SECTION 3

EFFECT OF DIET ON GLUCOSE SYNTHESIS

In ruminants, gluconeogenesis is a major biosynthetic process, since only in animals given a high proportion of starch in their diets could there be significant absorption of glucose from the alimentary tract (see literature survey). Estimates of the irreversible loss of glucose in fed sheep have varied from 1.2 to 4.3 mg/min/kg (Ford, 1965; Bergman et al., 1966). The variations in estimates may be in part attributed to differences in the feed or in the feeding regimen adopted in the various laboratories. For instance, in many studies animals were fed once or twice daily and the irreversible loss of glucose measured some time after the animal had eaten, this usually coincided with maximum VFA concentrations in the rumen. Ford (1965) has shown that the irreversible loss of glucose in sheep varied with the quantity and quality of food eaten and he suggested that there was an increase in gluconeogenesis as feed intake increased.

The experiments to be presented in this section were undertaken to explore some of these effects of diet on the irreversible loss of glucose and its synthesis from ruminal propionate.

PART A: THE CONTRIBUTION OF PROPIONATE TO GLUCOSE SYNTHESIS IN SHEEP GIVEN DIETS OF DIFFERENT GRAIN CONTENT

Introduction

Ruminal propionate has generally been considered the major substrate for gluconeogenesis in the fed animal. Leng et al. (1967) reported that in sheep given lucerne at hourly intervals, propionate produced in the rumen contributed 54% of the carbon of the glucose synthesized, whereas it was shown by Bergman et al. (1966) that absorbed propionate contributed 27% of the irreversible loss of glucose. As only a relatively small proportion of the propionate produced in the rumen was apparently converted into glucose, it appeared that on the diets of lucerne there are more glucose precursors absorbed from the alimentary tract than are required (Bergman et al., 1966; Leng et al., 1967). Because diet has a great effect on production of glucose precursors in the rumen and small intestine of sheep (i.e. propionate and amino acids) and since glucose may be absorbed as such on diets high in grain, the effects of the proportion of starch to roughage in a diet on the irreversible loss of glucose and the contribution of propionate to glucose has been investigated.

Experimental

Eighteen wethers were each fitted with a rumen cannula.

Groups of six sheep were given one of the rations shown in Table 3-1, and which maintained their weight over the experimental period.

Table 3-1. Composition, gross energy and crude protein content of rations

Ration identification	Ration (g/day) Maize	Lucerne chaff	Gross energy* (kcal/day)	Crude protein** (g/day)
A	400	-	1564	36
B	260	250	1977	74
C	200	400	2318	99

* These diets provided approximately similar digestible energy intakes (Morrison, 1957).

** Nitrogen x 6.25.

For the 6 days preceding administration of tracers, the sheep were given their ration in twelve equal amounts at hourly intervals from 07.00 h to 18.00 h. On the 5th day of this regimen the animals were infused intraruminally with [2-^{14}C]-propionate and on the 6th day [U-^{14}C]glucose infusions were made intravenously. Blood samples taken before infusion on the 6th day indicated negligible residual radioactivity in circulating glucose. For each tracer infused, only four animals were used from each group.

Constant infusions of [2-^{14}C]propionate (78 to 82 nCi/min) and [U-^{14}C]glucose (50 to 90 nCi/min) were commenced at 08.00 h and 11.00 h respectively and samples of blood and ruminal fluid were taken for analysis at 20 to 30 min intervals between 14.00 h and 17.00 h when the VFA concentrations in the rumen were approximately constant (Leng and Leonard, 1965a).

The proportion of plasma glucose synthesized from ruminal propionate was estimated by comparing the plateau SR values of glucose carbon and propionate carbon obtained with infusions of [2-^{14}C]propionate (Annison et al., 1963a; Leng et al., 1967). Conversion rate of propionate into glucose was calculated as described by Leng et al. (1967), assuming 2 moles propionate are required for the synthesis of 1 mole glucose.

Results

Irreversible Loss of Glucose

Estimates of the irreversible loss of glucose are shown in Table 3-2. Plasma glucose concentrations remained approximately constant throughout the experimental period and the glucose SR reached a plateau between 3 and 6 h after the start of each infusion. The variations in concentrations and SR were usually greater than those reported for sheep given roughage (Leng et al., 1967). The mean value of the irreversible losses of glucose (excluding two results where animals did not eat) were

Table 3-2. Irreversible loss of plasma glucose in sheep given different rations (see Table 3-1)

Mean values and standard errors for four to seven samples taken at 25 to 35 min intervals between the 3rd and 6th hour of an intravenous infusion at [U-^{14}C]glucose.

Sheep no.	Ration	Plasma glucose (mg/100 ml)	Plateau specific radioactivity (μCi/g glucose)	Irreversible loss of glucose (mg/min)	(mmol/min)	(mg/Kg$^{.75}$/min)
167	A	81 ± 4	24.0 ± 1.0	41.7	0.23	3.2
89*	A	69 ± 1	27.4 ± 0.6	36.5	0.20	3.1
329	A	90 ± 5	14.2 ± 1.0	70.4	0.39	4.8
34	A	74 ± 2	20.3 ± 1.0	49.3	0.27	4.3
37	B	90 ± 5	14.4 ± 0.8	69.4	0.39	5.1
68	B	59 ± 1	17.2 ± 1.0	58.1	0.32	3.9
174	B	87 ± 2	13.3 ± 0.7	75.2	0.42	6.4
329	B	70 ± 1	17.6 ± 0.9	56.8	0.32	3.7
6*	C	63 ± 3	24.4 ± 1.7	41.0	0.23	3.1
147	C	72 ± 1	20.3 ± 0.7	49.3	0.27	3.5
123	C	71 ± 4	16.4 ± 1.4	61.0	0.34	5.1
182	C	61 ± 2	15.1 ± 0.7	66.2	0.37	5.2

* Animal did not feed during experimental period.

not significantly different ($P > 0.05$) on the three rations.

