CHAPTER 8

QUANTIFYING METABOLIC CROSSOVER (5 POOL MODEL - LUCERNE CHAFF DIET)

8.1 INTRODUCTION

The experiment presented here was done in an attempt firstly, to test whether results similar to those obtained from the oaten straw chaff experiment were obtainable using a different diet, in this case lucerne chaff. In addition, as will be shown in the following sections, there are highly significant relationships between the apparent rate of propionate irreversible loss and the apparent propionate to glucose transfer quotients for both [1-^{14}C] and [2-^{14}C]propionate. Therefore, the transfer quotients of propionate to glucose can be predicted for both [1-^{14}C] and [2-^{14}C]propionate at any given rate of irreversible loss of propionate. This is the information necessary to solve the glucose ratio using propionate. Thus, it should be possible to quantify the effects of metabolic crossover at any rate of propionate irreversible loss.

8.2 THE RELATIONSHIP BETWEEN PROPIONATE IRREVERSIBLE LOSS AND PROPIONATE TO GLUCOSE TRANSFER QUOTIENT (USING [2-^{14}C]PROPIONATE)

A strong correlation between the rate of propionate irreversible loss in the rumen using [2-^{14}C]propionate (P2IL) and the propionate to

glucose transfer quotient using [2-^{14}C]propionate (P2GTQ) is apparent in data for the 800g/d lucerne chaff fed sheep in the literature (Judson and Leng 1973, Leng Steel and Luick 1967). The results of Judson and Leng (1973) and Leng et al. (1967) are presented in Table 8-1 and Figure 8-1.

The equation of the relationship (+/- standard error) is

$$P2GTQ = 1.07(+/-.104)P2IL + 6.25(+/-4.584)*$$

$$(r^2=0.96, RMS=5.22)$$

Bergman et al. (1966) investigated the proportion of the glucose pool being provided by the propionate pool using [2-^{14}C] propionate with sheep fed 800g/d of a pelleted lucerne hay. This diet is similar to that used by Judson and Leng (1973) and Leng et al. (1967) who fed 800g/d lucerne chaff and thus, would be expected to give similar results.

In the experiments of Judson and Leng (1973) and Leng et al. (1967), propionate was infused into the rumen and blood sampled from the jugular vein. Bergman et al. (1966) infused [2-^{14}C]propionate into a ruminal vein and sampled from the portal vein. Since propionate is largely removed by one pass through the liver, the rate of propionate absorption can be calculated. This calculation assumes

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* One result (propionate irreversible loss of 160gC/d and propionate to glucose transfer quotient of 87%) is outside the 95% confidence interval of the relationship between the other points. This result was obtained when a large amount of unlabelled propionate was infused into the rumen. At this level of propionate supply the capacity of the liver to extract propionate from the portal blood may have been exceeded. Propionate entering the peripheral circulation would be readily oxidized and thus would not be available for gluconeogenesis. Therefore, this result would not be expected to fit the relationship between the other points and was excluded from the regression calculations.

Table 8-1

The rates of propionate irreversible loss (IL) and the propionate to glucose transfer quotients (TQ) from [2-^{14}C]propionate infusions into the rumen [from Judson and Leng (1973) and Leng *et al.* (1967)].

	Propionate IL (gC/d)	Propionate Glucose TQ (%)
Judson and Leng (1973)	22.3	25
	46.1	58
	21.8	38
	23.3	31
	45.1	55
	28.0	36
	36.3[1]	46
	62.2[1]	73
	62.7[1]	83
	56.0[1]	60
	159.7[1]	87
Leng, Luick and Steel (1967)	51.3	57
	45.1	50

[1] cold propionate also infused into the rumen

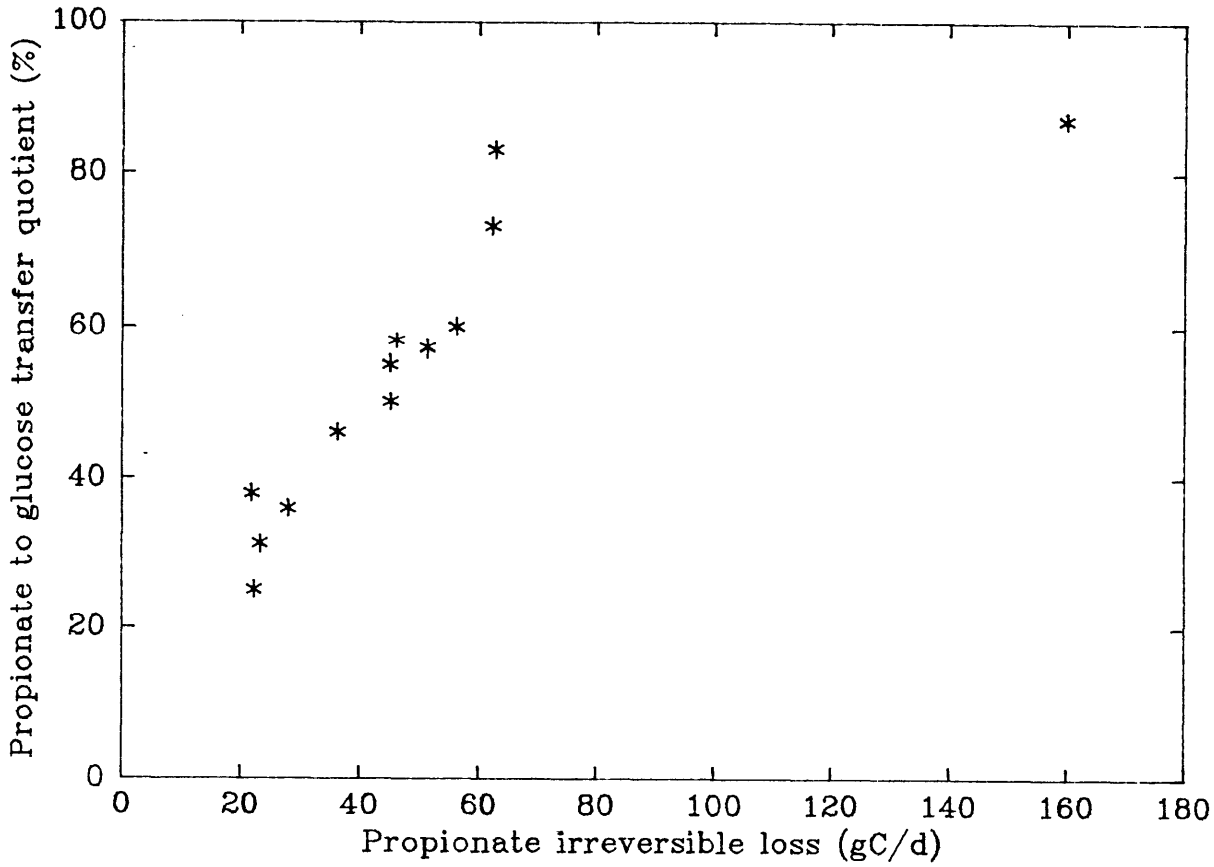

Figure 8-1

The relationship between the rate of propionate irreversible loss and the propionate to glucose transfer quotient estimated from intraruminal infusions of [2-^{14}C]propionate (the data is from Judson and Leng (1973) and Leng et al (1967)

that the amount of recirculating propionate and the amount produced in body tissues is negligible compared with the amount absorbed. They found that the arterial propionate concentration was only about 8% of the portal blood concentration.

The glucose specific radioactivity was also estimated on portal blood. This should not differ much from mixed venous blood because little or no glucose is absorbed from the gut of ruminants fed roughage based diets.

The results of Bergman et al. (1966) are presented in Table 8-2 and graphed in Figure 8-2. In the data of Bergman et al. (1966) also, there is a strong linear relationship (P<.001) between propionate to glucose transfer quotient and the rate of irreversible loss of propionate.

$$P2GTQ = 0.98(\pm .040)P2IL + 7.48(\pm 1.029)^*$$

$$(r^2=.99, RMS=1.49)$$

The equation of the line through the data of Bergman et al. (1966) is not significantly different from the line through the data of Judson and Leng (1973) and Leng et al. (1967). Both sets of data are graphed in Figure 8-3. The equation of the line through the combined data is

$$P2GTQ = 1.07(\pm .053)P2IL + 6.13(\pm 1.967)$$

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* The result (propionate irreversible loss of 72.8gC/d and a propionate to glucose transfer quotient of 62%) is outside the 95% confidence interval of the relationship between the other points. This result was obtained in a sheep infused with a large quantity of unlabelled propionate. As previously argued, possibly the capacity of the liver to extract propionate from the blood is exceeded at high rates of propionate infusion. Therefore, this point was excluded from the calculations.

Table 8-2

The rates of propionate irreversible loss (IL) and the propionate to glucose transfer quotients (TQ) from intraportal infusions of [2-^{14}C]propionate [from Bergman et al (1966)].

	Propionate IL (gC/d)	Propionate Glucose TQ (%)
Half ration	10.9	20
	11.9	18
	10.6	18
Full ration	24.2	33
	20.2	27
	16.6	24
	20.6	25
Full ration plus propionate infused	46.0	53
	42.7	49
	72.8	62

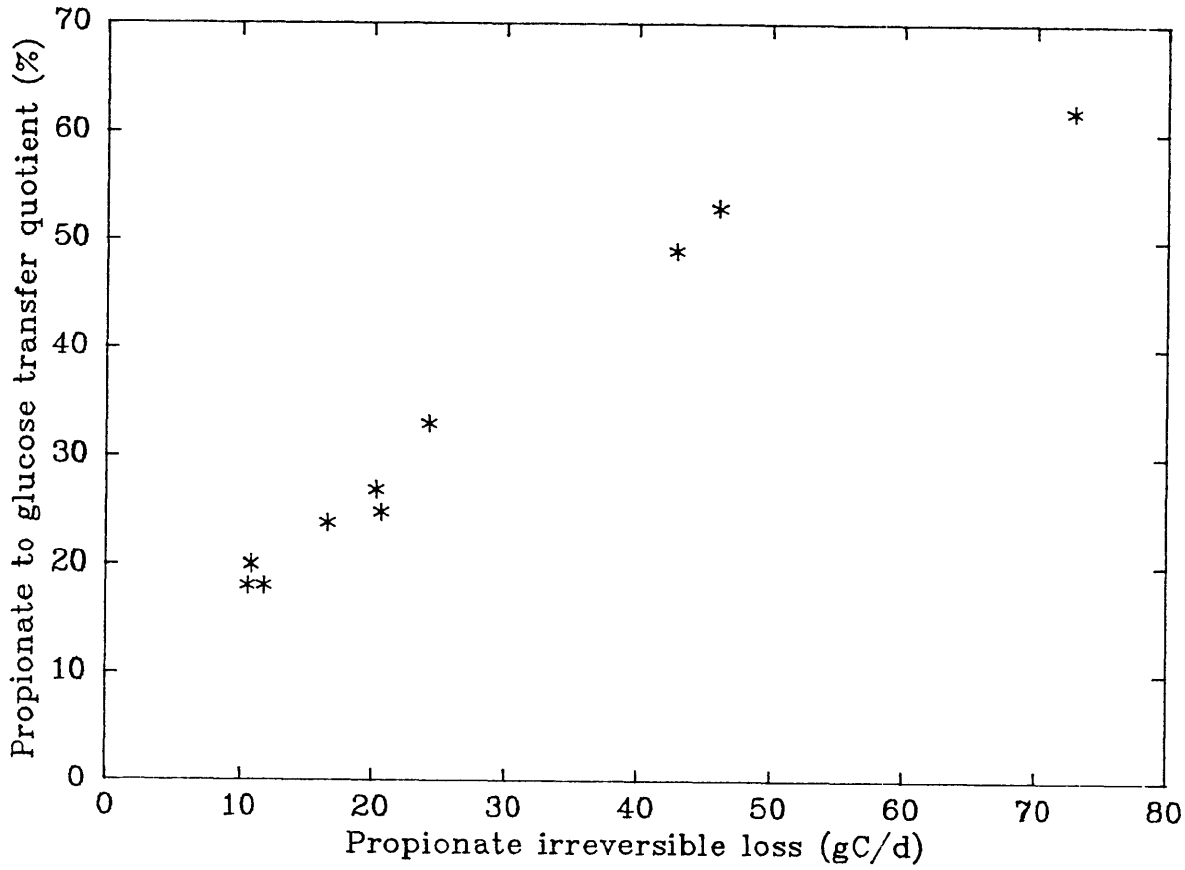

Figure 8-2

The relationship between the rate of propionate irreversible loss and the propionate to glucose transfer quotient estimated from intraportal infusions of [2-^{14}C]propionate (the data is from Bergman et al. (1966).

