycle. As a result, the measured flow of tracer from oxaloacetate to glucose will represent only 17% of the actual molecule flow. The percentage of glucose being provided by propionate corrected for metabolic crossover is

$$28.8 \times \frac{100}{17} = 167\%$$

Table 7-11

The estimates of the percentage of oxaloacetate arising from cycling of the tricarboxylic acid cycle obtained using values taken from the relevant 5 pool models to solve the CO_2 and glucose ratios using propionate, and the glucose ratio using acetate.

	Sheep H	Sheep I
CO_2 ratio (propionate)	84	68
Glucose ratio (propionate)	*	82
Glucose ratio (acetate)	90	84

* This value was negative and is therefore meaningless. Possible reasons as to why the negative value was obtained are given in the text.

This result suggests that the percentage of molecules in the oxaloacetate pool coming from cycling of the tricarboxylic acid cycle is overestimated by the glucose ratio.

The CO_2 ratio indicates that 68% of the oxaloacetate pool was provided by cycling of the tricarboxylic acid cycle. This estimate indicates that the measured tracer flow represents only 32% of the actual flow. Therefore, the percentage of glucose provided by propionate is

$$28.8\% \times \frac{100}{32} = 90\%$$

7.3.5.2 Sheep H -

The percentage of the glucose pool provided directly by the carboxyl carbon of propionate according to the 5 pool model (Figure 7-14) is 1.65%. Therefore, the carboxyl carbon of propionate provides 4.95% of glucose carbons 3 or 4. This value increased to 9.9% after correction for the loss of label in the decarboxylation reaction.

The glucose ratio using acetate indicates that 90% of the oxaloacetate pool was provided by cycling of the tricarboxylic acid cycle and thus only 10% was provided by net inputs. Therefore, the percentage of glucose provided by propionate is

$$9.9 \times \frac{100}{10} = 99\%$$

Again, the glucose ratio appears to overestimate the percentage of molecules in the oxaloacetate pool being provided by cycling of the tricarboxylic acid cycle.

The CO_2 ratio indicates that 84% of the oxaloacetate pool was provided by cycling of the tricarboxylic acid cycle and that 16% was provided by net inputs. Therefore, the percentage of glucose provided by the carboxyl carbon of propionate is

$$9.9 \times \frac{100}{16} = 62\%$$

This value is probably an underestimate because of the effects of the apparent large flow from rumen bicarbonate to glucose discussed earlier.

The above calculations indicate that the calculated percentage of glucose provided by propionate is very sensitive to the estimate of the percentage of molecules in the oxaloacetate pool being provided by cycling of the tricarboxylic acid cycle.

7.3.6 Effect Of Some Pathways Of Tracer Recycling On The Estimates Of Oxaloacetate From Cycling Of The Tricarboxylic Acid Cycle

7.3.6.1 Exchange Reactions Between Oxaloacetate And CO_2 Or Cycling Back To Oxaloacetate Via Pyruvate -

7.3.6.1.1 Solving The CO_2 And Glucose Ratios Simultaneously -

The effects of the above type of recycling on the incorporation of CO_2 into glucose and the glucose ratio are discussed in Section 6.5.4.4. As discussed in that Section only the tracer flows from the carboxyl carbons of oxaloacetate are affected. Therefore, the numerator and denominator of the CO_2 ratio will be decreased proportionally and thus, the effects will cancel out in the ratio. The value of the ratio and the estimate of the percentage of molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle

will not be affected.

If cycling of tracer via pyruvate and/or exchange reactions between oxaloacetate and CO_2 was the only type of tracer recycling occurring, then the differences between the estimates of the percentage of molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle given by the CO_2 and glucose ratios would be due to this recycling. That is, after correction for the effects of this recycling, the glucose ratio would give the same value of NI as the CO_2 ratio. Therefore, if the value of NI obtained from the CO_2 ratio is substituted into the glucose ratio, an estimate of the effects of the above form of recycling can be obtained.