Effects of Diet on the Concentrations and Molar Proportions of VFA in the Rumen

VFA concentrations in ruminal fluid were significantly greater ($P < 0.05$) in the animals consuming the greatest quantity of lucerne (Table 3-3); as the amount of starch in the ration increased the total VFA concentration decreased, but the proportion of propionate increased. As a result, the actual concentration of propionate was not markedly altered ($P > 0.05$).

Propionate Production Rates in the Rumen and the Conversion of Propionate into Glucose

Leng and Leonard (1965a) have shown with sheep given portions of their daily ration at hourly intervals that an approximately constant SR of propionate in ruminal fluid was obtained about 3 h after the start of an intraruminal infusion of labelled propionate. In this study a plateau SR of propionate was maintained between 6 and 9 h after the start of a [2-^{14}C]-propionate infusion. The mean production rates of propionate on the three rations (Table 3-4) were similar ($P > 0.05$).

Changes in glucose SR with time after the start of an infusion of [2-^{14}C]propionate were as described by Leng *et al.* (1967), except that the time taken for the glucose SR to plateau varied from 6 to 8 h.

Table 3-3. Total concentrations and molar percentages of volatile fatty acids (VFA) in ruminal fluid of sheep given different rations (see Table 3-1)

Mean values and standard errors for seven samples taken from 6 to 9 h after the start of a [2-^{14}C]propionate infusion.

Sheep no.	Ration	Total VFA (mmol/l)	Molar percentage of VFA as: Acetic	Propionic	Butyric	Isobutyric	Isovaleric	Valeric
167	A	69 ± 2	-	-	-	-	-	-
89	A	70 ± 2	52.5 ± 0.5	39.8 ± 1.0	3.9 ± .2	1.5 ± .2	1.4 ± .2	0.9 ± .1
329	A	63 ± 2	49.2 ± 0.9	38.8 ± 1.1	9.1 ± .4	1.0 ± .2	1.1 ± .0	0.7 ± .0
34	A	64 ± 2	52.2 ± 0.9	39.5 ± 0.6	5.0 ± .3	1.3 ± .2	0.7 ± .1	1.2 ± .2
37	B	71 ± 2	63.4 ± 0.3	19.5 ± 0.2	12.4 ± .3	1.5 ± .1	1.5 ± .1	1.7 ± .1
68	B	62 ± 3	61.6 ± 0.6	18.6 ± 0.5	13.8 ± .3	1.6 ± .1	2.5 ± .1	1.9 ± .1
16	B	87 ± 2	63.6 ± 1.1	21.6 ± 0.9	11.4 ± .2	1.3 ± .2	0.8 ± .1	1.4 ± .1
174	B	83 ± 10	61.6 ± 0.7	24.8 ± 0.6	10.3 ± .2	1.0 ± .3	1.1 ± .0	1.2 ± .0
6	C	109 ± 4	65.2 ± 0.8	15.8 ± 0.6	13.6 ± .5	1.3 ± .1	2.2 ± .1	2.0 ± .3
147	C	129 ± 3	61.5 ± 0.7	21.9 ± 0.3	11.4 ± .3	1.3 ± .2	1.8 ± .1	2.1 ± .2
119	C	93 ± 8	66.0 ± 0.6	22.0 ± 0.5	19.3 ± .3	1.2 ± .1	0.6 ± .2	0.9 ± .3
27	C	107 ± 6	61.5 ± 1.1	21.3 ± 0.6	13.2 ± .7	1.0 ± .2	1.3 ± .0	1.9 ± .1

Table 3-4. Production rate of propionate in the rumen of sheep given different rations (see Table 3-1) and the percentage contributed by propionate to glucose synthesis

Mean values and standard errors for four to seven samples taken at 25 to 35 min intervals between the 6th and 9th hour of an intraruminal infusion of [2-^{14}C]propionate.

Sheep no.	Ration	Propionate (mmol/l ruminal fluid)	Plasma glucose (mg/100 ml)	Plateau specific radioactivity (μCi/g C) Rumen propionate	Plasma glucose	Production rate of propionate (mmol/min)	Glucose synthesized from propionate (%)	Conversion of propionate into glucose** (mmol/min)	Propionate produced converted into glucose (%)
167	A	-	59 ± 4	35.6 ± 1.8	14.4 ± 1.4	0.78	40	0.24	31
89	A	28	59 ± 3	36.5 ± 2.7	9.8 ± 0.3	0.76	27	0.16	21
329	A	24	84 ± 2	37.5 ± 1.5	12.5 ± 0.5	0.74	33	0.20	27
34	A	25	79 ± 1	34.6 ± 1.7	14.5 ± 0.3	0.80	42	0.25	31
Mean		26				0.77	36	0.21	28
37	B	14	75 ± 5	31.9 ± 0.5	9.7 ± 0.8	0.87	30	0.22	25
68	B	12	69 ± 3	35.6 ± 1.7	13.9 ± 0.5	0.78	39	0.28	36
16*	B	19	65 ± 2	49.6 ± 2.7	16.5 ± 0.9	0.56	33	0.24	43
174	B	21	97 ± 6	35.1 ± 1.7	18.2 ± 1.3	0.79	52	0.37	47
Mean		17				0.75	39	0.28	38
6	C	17	69 ± 3	27.2 ± 3.8	12.4 ± 0.5	1.02	46	0.30	29
147	C	28	73 ± 3	29.8 ± 2.3	15.9 ± 0.8	0.93	53	0.35	38
119*	C	20	76 ± 6	44.0 ± 2.3	26.0 ± 2.9	0.63	59	0.39	62
27*	C	23	76 ± 4	33.8 ± 1.1	18.9 ± 0.7	0.82	56	0.37	45
Mean		22				0.85	54	0.35	44

* These sheep were not used for the determination of the irreversible loss of glucose (Table 3-2). Liveweight of sheep were: 16, 22.5 kg; 119, 21.5 kg; 27, 24.5 kg.