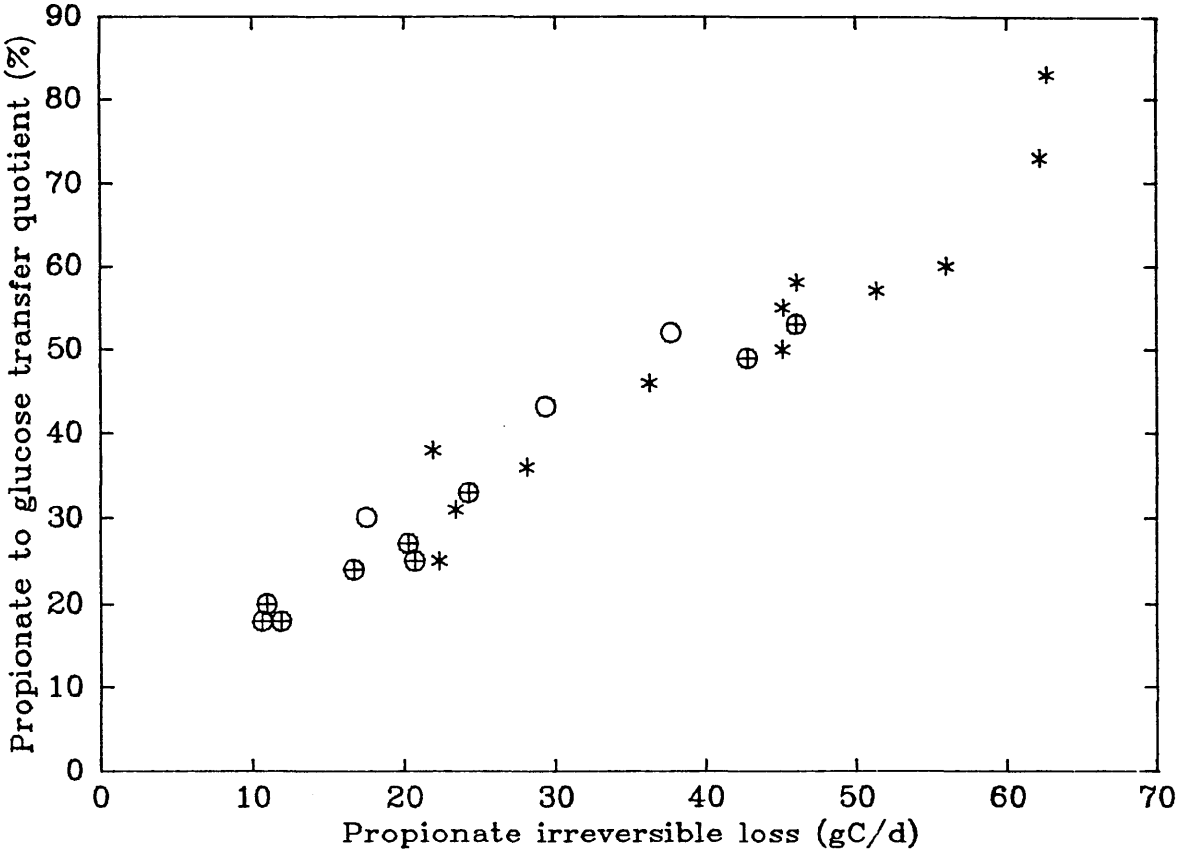

Figure 8-3

The relationship between the rate of propionate irreversible loss and the propionate to glucose transfer quotient (using [2-^{14}C]propionate) from the combined data of Judson and Leng (1973) (*), Leng et al (1967) (*) and Bergman et al (1966) (⊕). The data from the present experiment (O) are also shown in the figure but were not included in the calculation of the regression equation.

(r^2=.96,RMS=4.00)

When [^{14}C]propionate is infused into sheep, the bicarbonate pools become labelled. As bicarbonate is incorporated into glucose, there must be a flow of tracer from propionate to glucose via the bicarbonate pools. Correcting the data for this indirect flow will give a more correct estimate of the direct flow from propionate to glucose.

In the experiments of Bergman et al. (1966), the relationship between the propionate irreversible loss and the propionate to blood CO_2 transfer quotient ($PBCO_2TQ$) estimated using [2-^{14}C]propionate (figure 8-4) is

$$PBCO_2TQ = 0.17(\pm .007)P2IL + 0.37(\pm .182)^*$$

(r^2=.98,RMS=0.26)

This equation relating propionate irreversible loss to the propionate to blood CO_2 transfer quotient was used to estimate the propionate to blood CO_2 transfer quotient in the experiments of Judson and Leng (1973) and Leng et al. (1967) (Table 8-3). The measured propionate to blood CO_2 transfer quotients of Bergman et al. (1965) and the calculated propionate to blood CO_2 transfer quotients of Judson and Leng (1973) and Leng et al. (1967) were multiplied by the average CO_2 to glucose transfer quotient from the present experiments (13.2% Table 8-5) to estimate the percentage of the propionate tracer in glucose that passed through the CO_2 pool. The calculated values

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* The result corresponding to the result excluded from the regression of the rate of propionate irreversible loss against the transfer quotient of propionate to glucose was also excluded from this regression

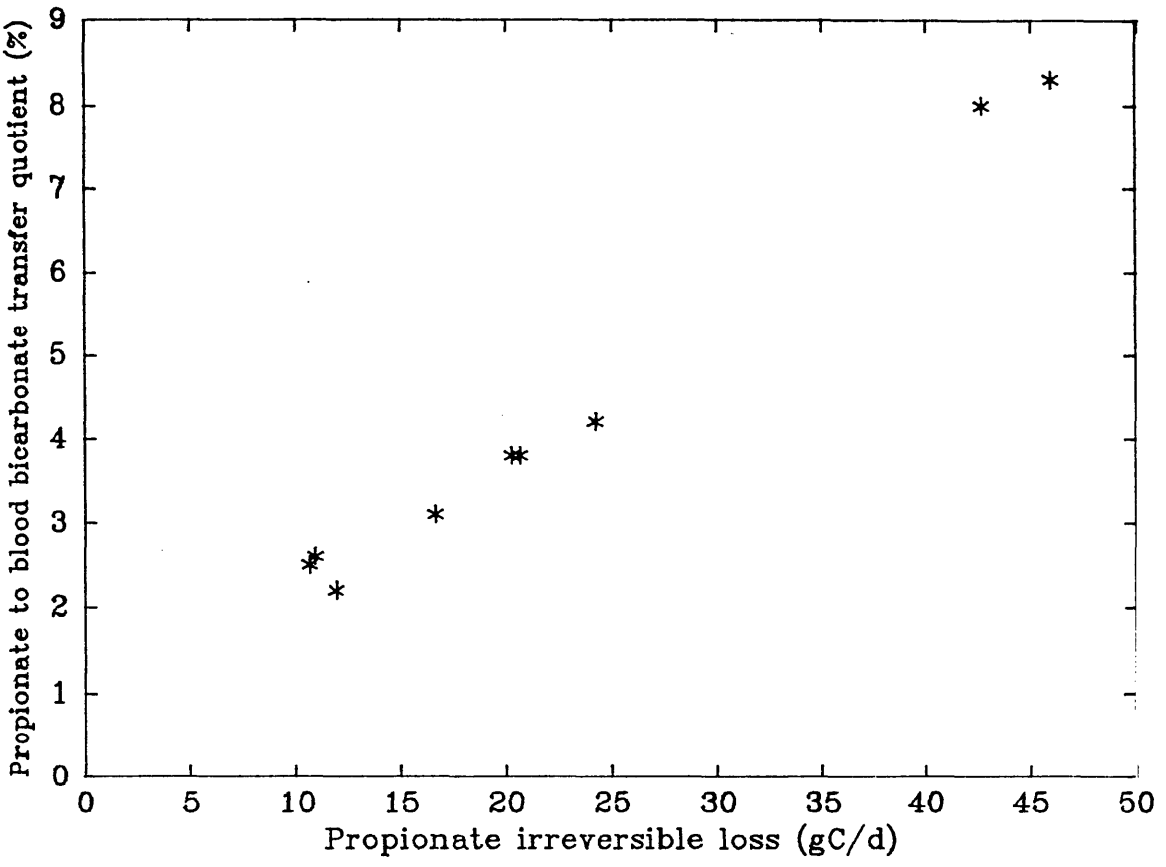


Figure 8-4

The relationship between the rate of propionate irreversible loss and the propionate to blood bicarbonate transfer quotient estimated from intraportal infusions of [2-^{14}C]propionate (the data is from Bergman et al (1966). The point excluded from the calculation of the regression equation of the data presented in Figure 8-2 has been excluded from this figure.

Table 8-3

The rates of propionate irreversible loss, the calculated (data from Judson and Leng (1973) and Leng et al. (1967)) or measured (data from Bergman et al. (1966)) propionate to blood bicarbonate transfer quotient (TQ), the percent of tracer in glucose that passed through the CO_2 pool, and the propionate to glucose transfer quotient corrected for this indirect flow.

propionate IL (gC/d)	Propionate Blood HCO_3^- TQ (%)	% of glucose from propionate via HCO_3^- (%)	Corrected Propionate Glucose TQ (%)
	calculated		
22.3	4.22	0.56	24.44
46.1	8.34	1.10	56.90
21.8	4.14	0.54	37.46
23.3	4.40	0.58	30.42
45.1	8.17	1.07	53.93
28.0	5.21	0.69	35.31
36.3	6.65	0.87	45.13
62.2	11.13	1.46	71.54
62.7	11.21	1.47	81.53
56.0	10.05	1.32	58.68
51.3	9.24	1.22	55.78
45.1	8.17	1.07	48.93
	Measured		
10.9	2.6	0.34	19.66
11.9	2.2	0.28	17.71
10.6	2.5	0.33	17.67
24.2	4.2	0.55	32.45
20.2	3.8	0.50	26.50
16.6	3.1	0.41	23.59
20.6	3.8	0.50	24.50
46.0	8.3	1.09	51.95
42.7	8.0	1.05	47.95

are presented in Table 8-3. These values were used to correct the propionate to glucose transfer quotients for the flow of tracer via the CO_2 pool (Table 8-3). The relationship between the rates of propionate irreversible loss and these corrected propionate to glucose transfer quotients (CP2GTQ) (Figure 8-5) is

$$CP2GTQ = 1.04(\pm.053)P2IL + 6.08(\pm1.966)$$

$$(r^2=.95, RMS=4.00)$$

The correction for the flow of tracer via CO_2 is relatively small. Therefore, any errors introduced by using average values should have little effect on the final result.