The value of NI obtained from the CO_2 ratio (0.46) from sheep I[1] was substituted into the glucose ratio corrected for the effects of recycling of tracer via pyruvate and/or exchange reactions between oxaloacetate and CO_2 (Section 6.5.4.4) and the equation solved for X, i.e.

$$X = 0.15$$

According to this interpretation cycling via pyruvate and/or exchange reactions between oxaloacetate and CO_2 (with subsequent equilibration of the carboxyl carbons in the dicarboxylic acid pools) replaced 15% of the carboxyl carbon that is not removed in the oxaloacetate to phosphoenolpyruvate reaction with carbon from CO_2. Therefore, it indicates that the tracer flow from CO_2 to glucose is

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[1] The data from sheep H were not used here because of doubts about the reality of the apparent large direct flow from rumen bicarbonate to glucose and because of the effects that this flow would induce in the calculated values for the other flows in the model.

118% ($^1/_{(1-x)}$), and the tracer flow from the carboxyl carbon of propionate 85%, of what they would have been without the recycling.

The percentage of glucose carbons 3 or 4 provided by the carboxyl carbon of propionate = 14.4%.

Correcting for the loss of tracer due to equilibration and subsequent loss of one carboxyl in the decarboxylation reaction

$$14.4 \times 2 = 28.8$$

Correcting for the recycling via phosphoenolpyruvate and pyruvate (or a form of cycling that has similar effects on the tracer flows)

$$28.8\% \times {}^1/_{0.85} = 34\%$$

Correcting this value for the dilution caused by metabolic crossover indicates that

$$34\% \times {}^1/_{0.32} = 106\%$$

of the glucose carbons 3 or 4 were provided by the carboxyl carbon of propionate.

7.3.6.1.2 Simultaneously Solving The Glucose Ratio And The Incorporation Of CO_2 Into Glucose Equation -

If the above form of recycling is the only form of recycling, simultaneously solving the equation for the glucose ratio and the equation for the incorporation of CO_2 into glucose should give an estimate of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle the same as given by solving the CO_2 ratio. However, using the data from sheep I (Figure 7-13), solving the glucose ratio and the equation describing the

incorporation of CO_2 into glucose simultaneously gives values of 3.0 for NI (25% of the oxaloacetate from cycling of the tricarboxylic acid cycle) and .42 for X (the proportional decrease in the carboxyl carbon specific radioactivity due to the effects of the recycling). This value (25%) for the percentage of molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle is quite different to the value (68%) expected from the CO_2 ratio. Therefore, it appears that recycling of tracer via pyruvate and/or exchange of CO_2 in the oxaloacetate to phosphoenolpyruvate reaction is not the only form of tracer recycling having a significient effect on the pattern of tracer flow.

7.3.6.2 Incorporating The Effects Of Tracer Recycling Via Acetyl-CoA Into The Interpretation -

As explained in Section 4.6.1, tracer from the middle carbons of oxaloacetate can return to the oxaloacetate pool via the pathway: oxaloacetate, phosphoenolpyruvate, pyruvate, acetyl-CoA, citrate and then back to oxaloacetate via the tricarboxylic acid cycle. The tracer that is returned is distributed equally on all four carbons. Thus it will increase the specific radioactivity of both the middle and carboxyl carbons of oxaloacetate. Tracer that was originally on the carboxyl carbons of oxaloacetate is not returned by this pathway.

This form of cycling will increase the denominator of the CO_2 ratio without affecting the numerator, thus, decreasing the value of the CO_2 ratio, decreasing NI and increasing the estimate of the percentage of molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle. The effect of this form of recycling on the glucose ratio is to increase the numerator of the glucose ratio

without affecting the denominator. Therefore, the value of the ratio will be increased, NI will be decreased and the estimate of the percentage of molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle will be increased.

Recycling of tracer via acetyl-CoA alone cannot explain the data because this form of tracer recycling will not increase the incorporation of tracer from CO_2 into glucose.

7.3.6.2.1 Effect On The Incorporation Of CO_2 Into Glucose -

Recycling via acetyl-CoA does not affect the incorporation of tracer from CO_2 into glucose. Therefore, the equation that describes the incorporation of tracer into glucose, as developed in Section 6.5.4.4., i.e.

$$^1/_{1+NI} = 1 - A(1-X)$$

still holds.