** Calculated from the mean values of irreversible loss of glucose found for the sheep when receiving the same ration on the next day, except that the mean results for irreversible loss of glucose in sheep not feeding have been excluded.

The percentage of the glucose entering the sampled pool that was derived from propionate, the net conversion rate of propionate into glucose and the percentage of the propionate produced in the rumen which was converted into glucose increased as the proportion of lucerne in the ration increased. This increase was significant ($P < 0.05$) between rations A and C for the percentage of the glucose derived from propionate and the net conversion rate of propionate into glucose.

Discussion

The glucose required by sheep to meet the demands for biosynthetic processes and for oxidation in specific organs cannot be estimated by the techniques used here since in sheep the irreversible loss of glucose and requirements for glucose are not necessarily the same. However, the glucose requirements of sheep may approximate the quantity synthesised on all-roughage diet where little glucose is absorbed from the alimentary tract. The need for gluconeogenesis in sheep is variable and depends on (a) the absorption of glucose from the intestinal tract and (b) on the requirements of the animal for glucose. Absorption of glucose from the intestinal tract in substantial quantities may reduce the necessity for gluconeogenesis, since the production of glucose from short chain intermediates in excess of requirements is an energy-requiring process. For instance, 4 moles ATP are

required to synthesize 1 mole glucose from propionate. In pregnancy and lactation, when the requirements for glucose are probably increased, the animal has to increase the synthesis of glucose from short chain intermediates. The sheep has to adapt itself to physiological circumstances, indicating that there must be a controlling mechanism regulating the rate of gluconeogenesis (Krebs, 1964a). In normal sheep given roughage diets there seems to be little reason to suppose that there will be a shortage of glucose precursors, since considerably more propionate is produced in the rumen than is apparently required for glucose production (see Table 1-5). However, Ford (1965) found that the irreversible loss of glucose in sheep was two to three times greater on freshly cut spring grass than on a diet of hay and oats. This was attributed to a higher intake of protein on the spring grass diet; it is not known how much glucose is absorbed from the intestines of sheep given these diets. The apparent increase in glucose synthesis at high levels of intake, especially where animals are fattening, may be due in part to the demand for glycerol for triglyceride synthesis. However, it has not been determined whether all glycerol synthesis occurs from glucose or whether some glycerol is synthesized directly from propionate. Adipose tissue synthesizes glyceride glycerol from

glucose since there is apparently little or no glycerol kinase present in this tissue (Vaughan, 1961; Khachadurian, Kamelian and Adrouni, 1967). However, the quantitative requirements for glycerol on these diets are not known.

The rate of fat deposition will depend on the ration given and also on the previous nutritional history of the animal. A sheep changing from a low to a high plane of nutrition may require more glucose as there may be an increased requirement for glycerol for fat deposition and of glucose for the synthesis of compounds such as glycolipids, nucleic acids and mucopolysaccharides. When studying glucose synthesis in animals a steady rate of glucose synthesis is desirable throughout an experimental period and is conveniently achieved by continuous feeding since, in animals fed once daily, glucose entry rates may be continuously changing.

Mean values of irreversible loss of glucose on all rations (excluding results where animals were not eating) were not significantly different, but the variation within each group was much greater than the variation of other results published from this laboratory (Leng et al., 1967).

The concentrations of VFA and their molar proportions were similar between sheep on the same ration. The proportion of propionate was highest on the high-grain diets but the actual concentrations of propionate and the production rate of propionate

in the rumen were similar in all sheep, demonstrating that the molar proportions of the acids in the rumen are not necessarily indicative of relative production rates of these acids.

Significant trends in the percentage of the glucose produced from propionate and the percentage of the propionate converted into glucose indicate that on the high starch diets less propionate was converted to glucose although the same amount of propionate was produced. The protein content of the diet was less with maize than with the maize-lucerne diet although this need not reflect the difference in the quantity of amino acids absorbed from the intestinal tract. When diets contain a large amount of readily fermentable carbohydrate, conditions for the reutilization of endogenous urea by ruminal organisms would be most favourable (Schmidt-Nielsen and Osaki, 1958; Houpt, 1959; Cocimano and Leng, 1967) and it has been reported that fermentation giving a high proportion of propionate may be associated with increased synthesis of microbial protein (Ishaque, Thomas and Rook, 1971). However, any differences in protein availability between diets was probably not great since the lower concentration of VFA in ruminal fluid on high-starch diets suggests that the production rate of total VFA was lower (see Leng, 1970b) and hence the quantity of energy available for microbial synthesis (Walker, 1965) was probably less than on the high-lucerne low-maize diet.