8.3 THE RELATIONSHIP BETWEEN PROPIONATE IRREVERSIBLE LOSS AND PROPIONATE TO GLUCOSE TRANSFER QUOTIENT (USING [1-^{14}C]PROPIONATE)

It is apparent in the data from the preceding experiments (Table 8-4) that there is a significant linear ($p<.001$) relationship between the rate of propionate irreversible loss estimated using [1-^{14}C]propionate (P1IL) and the propionate to glucose transfer quotient estimated using [1-^{14}C] propionate (P1GTQ) (Figure 8-6).

$$P1GTQ = 0.27(\pm .015)P1IL + 1.54(\pm .837)$$

$$(r^2=.99, RMS=.64)$$

The indirect flow of tracer to glucose via the bicarbonate pool can be estimated as explained in section 6.5.4.3. The percentage of the blood bicarbonate pool arising from [1-^{14}C]propionate and the percentage of the carbon in the glucose pool arising from bicarbonate can be estimated from the studies presented in sections 6.4 and 6.5. From these values the percentage of the tracer (and therefore carbon)

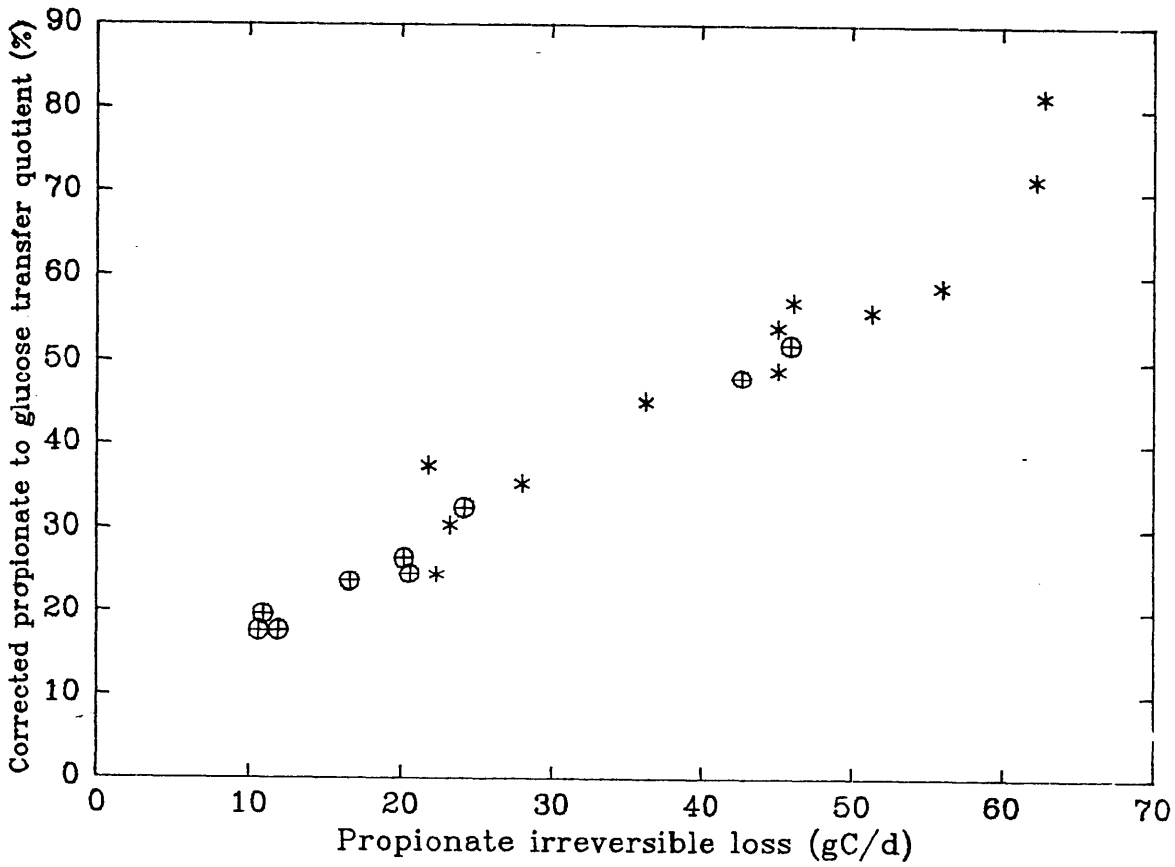


Figure 8-5

The relationship between the rate of propionate irreversible loss and the propionate to glucose transfer quotient, corrected for the indirect flow of tracer via the CO_2 pool (calculated as explained in the text and presented in Table 8-3) using [2-^{14}C]propionate (the original data is from Judson and Leng (1973), Leng et al. (1967) and Bergman et al. (1966).

Table 8-4

The rates of propionate irreversible loss (IL) and the propionate to glucose transfer quotients (TQ) from infusions of [1-^{14}C]propionate (data from Sections 6.4 and 6.5)

	Propionate IL (gC/d)	Propionate Glucose TQ (%)
Sheep C	37 (3.8)	11 (1.3)
Sheep D	57 (3.8)	17 (1.2)
Sheep E	39 (1.4)	12 (0.6)
Sheep F	53 (6.6)	15 (1.9)
Sheep G	90 (3.3)	26 (2.1)

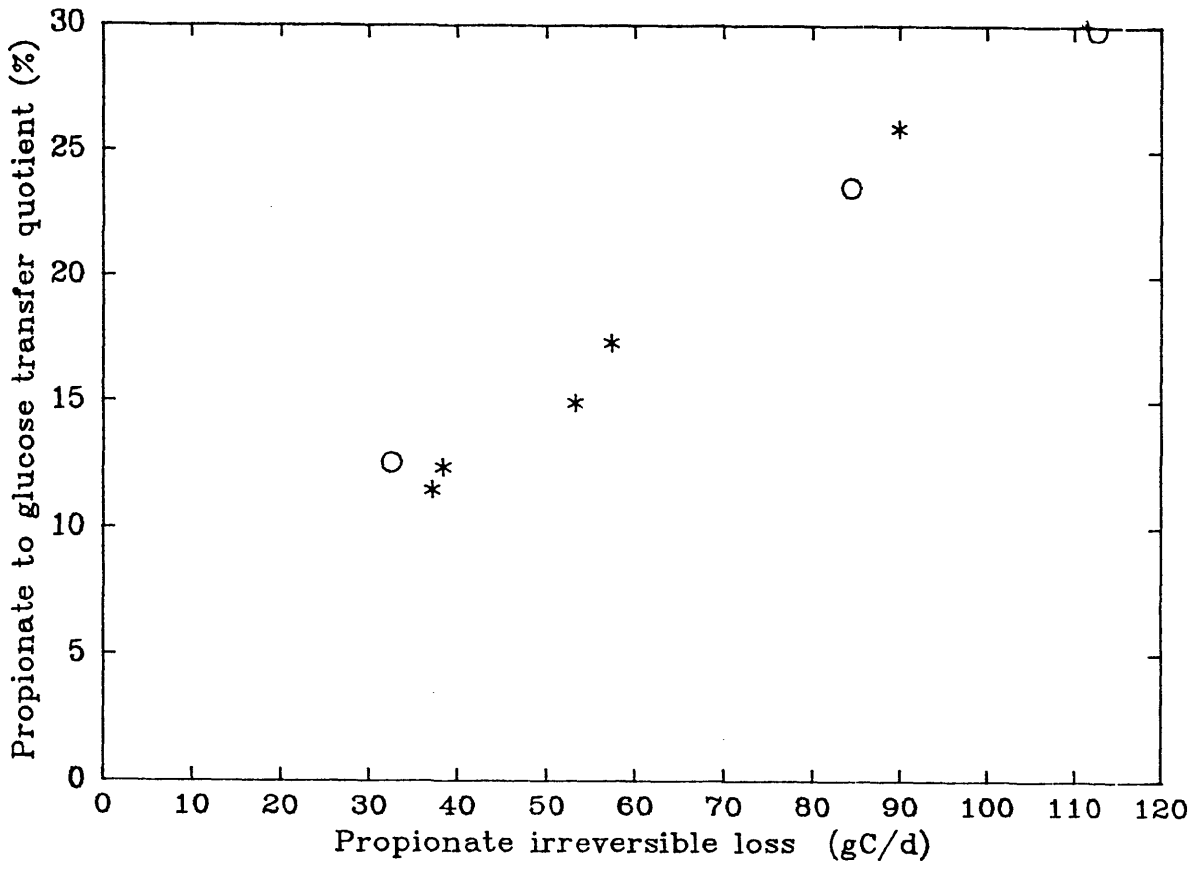


Figure 8-6

The relationship between the rate of propionate irreversible loss and the propionate to blood glucose transfer quotient using [1-^{14}C]propionate (from Table 8-4 *). The data from the present experiment (O) are also included in the figure but were not used in the calculation of the regression line.

in glucose that came from propionate via the bicarbonate pool can be estimated. The results are presented in Table 8-5. These results indicate that between 14% and 25% of the tracer in glucose when [1-^{14}C]propionate was infused intraruminally, passed through the bicarbonate pool before being incorporated into glucose.

Figure 8-7 illustrates the relationship between the propionate to glucose transfer quotient and this transfer quotient corrected for the flow of tracer via bicarbonate. The propionate to glucose transfer quotient corrected for the flow via bicarbonate is linearly ($p<.001$) related to the rate of propionate irreversible loss (estimated with [1-^{14}C]propionate) (figure 8-8).

The equation for this relationship is

$$CP1GTQ = .21(+/- .021)P1IL + 1.75(+/- 1.222)$$

$$(r^2=.97, RMS=.90)$$

8.4 COMPARISON OF THE RATES OF IRREVERSIBLE LOSS OF THE CARBOXYL AND MIDDLE CARBONS OF PROPIONATE

In the preceding sections equations relating the rates of propionate irreversible loss to the propionate to glucose transfer quotients have been developed for both [1-^{14}C] and [2-^{14}C]propionate. Therefore, the propionate to glucose transfer quotient at any level of propionate irreversible loss can be calculated for both [1-^{14}C] and [2-^{14}C]propionate. For values of the transfer quotients given by the above equations to be comparable, the rates of propionate irreversible loss measured by [1-^{14}C] and [2-^{14}C]propionate must be equatable.