7.3.6.2.2 Effect On The CO_2 Ratio -

This ratio is defined as ($^{14}CO_2$ derived from [1-^{14}C]acetate)/($^{14}CO_2$ derived from [2-^{14}C]acetate). Since the equilibrium specific radioactivity of the carboxyl carbons of oxaloacetate determines directly the amount of $^{14}CO_2$ expired, the above ratio can be written as:

$$\frac{\text{specific radioactivity of the carboxyl carbon of oxaloacetate derived from [1-}^{14}\text{C]acetate}}{\text{specific radioactivity of the carboxyl carbon of oxaloacetate derived from [2-}^{14}\text{C]acetate}}$$

The effects of recycling via pyruvate or backflow in the oxaloacetate to phosphoenolpyruvate reaction affect both the denominator and numerator equally and thus cancel out in the ratio. The effects of cycling via acetyl-CoA only affects the denominator.

Let Z be the proportional increase in the specific radioactivity of the carboxyl carbon of oxaloacetate due to recycling via acetyl-CoA. Therefore, the denominator of the CO_2 ratio has to multiplied by (1+Z) to account for this.

Let C equal the value of the CO_2 ratio. Therefore,

$$C = \left[{}^{ASR}/_{(2+2NI)}\right] / \left[{}^{ASR(1+Z)}/_{(2+2NI)\times(1+2NI)}\right]$$

$$= {}^{(1+2NI)}/_{(1+Z)}$$

7.3.6.2.3 Effect On The Glucose Ratio -

The glucose ratio is defined as: (^{14}C incorporated into glucose from [2-^{14}C]acetate)/(^{14}C incorporated into glucose from [1-^{14}C]acetate) by way of the pathway: acetate, tricarboxylic acid cycle, oxaloacetate, pyruvate glucose.

Therefore, in terms of the specific radioactivity of oxaloacetate carbons, the glucose ratio

$$= \frac{2 \times \left[\begin{array}{c}\text{specific radioactivity of} \\ \text{the middle carbons of} \\ \text{oxaloacetate} \\ \text{([2-}^{14}\text{C]acetate infused)}\end{array}\right] + \left[\begin{array}{c}\text{specific radioactivity of} \\ \text{the carboxyl carbons of} \\ \text{oxaloacetate} \\ \text{([2-}^{14}\text{C]acetate infused)}\end{array}\right]}{\begin{array}{c}\text{specific radioactivity of carboxyl carbons of oxaloacetate} \\ \text{derived from [1-}^{14}\text{C]acetate}\end{array}}$$

7.3.6.2.3.1 Methyl Carbon Of Oxaloacetate -

Recycling of tracer via pyruvate and/or exchange reactions between oxaloacetate and CO_2 does not affect the specific radioactivity of the middle carbons of oxaloacetate.

Recycling via acetyl-CoA returns the same amount of tracer to the middle and carboxyl carbons of oxaloacetate. As the specific radioactivity of the middle carbons of oxaloacetate is (2+2NI) times greater than that of the carboxyl carbons (before the effects of recycling), the proportional increase caused by this recycling will only increase the middle carbon specific radioactivity by $Z/(2+2NI)$.

Therefore, the specific radioactivity of the middle carbons of oxaloacetate

$$= [ASR/(1+2NI)] \times [1+(Z/(2+2NI))]$$

7.3.6.2.3.2 Carboxyl Carbon Of Oxaloacetate -

When [2-^{14}C]acetate is infused, the carboxyl carbon will be decreased by the recycling via pyruvate or backflow of the oxaloacetate to phosphoenolpyruvate reaction (multiply by (1-X)) and increased by the recycling via acetyl-CoA (multiply by (1+Z)). Therefore, the specific radioactivity of the carboxyl carbon of oxaloacetate when [2-^{14}C]acetate is infused

$$= ASR \times (1+Z)(1-X)/(2+2NI) \times (1+2NI)$$

When [1-^{14}C]acetate is infused there is no recycling of tracer via acetyl-CoA, therefore, the specific radioactivity of the carboxyl carbon only has to be corrected for the effects of recycling via pyruvate and/or exchange reactions between oxaloacetate and CO_2 (i.e.