A possible explanation for the reduction in the synthesis rate of glucose from propionate could be that some of the dietary starch entered the small intestine and was absorbed as glucose (see literature survey). Ørskov et al. (1969) suggested that the horny portion in the endosperm of raw maize (see Kerr, 1950) might reduce the breakdown of starch by ruminal organisms. Significant quantities of starch may also have entered the lower alimentary tract as storage polysaccharides of bacteria (Walker and Nadar, 1970) or as starch grains ingested by protozoa (Hungate, 1963). Increased concentrations of ciliate protozoa are associated with increased frequency of feeding (Moir and Somers, 1956; Putnam, Gutierrez and Davis, 1961).

An uptake of glucose from the alimentary tract in the present studies could have reduced gluconeogenesis from propionate and amino acids. The quantities of starch escaping fermentation could vary considerably between animals and this may be the reason for the variable rates of irreversible loss of glucose reported here.

It is now generally agreed that infusions of glucose to non-ruminants suppress endogenous production of glucose (Steele, Bishop, Dunn, Altszuler, Rathgeb and de Bodo, 1965; Steele, 1966; Hentenyi and Wrenshall, 1968). In starved sheep, Annison and White (1961) and West and Passey (1967) have shown that an

exogenous glucose load decreases the endogenous irreversible loss of glucose. It is possible, in these studies and the studies presented here, that the endogenous glucose output by the liver was decreased and the quantity of glucogen stored increased. However, the glycogen reserves could not be expected to increase markedly.

PART B: THE CONTRIBUTION OF PROPIONATE TO GLUCOSE SYNTHESIS IN SHEEP GIVEN ROUGHAGE DIETS OF DIFFERENT PROTEIN AND ENERGY CONTENT

Introduction

It was shown in the previous study that the irreversible loss of glucose was similar for sheep given diets containing different proportions of maize and lucerne. These findings agree with those of Ulyatt et al. (1970), who failed to detect any significant changes in the irreversible loss of glucose in sheep given barley, dried grass or hay. However, it is apparent from the studies presented in Part A, that diet may have a marked effect on the relative contribution of different substrates to glucose synthesis as indicated by the decrease in the synthesis rate of glucose from ruminal propionate as the starch content of the diet was increased.

Ford (1965) obtained substantial increases in the irreversible loss of glucose where rations of high protein content were fed to sheep and he suggested that the extra protein might have been the source of the increased amounts of glucose synthesized. In further studies, Ford and Reilly (1969) and Reilly and Ford (1971) were able to demonstrate a positive correlation between protein intake and the irreversible losses of plasma glucose and of amino acids by varying the quantity and quality of roughage fed to sheep.

The experiments reported here were undertaken to examine some effects of roughage diets of different protein and energy content on the irreversible loss of plasma glucose and the contribution of ruminal propionate to glucose synthesis.

Experimental

Ewes and wethers were used and they were usually fitted with rumen cannulas. Constituents of rations offered to sheep are shown in Table 3-5. For the 6 days before administration of tracers sheep were given their daily ration in 24 equal portions at hourly intervals. Intravenous infusions of [U-^{14}C]glucose (50 to 70 nCi/min) and intraruminal infusions of [2-^{14}C]propionate (50 to 80 nCi/min) were made on the 5th or 6th day of this feeding regimen. Blood and ruminal fluid were sampled at frequent intervals after the start of infusions of these tracers as described in Part A.

Table 3-5. Constituents of rations given to sheep

Constituents* (%)	Ration identification								
	A	B	C	D	E	F	G	H	I
Wheaten straw	33	25	-	-	-	-	-	-	-
Wheaten chaff	67	75	100	85	75	54	39	23	-
Lucerne chaff	-	-	-	15	25	46	61	77	100

* Percentages of individual constituents present on air-dry basis.

Results

Irreversible Loss of Glucose

Plasma glucose concentrations were approximately constant during the infusion of tracers and similar between sheep (see Tables 3-6 and 3-8). The change in the SR of plasma glucose with time after the start of a constant infusion of [U-^{14}C]glucose was similar to that obtained previously (Part A) and the plateau SR was maintained for intervals up to 7 h.

The irreversible loss of glucose increased linearly ($P < 0.01$) with the apparent digestible energy intake of sheep given roughage rations I or E (see Figure 3-1). An increase in intake of apparently digestible energy was usually associated with an increase in intake of digestible protein. The relationships between the irreversible loss of glucose (Y, mg/min) and digestible energy (X_E kcal/day) and digestible protein intake (X_P g/day), were:

$$Y = 16.8 + 0.0218\ X_E \quad [RSD = \pm 4.78,\ r = 0.930] \quad \ldots\ 3\text{-}1$$

$$Y = 32.2 + 0.262\ X_P \quad [RSD = \pm 9.44,\ r = 0.688] \quad \ldots\ 3\text{-}2$$

where, RSD = residual standard deviation, and

r = correlation coefficient.

An effect of quality of diet on glucose synthesis is also indicated by the positive relationship ($P < 0.05$, $r = 0.846$)

Figure 3-1. Relationship between the irreversible loss of glucose and the apparently digestible energy intake. Sheep were given either 250, 600, 800 or 1,000 g lucerne chaff daily. Two further sheep were given 200 g wheaten chaff and 600 g lucerne chaff daily.