Table 8-5

Calculation of the per cent of glucose that came from propionate via the bicarbonate pool. Calculated as explained in the text

Sheep	C	D	E	F	G
% glucose from propionate	11.46	17.31	12.33	14.92	25.89
	(1.27)	(1.18)	(0.56)	(1.90)	(2.04)
% bicarbonate from propionate	20.93	24.32	15.06	21.13	35.76
	(3.42)	(1.86)	(0.89)	(3.12)	2.97)
% glucose from bicarbonate	13.46	10.99	12.46	13.27	15.55
	(2.10)	(1.51)	(1.20)	(1.15)	(1.30)
% glucose from propionate via bicarbonate	2.82	2.67	1.88	2.80	5.56
	(0.64)	(0.42)	(0.21)	(0.48)	(0.66)
% of the tracer in glucose that came via bicarbonate	24.6	15.4	15.2	18.8	21.5
	(6.2)	(2.6)	(1.8)	(4.0)	(3.1)
Propionate to glucose transfer quotient corrected for the flow of tracer via bicarbonate	8.64	14.64	10.45	12.12	20.33

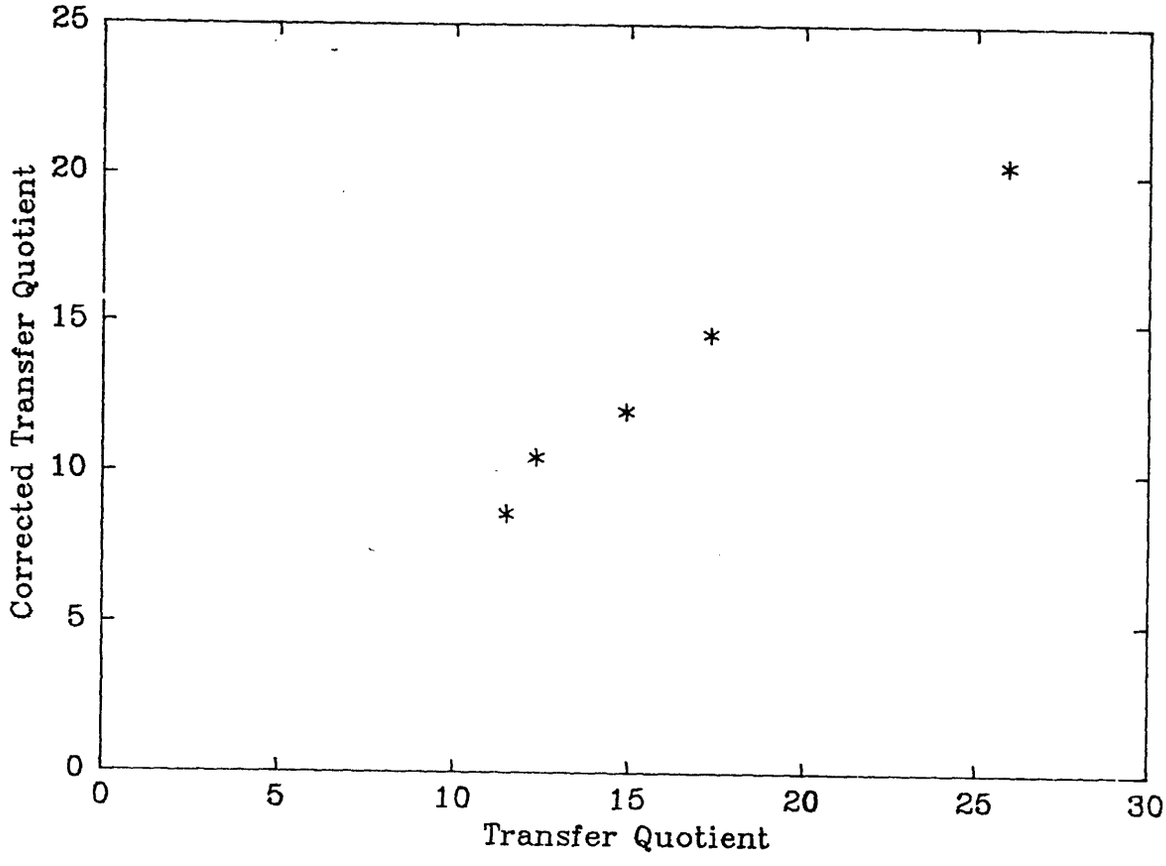


Figure 8-7

The relationship between the propionate to glucose transfer quotient (using [1-^{14}C]propionate) and this transfer quotient corrected for the indirect flow of tracer via the blood bicarbonate pool

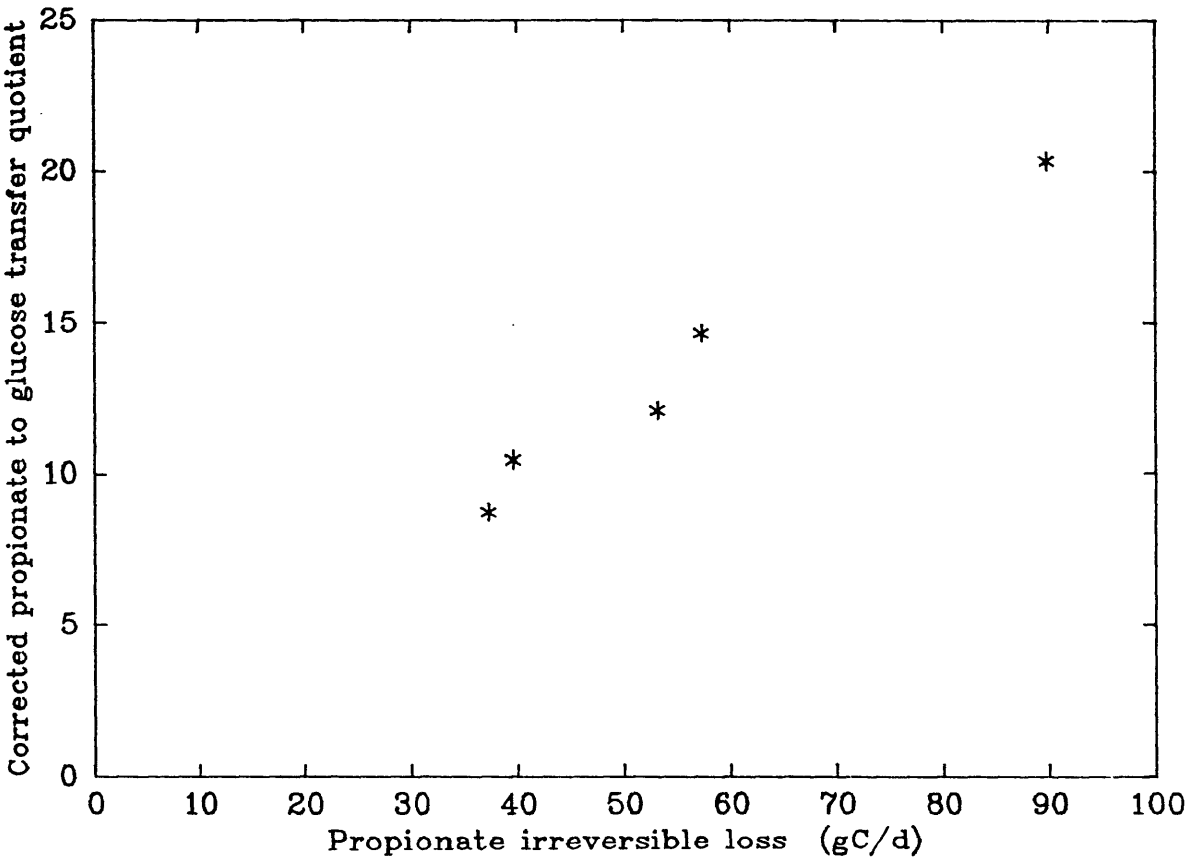


Figure 8-8

The relationship between the rate of propionate irreversible loss and the propionate to blood glucose transfer quotient, corrected for the indirect flow of tracer to glucose via the bicarbonate pool, using [1-^{14}C]propionate

Knox et al. (1961) found that in lactating dairy cows 17.7% of injected [1-^{14}C]propionate tracer was recovered in rumen CO_2 in 5h. However, only 1.1% of the [2-^{14}C]propionate was recovered in rumen CO_2 over the same time period. In the experiments presented in Chapter 7, using oaten straw chaff, it appears that the carboxyl carbon of propionate is metabolised in the rumen but the methyl carbon is not. Rowe at al. (1981) reported that ^{14}C-bicarbonate was incorporated into the carboxyl position of propionate, indicating turnover of the carboxyl carbon in the rumen. Therefore, the apparent propionate irreversible loss measured using [1-^{14}C]propionate will be higher than the apparent propionate irreversible loss measured by [2-^{14}C]propionate.

Mayes et al. (1981) found that the rate of propionate irreversible loss estimated using [1-^{14}C]propionate was about double the estimate found using [2-^{14}C]propionate. In the work of Leng et al. (1967) one animal had the rate of propionate irreversible loss estimated using both [1-^{14}C] and [2-^{14}C]propionate. The estimate using [2-^{14}C]propionate was only 60% of the estimate using [1-^{14}C]propionate. Also, in the experiments presented in Chapter 7 the rate of irreversible loss using [1-^{14}C]propionate was higher than the estimate using [2-^{14}]propionate. Therefore, the irreversible loss of the carboxyl carbon of propionate is different to the irreversible loss of the middle carbon. In order to compare the transfer quotients given by the equations it is necessary to know the relationship between the rates of irreversible loss measured using [1-^{14}C] and [2-^{14}C]propionate.

Therefore, in the studies presented now, all the data necessary to solve the 5 pool model (propionate middle carbon, rumen bicarbonate, blood bicarbonate, glucose and propionate carboxyl carbon) were obtained. This allows a comparison of the rates of irreversible loss of the carboxyl and middle carbons of propionate. It also allows a comparison of the percentage of the carbon atoms in the glucose pool that originated in the propionate pool (i.e. the transfer quotient) with the percentage of the carbon atoms in the glucose pool provided by pathways that did not involve passage through the bicarbonate pools.

8.4.1 Materials And Methods

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

The three Merino sheep used in this experiment were on the same diet (800g air dry lucerne chaff) and feeding regime as in earlier experiments. The animals were well accustomed to being handled and appeared not to be stressed by the experimentation.

The animals were infused intraruminally with [1-^{14}C]propionate (0.2μCi/min), [2-^{14}C]propionate (0.15μCi/min) and ^{14}C-bicarbonate (0.3μCi/min), and intravenously with [U-^{14}C]glucose (0.4μCi/min) and ^{14}C-bicarbonate (0.15μCi/min). All infusions were of 14h duration and were timed to finish at 2000h. At least 1 day was allowed to elapse between the infusions to allow residual radioactivity in the animals to dissipate. Pre-infusion samples were taken before all infusions.

Samples of blood and rumen fluid were taken at hourly intervals over the last 6h of each infusion. Blood samples were assayed for glucose and bicarbonate specific radioactivities. Rumen fluid samples

were assayed for bicarbonate and propionate specific radioactivities. To obtain the mean specific radioactivity of rumen propionate, a subsample from each set of rumen fluid samples was bulked and analysed for propionate specific radioactivity by the high pressure liquid chromatography system.

The 5 pool models were constructed in the same way as those in the preceding chapter.

8.4.2 Results

The specific radioactivities of the primary and secondary pools for each infusion are presented in Tables 8-6 to 8-10. In the case of the specifically labelled propionates, the specific radioactivities are expressed as the specific radioactivity of the tracee atom. The transfer quotients represent the proportion of the secondary pools provided by the tracee atom. The rates of irreversible loss of the studied metabolites are presented in Table 8-11.

The 5 pool models (propionate middle carbon, rumen bicarbonate, blood bicarbonate, glucose and propionate carboxyl carbon) are presented in Figures 8-9 and 8-11. The direct flow of carbon from rumen bicarbonate to glucose was small or negative in all models and was therefore excluded.