multiply by (1-X)). The specific radioactivity of the carboxyl carbon of oxaloacetate when [1-^{14}C]acetate is infused

$$= {}^{ASR(1-X)}/_{(2+2NI)}$$

7.3.6.2.3.3 The Corrected Glucose Ratio -

The equations for the specific radioactivities of the carbons of oxaloacetate (corrected for the above forms of recycling) were used to formulate the glucose ratio. B (the glucose ratio)

$$= \frac{[2({}^{ASR}/_{(1+2NI)} \times (1+{}^{Z}/_{(2+2NI)})]+[{}^{ASR\times(1+Z)\times(1-X)}/_{(2+2NI)\times(1+2NI)}]}{{}^{ASR(1-X)}/_{(2+2NI)}}$$

7.3.6.3 Correction For The Effects Of Both Forms Of Recycling Simultaneously -

The parameters A (from the equation for the incorporation of CO_2 into glucose), B (from the glucose ratio) and C (from the CO_2 ratio) can be measured experimentally. This means that there are 3 unknowns (NI,X,Z) in the 3 equations and that the equations can be solved simultaneously.

By substituting the equation for the incorporation of CO_2 into glucose and the CO_2 ratio into the glucose ratio and solving for NI the following quadratic equation is obtained,

$$NI^2x[4AC+4A+2-2BC] + NIx[6AC+6A+1-BC] + [2AC+2A] = 0$$

$$(2NI+1) \times [NI(2CA+2A+1-BC)+(2CA+2A)] = 0$$

One root of the equation will always be the nonsensical value $-{}^{1}/_{2}$. Therefore,

$$NI = [2A(C+1)]/[BC-1-2A(C+1)]$$

Using the values of A, B and C obtained from sheep I[1] a negative value of NI is obtained. Therefore, at no positive value of NI are the data for the three equations internally consistent under the interpretation developed above.

7.3.6.4 Conclusion -

The above interpretation does not explain all the data simultaneously and suggests that another factor is influencing the results. The equation describing the incorporation of CO_2 into glucose gives values greater than expected from the theory even though because of the assumptions involved, the estimate should be an underestimation. Therefore, it appears that the theory used to describe the incorporation of CO_2 into glucose is not adequate to explain the data. There appears to be a pathway that increases the incorporation of CO_2 into glucose that is not included in the present theory. A pathway that increases the incorporation of CO_2 into glucose must decrease the incorporation of carbon from other precursors. Therefore, this proposed unknown pathway may also affect the glucose and CO_2 ratios. Thus, the estimates of the percentage of molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle obtained from the interpretations above will be incorrect. However, data from more animals are needed before definite conclusions can be drawn.

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[1] The data from sheep H were not used here because of doubts about the reality of the apparent large direct flow from rumen bicarbonate to blood glucose and because of the effects that this flow would induce in the other flows in the model.

The incorporation of CO_2 was higher than expected. Could this simply be due to the specific radioactivity of CO_2 in mixed venous blood being different from the intracellular specific radioactivity of CO_2 in the liver? Kornberg, Davies and Wood (1952) using cats found that the specific radioactivity of the carbon in urea (which is synthesised in the liver) was the same as that of blood bicarbonate carbon. This indicates that the intracellular bicarbonate in the liver is equilibrating with the bicarbonate of the blood passing through it. In the ruminant, perhaps absorption of CO_2 from the rumen is lowering the bicarbonate specific radioactivity in the portal blood presented to the liver. However, if this were so, then the present estimate of CO_2 incorporation into glucose (which is already unrealistically high under the present interpretation) would be an underestimate.

A major problem with this study is the possible compounding of errors due to the extensive mathematical treatment of the data. This is especially a problem using ratios because quite small changes in the denominators can cause large changes to the values of the ratios. However, if the errors in the original data (25 parameters in the 5 pool models) are random then possibly some of the error may cancel.

Another difficulty with this experiment was the amount of experimentation performed on each animal. Seven infusions were needed to obtain all the data needed to solve all the models. This amount of experimentation can be very stressful on the experimental animals. To overcome this, one week was left between infusions to allow the animals to recover from the experimentation. Leaving this amount of time between infusions had the added advantage of ensuring that all residual activity in the animals had dissipated. However, it took

several weeks to get all the data necessary to solve the models. This is a very long time to try to keep the animals in one condition (i.e. steady state) and the animals may have changed during the experimental period. In the following experiment less time was left between infusions and thus all the data collected in a shorter time span.