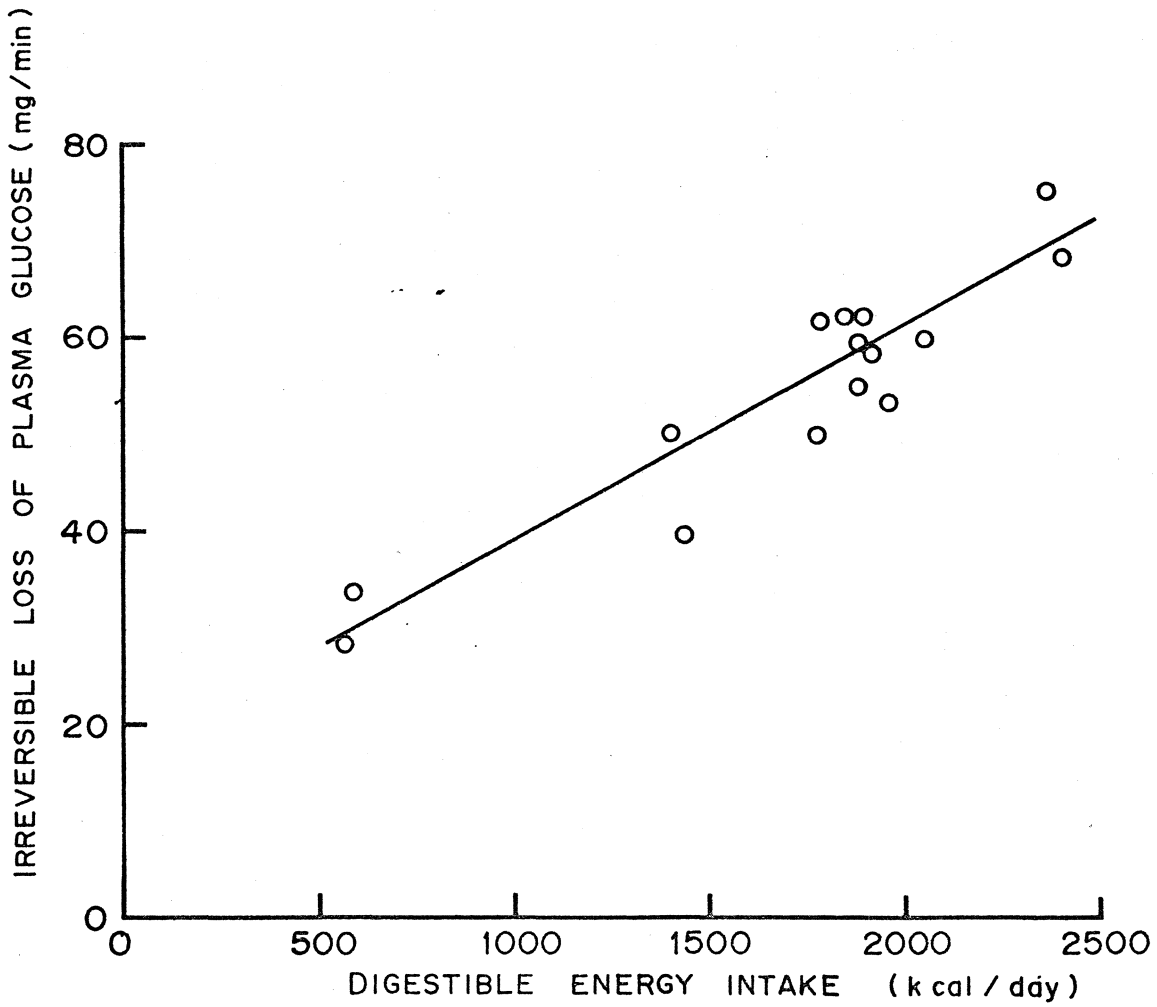

IRREVERSIBLE LOSS OF PLASMA GLUCOSE (mg/min)
80
60
40
20
0
0
500
1000
1500
2000
2500
DIGESTIBLE ENERGY INTAKE (k cal / day)

Table 3-6. Irreversible loss of plasma glucose in sheep given different roughage rations (see Table 3-5) of 800 g daily

Mean values and standard errors for four to seven samples taken at 25 to 35 min intervals from 3 to 6 h after the start of an intravenous infusion of [U-^{14}C]glucose.

Sheep no.	Ration	Protein intake (g/day)	Plasma glucose (mg/100 ml)	Plateau specific radioactivity (μCi/g glucose)	Irreversible loss of glucose (mg/min)	(mmol/min)	(mg/Kg$^{.75}$/min)
122*	A	33	59 ± 3	19.6 ± 0.6	51.0	.28	4.5
125	B	48	58 ± 3	20.5 ± 0.5	48.8	.27	4.2
122	C	62	70 ± 1	18.8 ± 0.5	53.2	.30	4.2
179	E	68	63 ± 1	17.1 ± 0.3	58.5	.32	4.2
46	E	68	61 ± 1	16.3 ± 0.1	61.3	.34	4.6
125	D	77	66 ± 1	16.6 ± 0.4	60.2	.33	5.0
81	F	110	60 ± 1	15.3 ± 0.3	65.4	.36	4.8
181	G	119	66 ± 2	16.5 ± 1.2	60.6	.34	4.5
162	H	123	61 ± 3	12.5 ± 0.0	80.0	.44	6.2
162	I	138	66 ± 0	16.8 ± 0.3	59.5	.33	4.4
181	I	181	60 ± 4	11.4 ± 0.1	87.7	.49	6.5

* Sheep given 600 g ration A daily.

between the irreversible loss of glucose and protein intake for sheep given similar quantities of roughage (Table 3-6). The contribution of ruminal propionate to glucose synthesis was also estimated with sheep given these diets.

Effect of Diet on the Concentration and Molar Proportions of VFA in Ruminal Fluid

The total concentration of VFA in ruminal fluid which was approximately constant during infusions of [2-^{14}C]propionate was positively correlated ($P < 0.05$, $r = 0.641$) with protein intake (Table 3-7). The irreversible loss of plasma glucose also increased with total VFA concentration but this relationship was not significant ($P > 0.05$, $r = 0.444$). The molar percentages of the individual VFA were similar between sheep (Table 3-7).