8.4.3 Discussion

To solve the glucose ratio, an estimate of the direct flow from the middle carbon of propionate to glucose is required.

Table 8-6

The specific radioactivity (SR) of the middle carbon of propionate and the 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 arose from the pool into which the tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep P		Sheep Q		Sheep R	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Propionate middle carbon	15.0	-	21.3	-	31.2	-
Rumen HCO_3^-	0.20 (0.009)	1.3 (0.16)	0.22 (0.013)	1.0 (0.20)	0.23 (0.007)	0.7 (0.10)
Blood HCO_3^-	0.58 (0.021)	3.87 (0.55)	0.55 (0.016)	2.6 (0.34)	0.46 (0.009)	1.5 (0.17)
Glucose	2.60 (0.065)	17.3 (2.12)	3.06 (0.092)	14.4 (1.88)	3.15 (0.061)	10.0 (1.15)

The values in ( ) are the standard errors

The standard deviation of the propionate specific radioactivity was assumed to be 10% of the value

Table 8-7

The specific radioactivity (SR) of blood bicarbonate 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 the tracer was infused [i.e. the transfer quotient (TQ)] are also presented.

	Sheep P		Sheep Q		Sheep R	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Blood HCO_3^-	1.61 (0.088)	-	1.31 (0.102)	-	1.29 (0.080)	-
Propionate middle carbon	0.53	33.1 (6.07)[1]	0.23	17.9 (4.32)	0.36	28.3 (5.73)
Rumen HCO_3^-	0.78 (0.037)	48.0 (9.81)	0.31 (0.033)	23.6 (8.54)	0.56 (0.033)	43.8 (10.6)
Glucose	0.31 (0.027)	19.1 (5.65)	0.28 (0.026)	21.5 (7.38)	0.24 (0.025)	18.4 (6.41)

The values in ( ) are the standard errors

[1] The standard deviation of the propionate specific radioactivity was assumed to 10% of the value

Table 8-8

The specific radioactivity (SR) of glucose and the specific radioactivities of the secondary pools during an intravenous infusion of [U-^{14}C]glucose. 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 P		Sheep Q		Sheep R	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Glucose	20.8 (0.146)	-	23.8 (0.052)	-	17.9 (0.671)	-
Propionate middle carbon	0.52	2.5 (0.25)[1]	0.43	1.8 (0.20)	0.39	2.2 (0.31)
Rumen HCO_3^-	0.87 (0.044)	4.2 (0.57)	0.76 (0.023)	3.2 (0.30)	0.43 (0.020)	2.4 (0.35)
Blood HCO_3^-	2.31 (0.087)	11.1 (1.13)	1.98 (0.053)	8.3 (0.73)	1.34 (0.038)	7.51 (0.93)

The values in ( ) are the standard errors

[1] The standard deviation of the propionate specific radioactivity was assumed to 10% of the value

Table 8-9

The specific radioactivity (SR) of the carboxyl carbon of propionate and the specific radioactivities of the secondary pools during an intraruminal infusion of [1-^{14}C]propionate. 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 P		Sheep Q		Sheep R	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Propionate carboxyl carbon	8.1	-	10.4	-	29.9	-
Rumen HCO_3^-	1.10 (0.066)	13.6 (2.55)	1.54 (0.141)	14.8 (3.87)	1.97 (0.052)	6.6 (0.80)
Blood HCO_3^-	1.11 (0.039)	13.7 (1.81)	1.07 (0.062)	10.2 (1.87)	1.33 (0.026)	4.5 (0.50)
Glucose	0.88 (0.035)	10.8 (1.58)	0.82 (0.050)	7.9 (1.48)	1.25 (0.031)	4.2 (0.50)

The values in ( ) are the standard errors

[1] The standard deviation of the propionate specific radioactivity was assumed to 10% of the value

Table 8-10

The specific radioactivity (SR) of rumen bicarbonate and the specific radioactivities of the secondary pools during an intraruminal infusion of $H^{14}CO_3^-$. 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 P		Sheep Q		Sheep R	
	SR μCi/gC	TQ %	SR μCi/gC	TQ %	SR μCi/gC	TQ %
Rumen HCO_3^-	5.2 (0.23)	-	7.1 (0.38)	-	10.4 (0.40)	-
Propionate carboxyl carbon	4.99	95.4 (13.35)[1]	3.99	56.3 (9.72)	6.86	66.0 (9.42)
Blood HCO_3^-	3.13 (0.309)	59.9 (16.68)	3.53 (0.067)	49.8 (7.46)	3.65 (0.076)	35.1 (4.07)
Glucose	0.81 (0.053)	15.6 (3.09)	0.86 (0.027)	12.2 (1.99)	1.05 (0.022)	10.1 (1.17)

The values in ( ) are the standard errors

[1] The standard deviation of the propionate specific radioactivity was assumed to 10% of the value

Table 8-11

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

	Sheep P gC/d	Sheep Q gC/d	Sheep R gC/d
Propionate middle carbon	12.6 (1.28)[1]	9.8 (1.00)	5.8 (0.59)
Blood HCO_3^-	157 (24.3)	176 (38.8)	150 (26.5)
Glucose	32.5 (0.86)	26.1 (1.44)	29.1 (2.94)
Propionate carboxyl carbon	37.5 (3.83)	28.1 (2.86)	10.8 (1.10)
Rumen HCO_3^-	170 (11.8)	123 (8.5)	88 (9.1)

The values in ( ) are the standard errors

[1] The standard deviation of the plateau specific radioactivity of the propionate carbon pools was assumed to be 10% of the value