Propionate Production Rate and the Conversion of Propionate into Glucose

The SR of ruminal propionate was approximately constant between 6 and 9 h and glucose SR plateaued from 6 to 8 h after the start of a constant infusion of [2-^{14}C]propionate. Protein intake was not significantly correlated ($P > 0.05$) with either the percentage of glucose derived from propionate ($r = 0.011$) or the percentage of propionate produced in the rumen that was converted into glucose ($r = 0.536$) (Table 3-8).

Table 3-7. Total concentrations and molar percentages of volatile fatty acids (VFA) in ruminal fluid of sheep given different roughage diets (see Table 3-5) 800 g daily

Mean values and standard errors for seven samples taken from 6 to 9 h after the start of a [2-^{14}C]propionate infusion.

Sheep no.	Ration	Total VFA (mmol/l)	Molar percentage of VFA as: Acetic	Propionic	Butyric	Isobutyric	Isovaleric	Valeric
122*	A	63 ± 1	72.3 ± 2.6	17.1 ± 1.5	7.9 ± .9	0.4 ± .0	0.9 ± .1	1.4 ± .1
125	B	63 ± 2	69.0 ± 0.8	18.3 ± 0.5	9.6 ± .4	0.8 ± .1	1.5 ± .2	0.9 ± .1
122	C	80 ± 4	67.4 ± 0.8	20.8 ± 0.5	9.6 ± .3	0.5 ± .1	0.6 ± .0	1.1 ± .1
179	E	114 ± 6	67.4 ± 0.6	20.7 ± 0.4	9.1 ± .4	0.9 ± .1	0.5 ± .1	1.3 ± .1
46	E	70 ± 1	64.1 ± 0.6	20.5 ± 0.3	11.7 ± .3	1.2 ± .1	1.2 ± .1	1.5 ± .2
125	D	69 ± 4	67.8 ± 0.8	18.2 ± 0.4	11.3 ± .4	0.5 ± .1	0.8 ± .1	1.4 ± .0
81	F	103 ± 6	70.7 ± 1.0	16.6 ± 0.6	9.6 ± .3	0.6 ± .1	1.0 ± .1	1.5 ± .1
181	G	90 ± 3	70.1 ± 0.8	16.5 ± 0.7	9.4 ± .3	0.6 ± .1	1.6 ± .0	1.8 ± .1
162	H	103 ± 3	68.1 ± 1.3	20.9 ± 1.0	7.5 ± .3	0.5 ± .2	1.5 ± .1	1.5 ± .1
162	I	122 ± 3	67.9 ± 0.6	22.7 ± 0.4	4.9 ± .4	1.5 ± .1	1.5 ± .1	1.5 ± .1
181	I	97 ± 4	71.9 ± 1.1	18.3 ± 0.6	7.9 ± .4	0.8 ± .1	1.2 ± .1	1.9 ± .1

* Sheep given 600 g ration A daily.

Table 3-8. Production rate of propionate in the rumen of sheep given different roughage rations (see Table 3-5) and the percentage contributed by propionate to glucose synthesis

Mean values and standard errors for four to seven samples taken at 25 to 35 min intervals between the 6th and 9th hour of an intraruminal infusion of [2-^{14}C]propionate.

Sheep no.	Ration	Propionate (mmol/l ruminal fluid)	Plasma glucose (mg/100 ml)	Plateau specific radioactivity (μCi/g C) Rumen propionate	Plasma glucose	Production rate of propionate (mmol/min)	Glucose synthesized from propionate (%)	Conversion of propionate into glucose (mmol/min)	Propionate produced converted into glucose (%)
122*	A	11	59 ± 2	57.1 ± 1.36	28.2 ± 1.02	.49	49	.27	55
125	B	12	49 ± 1	51.7 ± 2.93	24.0 ± 0.33	.54	46	.25	46
122	C	17	70 ± 1	44.2 ± 0.35	27.4 ± 0.29	.63	62	.37	59
179	E	24	67 ± 1	36.1 ± 2.76	22.9 ± 0.72	.77	63	.40	52
46	E	14	64 ± 1	38.5 ± 2.05	18.9 ± 0.49	.72	49	.33	46
125	D	13	71 ± 2	42.7 ± 1.79	28.7 ± 0.04	.65	67	.44	68
81	F	17	64 ± 1	48.0 ± 2.09	22.5 ± 0.28	.58	47	.34	59
181	G	15	76 ± 1	45.2 ± 1.17	21.8 ± 0.33	.61	48	.33	54
162	H	22	64 ± 2	34.3 ± 4.02	21.9 ± 0.53	.81	64	.56	69
162	I	28	69 ± 1	28.0 ± 1.87	21.4 ± 0.05	.99	76	.50	51
181	I	16	67 ± 0	47.0 ± 0.84	20.1 ± 0.55	.59	43	.42	71
							—		—
Mean							56		57
Standard error							±3.3		±2.7

* Sheep given 600 g ration A daily.

The concentration of propionate in ruminal fluid (X_E, mmol/l) increased linearly ($P < 0.01$) with propionate production rate (Y_P, X_P, mmol/min) as did the percentage of plasma glucose derived from ruminal propionate (Y_G). These relationships were:

$$Y_P = 0.263 + 0.0237\ X_C \quad [RSD = \pm 0.069,\ r = 0.888] \quad \ldots\ 3\text{-}3$$

$$Y_G = 15.3 + 6.03\ X_P \quad [RSD = \pm 6.99,\ r = 0.793] \quad \ldots\ 3\text{-}4$$

The irreversible loss of plasma glucose also increased with propionate production rate but this relationship was not significant ($P > 0.05$, $r = 0.186$)

Discussion

The marked response in the irreversible loss of glucose to changes in the quantity and quality of the roughage diets fed to sheep in these experiments is in accord with the findings of Ford (1965), Ford and Reilly (1969) and Reilly and Ford (1971). In this study, the irreversible loss of glucose was positively correlated with the digestible energy intake as well as the digestible protein intake. Similarly in sheep given the roughage diets of different protein content (see Table 3-6) it is likely that energy intake increased with protein intake as indicated by the positive relationship between protein intake and total VFA concentration in ruminal fluid. This relationship is consistent

with the observations of Gray et al. (1967) who found that the energy available as VFA was a constant proportion of the digestible energy intake of roughage rations similar to those used in this study.

There was apparently little variation in the proportion of glucose entering the sampled pool that was derived from ruminal propionate or the proportion of propionate produced in the rumen that was converted into glucose with increasing protein intake (Table 3-8). The mean value of 57% for ruminal propionate converted into glucose was greater than the mean value of 32% reported by Leng et al. (1967) but only slightly greater than the mean value of 49% reported by Bergman et al. (1966) for absorbed propionate converted into glucose for sheep given roughage rations similar to those used by Leng et al. (1967). The lower value reported by Leng and associates may have been due to the feeding regimen adopted. The daily ration was given over 12 h instead of over 24 h as used by Bergman et al. (1966) and the synthesis of glucose from propionate was determined when the production rate of ruminal propionate was maximal (Leng and Leonard, 1965a). However, the estimated percentages of plasma glucose derived from ruminal propionate of 56 observed here and of 54 reported by Leng et al. (1967) are in good agreement which suggests that the relative

contribution of ruminal propionate and amino acids (see below) to glucose synthesis was similar for the different diets and feeding regimens. The positive relationship between the propionate production rate and the percentage of glucose synthesized from propionate (equation 3-4) may reflect small alterations in the relative availability of these substrates.

With the roughage rations used, amino acids absorbed from the digestive tract were probably largely derived from microbial protein (Weller, Gray and Pilgrim, 1958; Weller, Pilgrim and Gray, 1962). The amount of protein made available to the animal as amino acids may be related to the production of VFA since the energy for the synthesis of bacterial cells to replace those leaving the rumen arises during the fermentative process (Walker, 1965; Hungate, 1966).

Table 3-9 gives estimates of the potential synthesis of plasma glucose from microbial protein for the different roughage rations (Table 3-6). These calculations were based on the following assumptions:

(1) Ruminal fermentation of 1 mole hexose polysaccharide yields 1.0 mole butyrate or 0.5 mole propionate + 0.5 mole acetate. Propionate production rate was obtained from Table 3-8 and the net production rates of the other

acids were predicted from their concentrations in ruminal fluid (Table 3-8) using the relationships derived by Leng (1970b).

(2) For every 100 g hexose polysaccharide fermented, 22 g microbial protein passes from the rumen to the alimentary tract (Hogan and Weston, 1970).

(3) Digestibility of microbial protein in the small intestine was 80% (Bergen, Purser and Cline, 1968; Hoogenraad et al., 1970).

(4) That 57 g glucose were synthesized from 100 g digested protein (Krebs, 1964b).

The approximate estimates of the potential contribution of microbial protein to glucose synthesis were similar for the different diets (Table 3-9) but the mean value of 49% is considerably less than the potential synthesis of glucose from ruminal propionate (Table 3-8). Wolfe, Bergman and Williams (1970) reported that the net hepatic uptake of amino acids in fed sheep was sufficient to provide at the most about 25% of the glucose produced. The complete conversion of all the carbon residues of glucogenic amino acids into glucose appears unlikely since only about one-half of the propionate removed by liver appears as glucose (Bergman et al., 1966). However, it is probable that the contribution of propionate to glucose synthesis was greater than that obtained with

*Table 3-9. Theoretical calculation of the potential synthesis of plasma glucose from microbial protein, formed in the rumen of sheep given different roughage rations (see Table 3-5) of 800 g daily**

Sheep no.	Ration	Protein intake (mg/min)	Hexose polysaccharides digested in rumen (mg/min)	Synthesis of microbial protein (mg/min)	Potential synthesis of glucose from microbial protein: (mg/min)	(% of glucose produced)
122**	A	23	200	46	21	41
125	B	33	202	46	21	43
122	C	43	257	59	27	51
179	E	47	365	84	38	65
46	E	47	235	54	25	41
125	D	53	233	54	25	42
81	E	76	331	76	35	54
181	G	83	291	67	31	51
162	H	85	330	76	35	44
162	I	92	384	88	40	67
181	I	126	309	71	32	36
Mean						49

* Assumptions are given in the text.

** Sheep given 600 g ration A daily.

the techniques used in this study or in the study by Bergman and associates (1966). The proportion of plasma glucose derived from propionate as calculated neglects the possible mixing of carbon-14 in the tricarboxylic acid cycle (see literature survey) and the irreversible loss of glucose provides only an approximate estimate of the total rate of glucose synthesis (see Section 5). Further, the irreversible loss of glucose, measured with constant infusions of [U-^{14}C]glucose, probably excludes to some extent resynthesis of glucose carbon-14 from substrate pools which turnover slowly relative to the glucose pool. By using the a and m values, which describe the disappearance of [U-^{14}C]glucose from plasma (Section 5), in equation 1-7 it may be shown that at 5 h after the start of an infusion of [U-^{14}C]glucose, the SR of plasma glucose is about 91% of the theoretical maximum (mean value for sheep). Hence, the irreversible loss of glucose as measured in the present experiments probably overestimates the rate at which glucose carbon is replenished by exogeneous substrate.