Figure 8-9

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

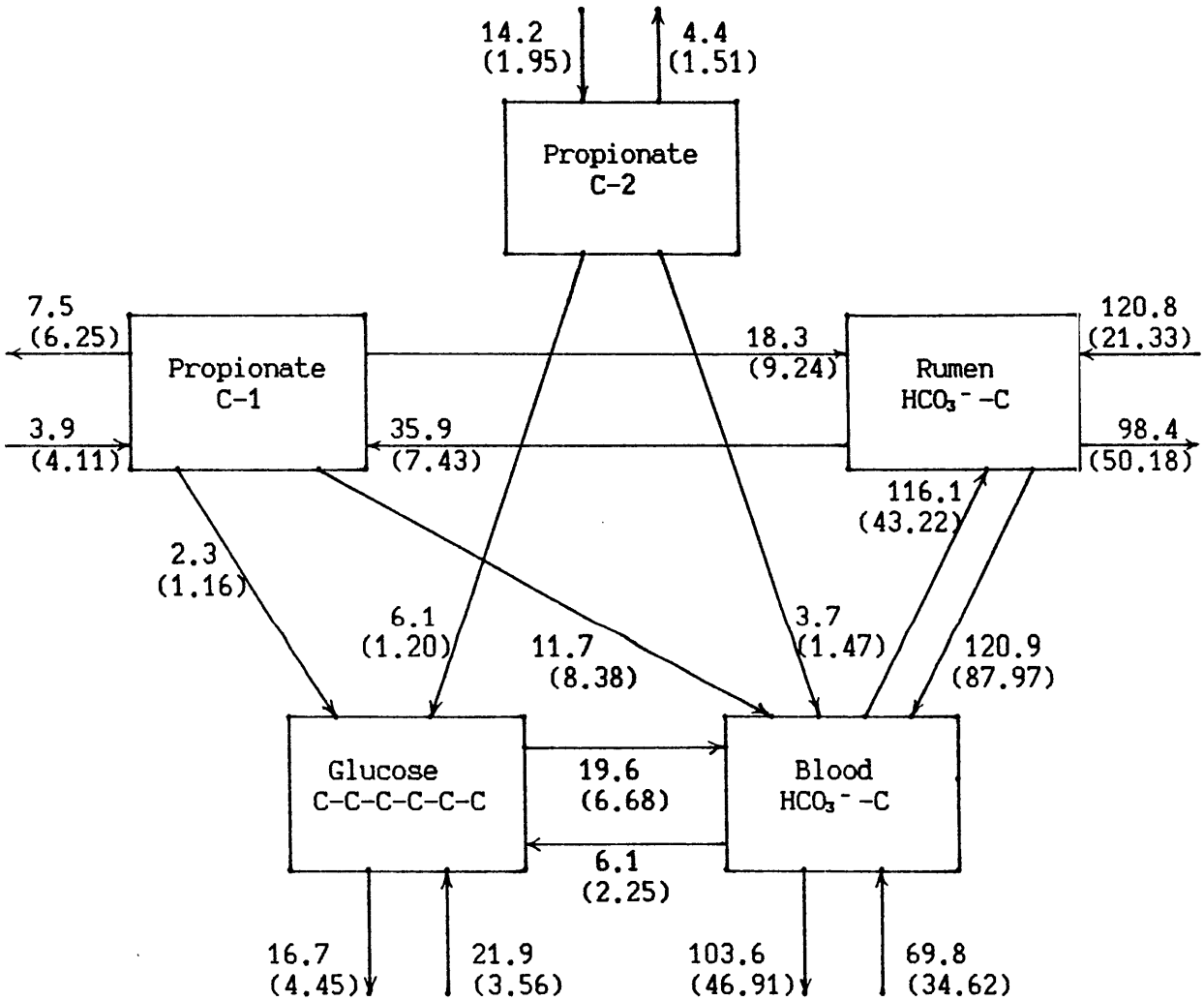


Figure 8-10

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

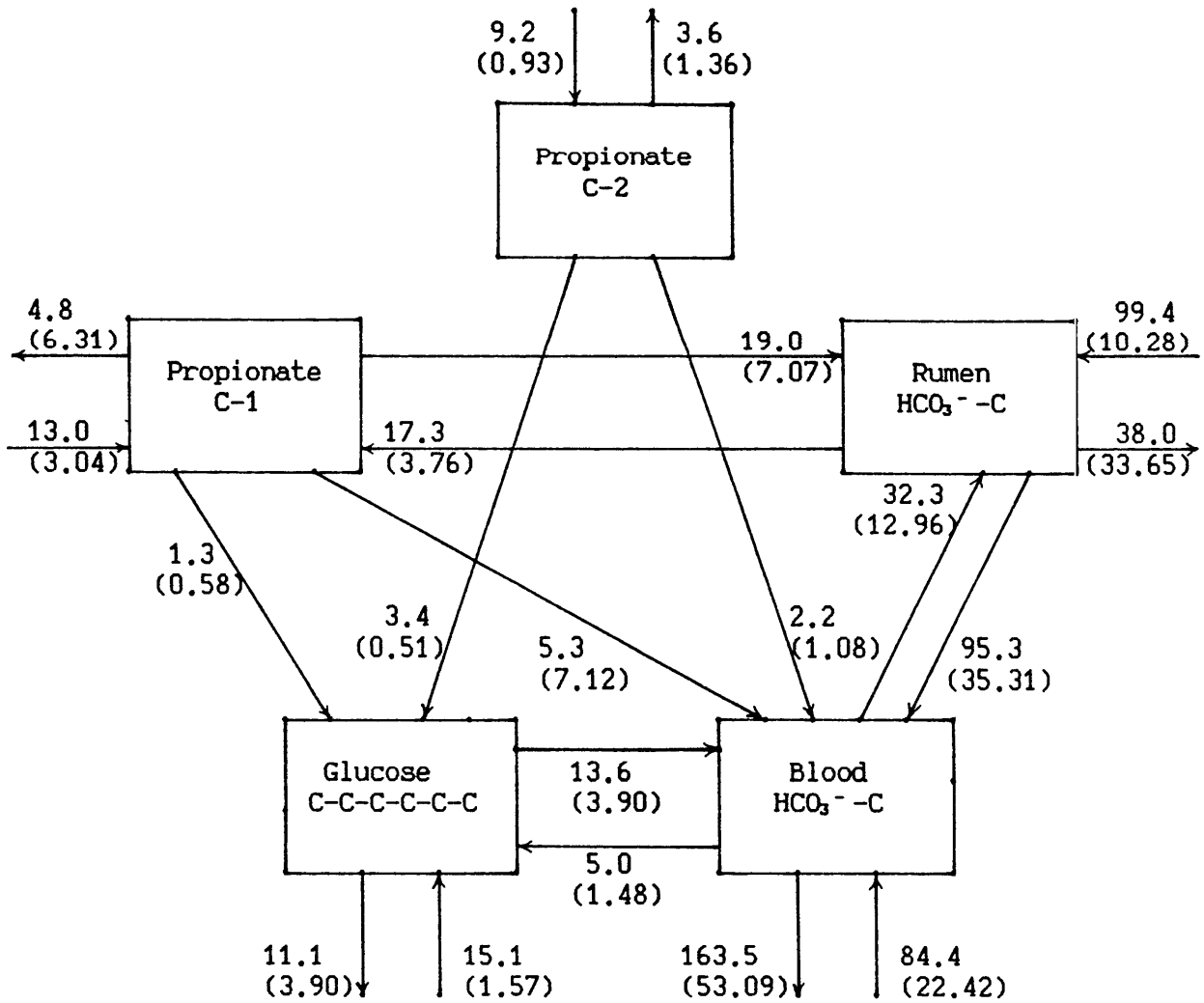
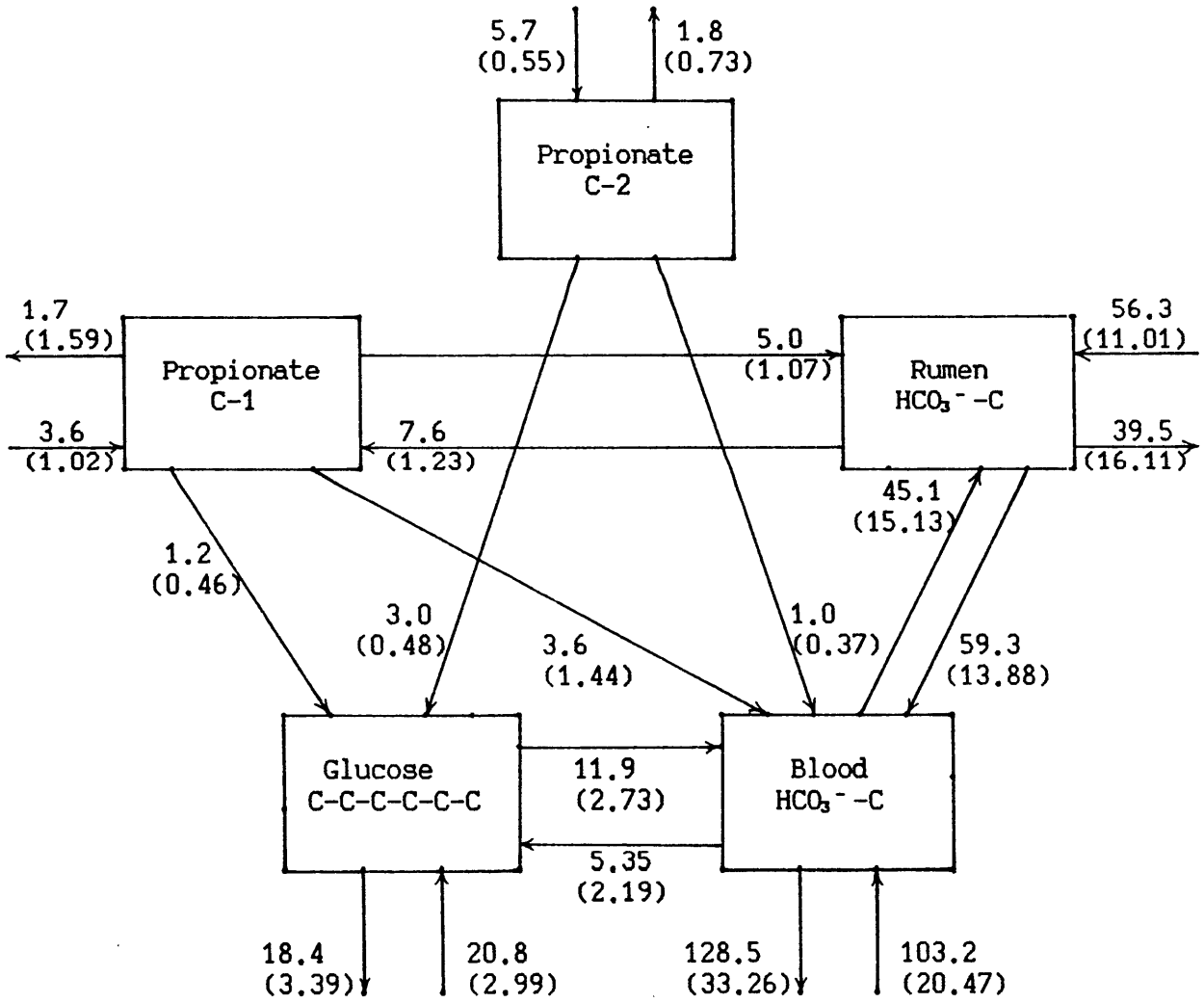


Figure 8-11

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



The relationship between the transfer quotient of the middle carbon of propionate to glucose (MPGTQ) and this value corrected for the indirect flow of tracer via the bicarbonate pools (CMPGTQ) (taken from the 5 pool model) is illustrated Figure 8-12.

The equation of the relationship is

$$CMPGTQ = 0.965(+/- .003)MPGTQ$$

$$(r^2=.999, RMS=0.75)$$

An estimate of the flow (corrected for the flow via the bicarbonate pools) from the carboxyl carbon of propionate to glucose is also required to solve the glucose ratio.

The relationship between the transfer quotient of propionate carboxyl carbon to glucose (CPGTQ) and this value corrected for the flow of tracer via the bicarbonate pools (CCPGTQ) (taken from the 5 pool models) is illustrated in Figure 8-13. The equation of the relationship is

$$CCPGTQ = 0.674(+/- .069)CPGTQ$$

$$(r^2=.979, RMS=.926)$$

These equations indicate that 3.5% ([2-^{14}C]propionate infused) and 32.6% ([1-^{14}C]propionate infused) of the tracer in glucose was there due to interaction with the bicarbonate pools.

These equations were used to correct the transfer quotients of propionate to glucose given in Tables 8-1,8-2 and 8-4. The rates of propionate irreversible loss are linearly related to the transfer quotients corrected for the indirect flow of tracer via the bicarbonate pools. The equations of the lines are

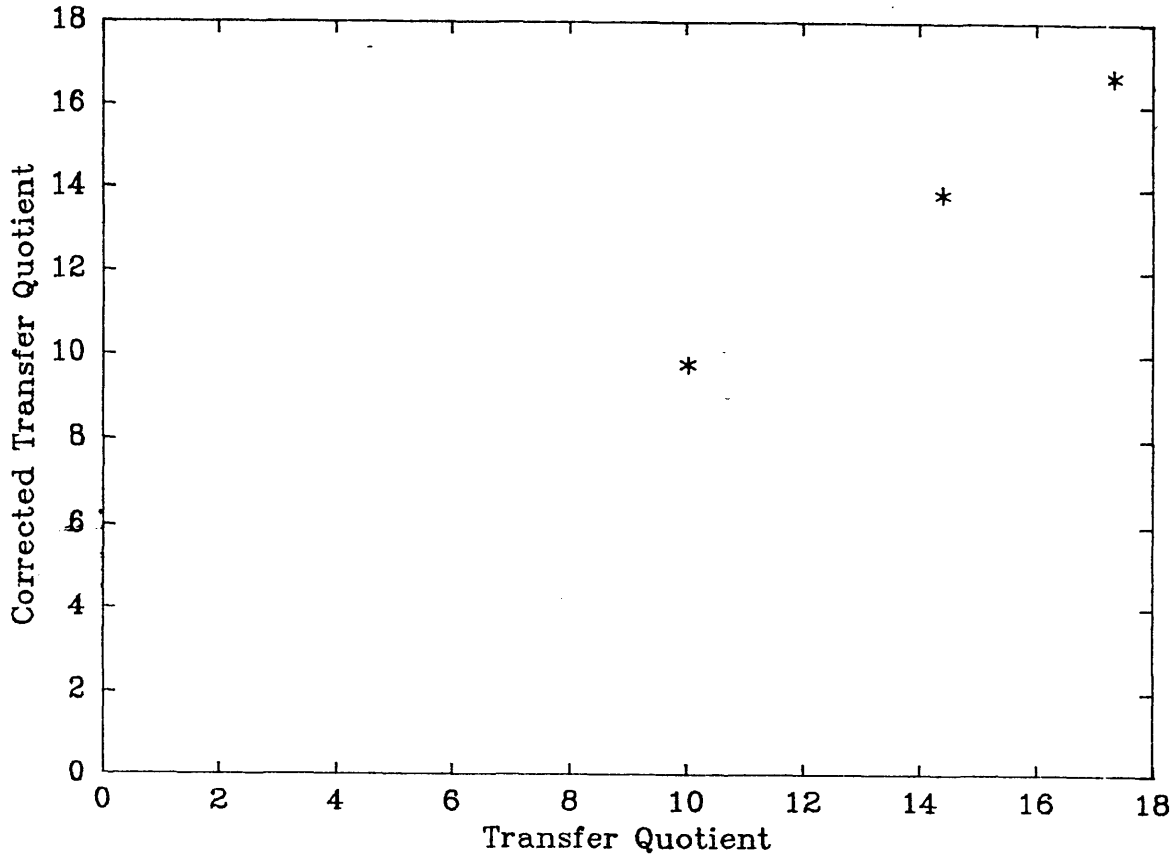


Figure 8-12

The relationship between the transfer quotient of the middle carbon of propionate to glucose, and the percentage of the glucose pool direct from the propionate middle carbon pool in the 5 pool models

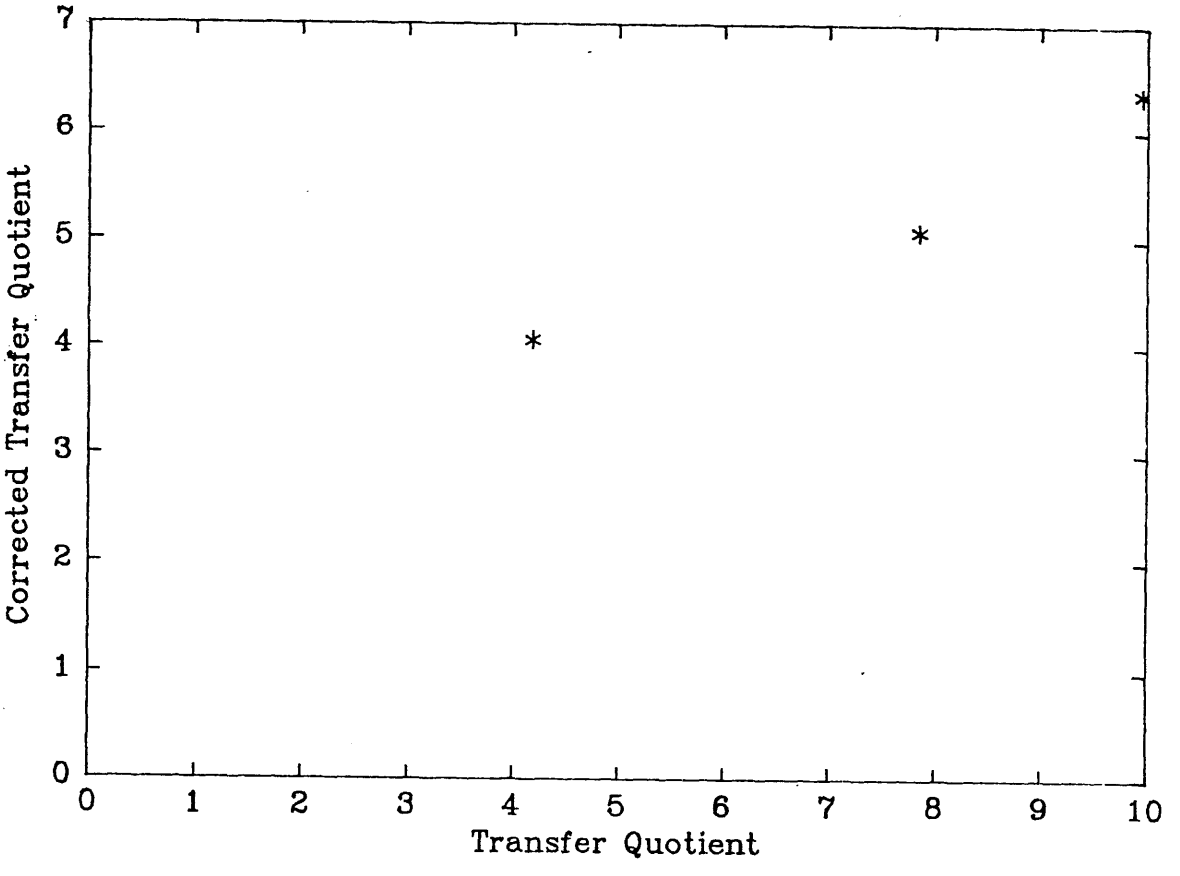


Figure 8-13

The relationship between the transfer quotient of the carboxyl carbon of propionate to glucose, and the percentage of the glucose pool direct from the propionate carboxyl carbon pool in the 5 pool models

$$CP2GTQ^{1} = 0.995(\pm .059)P2IL + 5.995(\pm 2.234)$$

$$(r^2=.929, RMS=4.620)$$

$$CP1GTQ^{1} = 0.131(\pm .023)P1IL + 4.454(\pm 1.597)$$

$$(r^2=.841, RMS=1.796)$$

The original reason for the above analysis was to obtain the appropriate proportions of the glucose pool provided by the carboxyl and middle carbons of propionate to solve the glucose ratio. Therefore, the transfer quotients of propionate to glucose (corrected for the indirect flow via the bicarbonate pools) were regressed against the rates of irreversible loss of propionate so that, at a specified irreversible loss, the transfer quotients of propionate to glucose for both [1-^{14}] and [2-^{14}C]propionate could be calculated. However, from the data obtained in this experiment, the relationship between the proportion of glucose provided by the carboxyl and middle carbons of propionate can be obtained directly without having to use an intermediate parameter. The relationship between the proportion of glucose provided by the middle carbon of propionate (CMPGTQ) and the proportion of glucose provided by the carboxyl carbon of propionate (CCPGTQ) (both corrected for the indirect flow of tracer via the

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[1] CP2GTQ = the transfer quotient of propionate to glucose corrected for the indirect flow of tracer via the bicarbonate pools using [2-^{14}C]propionate

P2IL = the apparent rate of propionate irreversible loss estimated using [2-^{14}C]propionate

CP1GTQ = the transfer quotient of propionate to glucose corrected for the indirect flow of tracer via the bicarbonate pools using [1-^{14}C]propionate

P1IL = the apparent rate of propionate irreversible loss estimated using [1-^{14}C]propionate

bicarbonate pools, i.e. values from the 5 pool models) (Figure 8-14) is

$$CCPGTQ = 0.325(\pm .055)CMPGTQ + 0.793(\pm .756)$$

$$(r^2=.999, RMS=.271)$$

This equation was used to estimate the propionate carboxyl carbon to glucose transfer quotients at various assumed values for the propionate middle carbon to glucose transfer quotients. These values were then used to solve the glucose ratio. The following parameters are presented in Table 8-12,

A. various proportions of the glucose pool provided by the middle carbon of propionate (corrected for the indirect flow via the bicarbonate pools, i.e. the figure from the 5 pool models)

B. the corresponding proportion of the glucose pool provided by the carboxyl carbon of propionate (calculated from the above equation)

C. NI (the rate of influx of 4 and 5 carbon compounds into the tricarboxylic acid cycle relative to the rate of condensation of acetyl-CoA with oxaloacetate, which is taken as unity) calculated by solving the glucose ratio using the values from A and B

D. the percentage of the molecules in the oxaloacetate pool that arose from cycling of the tricarboxylic acid cycle (calculated from the value of NI in C)

E. the percentage of glucose provided by propionate, i.e. the proportion of the glucose pool provided by the carboxyl carbon of propionate (from the 5 pool model) corrected for the loss of tracer due to equilibration about the symmetrical dicarboxylic acids and the

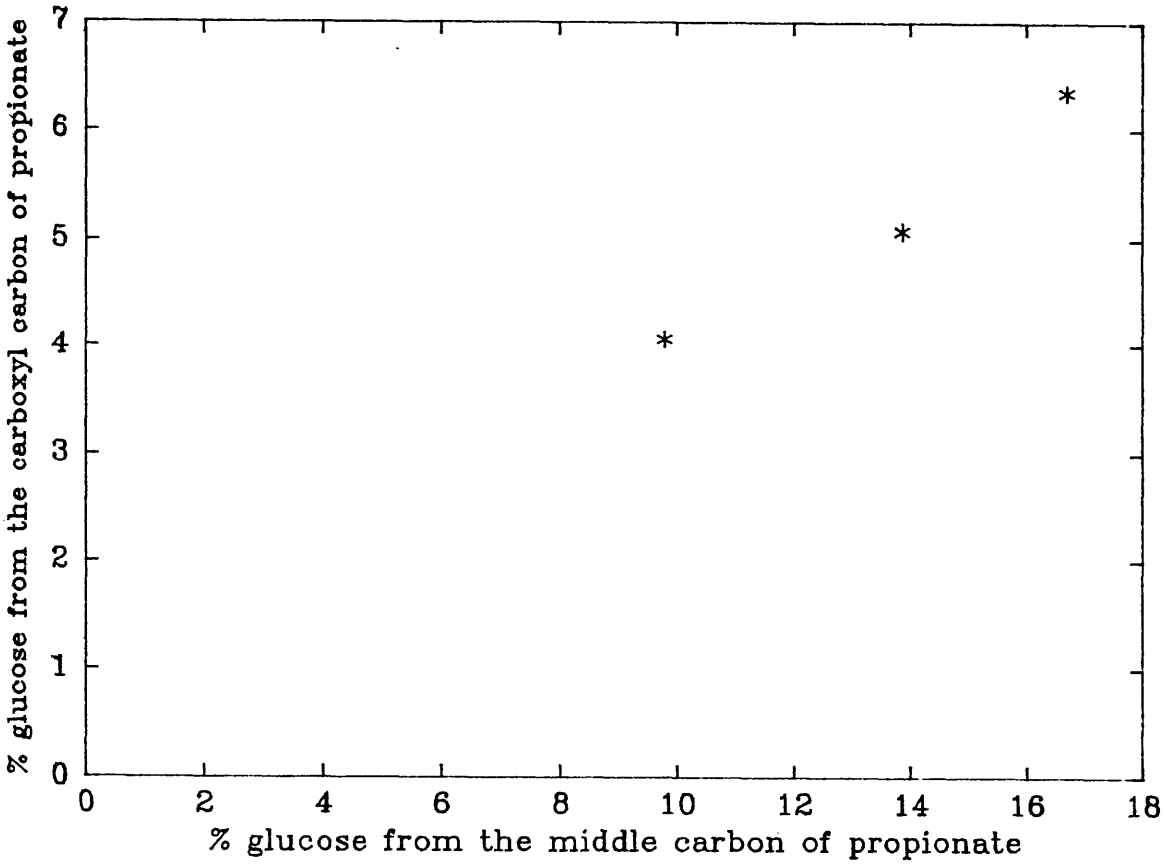

Figure 8-14

The relationship between the percentage of the glucose pool provided by the carboxyl and middle carbons of propionate (values taken from the 5 pool models)

Table 8-12

Calculation of the percentage of glucose provided by propionate

A	B	C	D	E
10	4	2.65	27	11
20	7.3	1.51	39	24
30	10.5	1.27	44	38
40	13.8	1.17	46	51
50	17.0	1.11	47	65
70	23.5	1.04	49	92

A = the percentage of the glucose pool provided directly fron the propionate middle carbon pool in the 5 pool models

B = the percentage of the glucose pool provided directly by the propionate carboxyl carbon pool calculated from A

C = NI (the rate of influx of 4 and 5 carbon unlabelled compounds into the tricarboxylic acid cycle relative to the rate of condensation of acetyl-CoA with oxaloacetate, which is defined as unity) calculated using A and B in the glucose ratio

D = the percentage of the oxaloacetate pool calculated from C

E = the percentage of the glucose pool provided by propionate calculated by correcting B for the effects of the decarboxylation reaction and metabolic crossover

subsequent decarboxylation reaction (multiply by 2) and the dilution caused by metabolic crossover (divide by 1 minus the percentage from cycling of the tricarboxylic acid cycle). Parameters D and E are graphed against A in Figure 8-15.

It was expected that as the transfer quotients of propionate to glucose increased, the flow from propionate to oxaloacetate would increase and the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle would decrease. The calculations suggest that the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle increased with increasing propionate to glucose transfer quotients. However, the calculated values for the percent of molecules in the oxaloacetate pool from cycling of the tricarboxylic cycle and the percent of glucose from propionate are very sensitive to changes in the regression equation relating the transfer quotients of the propionate carboxyl and middle carbons to glucose. With only 3 points in the regression equation relating the transfer quotients of the propionate carboxyl and middle carbons to glucose, this equation could be very different from the generalized relationship between them. Therefore, more points need to be added to the regression lines before much confidence can be put in the conclusions.

The above interpretation relies on the estimate of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle obtained from solving the glucose ratio. In the results presented in Chapter 7, the incorporation of the middle and carboxyl carbons of propionate into glucose (the glucose ratio) was not compatible with the entry of these carbons into the blood bicarbonate pools or the incorporation of CO_2 into glucose. For the

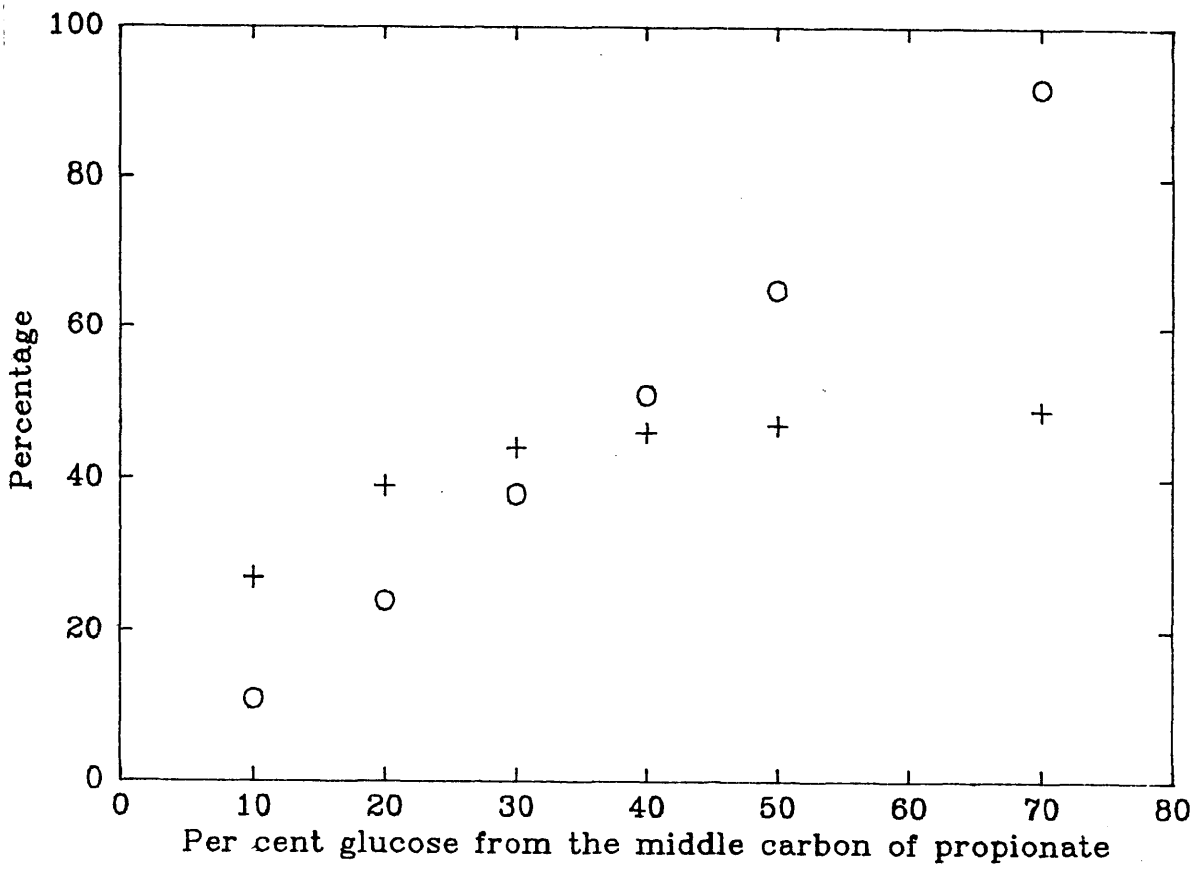

Figure 8-15

The percentage of oxaloacetate from cycling of the tricarboxylic cycle (+) and the percentage of glucose provided by propionate (O) (data presented in Table 8-12) plotted against the percentage of the glucose pool from the middle carbon of propionate (in the 5 pool models).

interpretation given above to be correct the estimate of the percentage of the molecules in the oxaloacetate pool must be correct. Therefore, the values given by the different methods of estimation were compared.

The estimates of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle obtained from solving the glucose and CO_2 ratios and the apparent percentage arising from pathways involving a carboxylation reaction are shown in Table 8-13.

As with the sheep fed oaten straw chaff, the incorporation of CO_2 into glucose is higher than that theoretically possible without postulating forms of tracer recycling. Therefore, some form of recycling must be occurring. However, unlike the situation with the oaten straw fed sheep, in this case the estimates of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle using the CO_2 ratio are higher than the estimates using the glucose ratio. Postulating recycling of tracer via pyruvate and/or exchange of CO_2 in the oxaloacetate to phosphoenolpyruvate reaction will not explain the results because this form of cycling will decrease the estimate of percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle given by the glucose ratio without affecting the estimate from the CO_2 ratio, thus increasing the difference between the two estimates.

Recycling via oxaloacetate, phosphoenolpyruvate, pyruvate, acetyl-CoA, citrate and back to oxaloacetate via the tricarboxylic acid cycle will not increase the incorporation of CO_2 into glucose and therefore, cannot be used alone to explain the results.

Table 8-13

The percentages of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle estimated from the CO_2 ratio, the glucose ratio and the incorporation of CO_2 into glucose (using the relevant data from the 5 pool models).

	CO_2 ratio %	Glucose ratio %	From CO_2 incorporated into glucose %
Sheep P	48	36	100
Sheep Q	59	34	121
Sheep R	43	29	106

Postulating that both forms of recycling occur simultaneously does not explain the results because according to the equation developed in the previous Chapter at no positive value of NI (therefore no positive value for the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle) are all the data internally consistant. Again, the problem appears to be that the incorporation of CO_2 into glucose is higher than expected from theory. Because any explanation that increases the incorporation of CO_2 into glucose will also affect the incorporation of tracer from other precursors of glucose it is probable that the CO_2 and glucose ratios also have to be adjusted.

Even though the errors associated with the results from each animal are probably large owing to the compounding effect of the extensive mathematical manipulation of the data, the same conclusions are drawn from the results of each animal. This allows confidence in the conclusions.

The results from this experiment support the conclusions drawn from the experiment presented in chapter 7. It appears that the present interpretation is not adequate to explain the data and that some unaccounted for factor is influencing the results.

CHAPTER 9

CONCLUSION

As discussed in Chapter 3, to obtain an estimate of the percentage of glucose being provided by propionate, the effects of interaction with the tricarboxylic acid cycle (i.e. metabolic crossover) have to be accounted for. However, accounting for metabolic crossover has proved more difficult than first thought.

One factor not appreciated at the start of the studies was the significant proportion of the tracer in the glucose pool, when propionate or acetate was infused intraruminally, that was there due to interaction with the bicarbonate pools. This necessitated the development of the 5 pool models to account for these indirect flows via the bicarbonate pools.

It is demonstrated that large errors in interpretation can be made if results obtained from infusions of specifically labelled compounds are extrapolated to the whole molecule. For example, the rates of irreversible loss of the carboxyl and middle carbons of propionate are different due to turnover of the carboxyl carbon of propionate with bicarbonate in the rumen. As a corollary of this, the information obtained using uniformly labelled compounds cannot necessarily be extrapolated to the individual atoms of that compound.

The differences between the incorporation of tracer from [1-^{14}C] and [2-^{14}C]propionate into blood glucose (after correction for the randomization of tracer about the carbons of the symmetrical dicarboxylic acids) could be explained by cycling of tracer in the tricarboxylic acid cycle (from the glucose ratio). However, the value of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle that explained the incorporation of tracer into glucose could not explain the entry of tracer from [1-^{14}C] and [2-^{14}C]propionate into the blood bicarbonate pool (the CO_2 ratio). Nor could it explain the incorporation of CO_2 into glucose.

The pathway of tracer recycling; oxaloacetate, phosphoenolpyruvate, pyruvate to oxaloacetate (and/or exchange reactions between oxaloacetate and CO_2) was postulated because this pathway increases the incorporation of CO_2 into glucose. In the sheep fed lucerne chaff, adding this form of recycling to the interpretation of the results, could not explain the incorporation of tracer from the middle and carboxyl carbons of propionate into glucose and their entry into blood bicarbonate simultaneously. However, in the sheep fed oaten straw chaff, this form of recycling could explain the incorporation of tracer from propionate into glucose and the entry into the blood bicarbonate pools simultaneously. But, the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle that explained the glucose and CO_2 ratios simultaneously would not explain the incorporation of CO_2 into glucose.

A further form of recycling (oxaloacetate, phosphoenolpyruvate, pyruvate, acetyl-CoA, citrate and back to oxaloacetate via the tricarboxylic acid cycle) was then incorporated into the interpretation of the results. However, even with this extra form of recycling all the results could not be explained simultaneously.

There appears to be at least one other form of recycling, that is not included in the theory used to explain the data, operative. The results fail to support the current concept of gluconeogenesis and are consistent with the suggestion that undiscovered enzyme reactions or compartmentation phenomena, or both, are operative. This is a similar conclusion to that of Mullhofer et al. (1977a,b) and Veneziale (1971,1972)

The unaccounted form or forms of recycling appear to increase the incorporation of CO_2 into glucose. Proposing a form of recycling that increases the CO_2 incorporation into glucose at the expense of the carboxyl carbons of oxaloacetate cannot be the complete answer, because this would cause a divergence of the estimates of the percentage of the molecules in the oxaloacetate pool from cycling of the tricarboxylic acid cycle given by the glucose and CO_2 ratios in the sheep given lucerne chaff. However, postulating a pathway that increased the CO_2 incorporated into glucose at the expense of the middle carbons of oxaloacetate could explain these results. It is probable that pathways affecting both the middle and carboxyl carbons of oxaloacetate are occurring simultaneously, with the effects on the carboxyl carbon being greater in the sheep fed oaten straw chaff and the effects on the middle carbon of oxaloacetate greater in the sheep fed lucerne chaff.

A possible clue as to how CO_2 enters the middle carbons of oxaloacetate is that the values of propionate irreversible loss and propionate to glucose transfer quotient using [3-^{14}C]propionate (data from Leng et al., 1967) lie below the regression relating the rate of propionate irreversible loss to the transfer quotient of propionate to glucose using [2-^{14}C]propionate (Figure 8.3). Could this be due to turnover with rumen bicarbonate? If so, the tracer from rumen bicarbonate that is incorporated into position 3 of propionate would be incorporated into glucose with a transfer quotient similar to that of [2-^{14}C]propionate. This possibility needs to be investigated.

Another avenue of research that might provide some insight into how the extra CO_2 is incorporated into glucose would be to assay the ^{14}C labelling patterns of cytosolic and mitochondrial metabolites separately. This may determine if there were unaccounted for mechanisms of getting material out of the mitochrondria which affect the pattern of tracer flow.

In the present interpretation it is assumed that the specific radioactivity of glucose accurately reflects the specific radioactivity of oxaloacetate. This assumption could be a source of error if the participation of glucose in other pathways of metabolism (e.g. the pentose phosphste pathway) affects the relationship between the specific radioactivities of glucose and oxaloacetate. This possibility needs to be investigated.

It has been demonstrated that the present interpretation is not adequate to allow the percentage of the molecules in the oxaloacetate pool arising from cycling of the tricarboxylic acid cycle to be calculated. Therefore, it has not been possible to calculate the effects of metabolic crossover and thus, the extent by which the

propionate to glucose transfer quotient underestimates the true net contribution of propionate to glucose. More basic research is needed to identify all forms of tracer recycling and to quantify their effects on tracer flows. Until this has been achieved, it will not be possible to convert flows calculated from the transfer of tracer into true net flows. As it is not possible, at this stage, to quantify the contribution of propionate to glucose it is not possible to quantify what has to be provided by other precursors.

CHAPTER 10

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