The influence of ruminal fermentation on dietary substrates may explain the lower correlation observed with the regression of the irreversible loss of plasma glucose on digestible protein intake than a digestible energy intake. That is, the amount of protein which passes from the rumen to the lower intestinal tract

is to a large degree independent of the protein intake (Clarke, Ellinger and Phillipson, 1966; Hogan and Weston, 1967a, 1967b; Weston and Hogan, 1968b) and thus it is considered that protein intake was not causally related to the irreversible loss of plasma glucose. This is also apparent from the experiments in Part A and those of Ulyatt et al. (1970) where the irreversible loss of glucose was similar for sheep given diets of different protein content but of similar digestible energy content. In sheep given other diets in which substantial quantities of dietary protein (Hogan and Weston, 1969) or starch (see Armstrong and Beever, 1969) escape fermentation in the rumen this relationship may not be valid.

SECTION 4

EFFECT OF SELECTED SUBSTRATES ON GLUCOSE SYNTHESIS

In ruminants, gluconeogenesis increases during pregnancy and lactation and decreases during starvation. This apparent adaptation of gluconeogenesis to different physiological conditions suggests that ruminants possess mechanisms which regulate its rate. Previous studies reported herein have indicated that diet may be an important factor in the regulation of gluconeogenesis in sheep.

Investigations of the regulation of gluconeogenesis have relied largely on studies with *in vitro* preparations of liver and kidney from the rat. The ruminant provides an excellent species for the study of gluconeogenesis since this process is continuous in the fed animal and it can be brought into a relatively steady state by continuous feeding. This facilitates studies in which perturbation of the system can be accomplished by provision of substrates or effectors by intravenous infusions or by other means of controlled input.

The object of the present studies was to measure short-term effects of substrate infusions on gluconeogenesis in fed sheep, as indicated by changes in (1) the irreversible loss of plasma glucose, (2) the synthesis of glucose from ruminal propionate and (3) the fixation of blood bicarbonate carbon into

glucose. The latter technique has been used previously as an index of gluconeogenesis in the intact animal (see Wagle and Ashmore, 1963; Black and Anand, 1966). Intravenous infusions of [6-^{3}H]glucose were also made with infusions of NaH$^{14}CO_{3}$ or [2-^{14}C]propionate when estimates of glucose irreversibly lost and the contribution of bicarbonate carbon or propionate carbon were required simultaneously. Estimates of the irreversible loss of glucose obtained with infusions of [6-^{3}H]glucose or [U-^{14}C]glucose were found to be approximately similar although consistently greater for the former tracer. These results are given in Section 5.

PART A: SHORT-TERM EFFECTS OF GLUCOSE INFUSIONS

Introduction

In previous findings with sheep on diets of varying maize content, it was shown that glucose synthesized from ruminal propionate decreased as the maize content of the diet increased even though the propionate produced in the rumen was similar (Section 3, Part A). It was suggested that glucose absorption from the gastro-intestinal tract may have increased as the grain content of the diet was increased and that this suppressed gluconeogenesis from propionate. Glucose infusions have previously been shown to abolish or markedly inhibit glucose synthesis in ruminants (see literature survey). However, these animals were usually without feed for approximately 24 h and the glucose infused was equivalent to or greater than the endogenous production rate of glucose.

The purpose of this experiment was to determine whether the fed sheep has the ability to alter the gluconeogenic rate in response to glucose infusions.

Experimental

Twelve sheep were used and they were given either 800 g lucerne chaff (ration A) or 400 g wheat with 50 g lucerne chaff and 5 g limestone (ration B) daily. The respective digestible

energy and crude protein contents were approximately 1900 kcal and 97 g for ration A (see Section 3) and 1386 kcal and 45 g for ration B.

At least 5 days before administration of radioactive tracers the animals were given their daily ration in 24 equal quantities at hourly intervals. [U-^{14}C]glucose (0.06 to 0.08 μCi/min), [6-^{3}H]glucose (0.11 to 0.47 μCi/min), or $NaH^{14}CO_3$ (0.40 to 1.93 μCi/min) were infused intravenously on the 5th or 7th day of the interval-feeding regimen. [2-^{14}C]propionate (0.11 to 0.13 μCi/min) was infused intraruminally and simultaneously with intravenous infusions of [6-^{3}H]glucose.

Tracer infusions were commenced between 05.00 h and 07.00 h and usually consisted of two or three successive periods, each of between 3 and 8 h duration. The first period was used to obtain control values for the concentration and SR of metabolites isolated from blood. Between 4 and 140 mg glucose/min were infused intravenously during the second and third period. The amount of glucose was varied by infusing from 2 to 40% solutions of glucose at rates between 0.18 and 0.37 ml/min. Separate catheter leads were used to convey the infused glucose and tracer to the animal but were joined by a Y-piece to the catheter in the vein. Samples of blood and ruminal fluid were taken at 20 to 30 min intervals, 2 to 3 h immediately before and during a glucose

infusion.

Results

Irreversible Loss of Plasma Glucose

Sheep received constant infusions of [U-^{14}C]- or [6-^{3}H]glucose for approximately 12 h. The SR of plasma glucose plateaued from 3 to 4 h after the start of this infusion. In the second 6 h of the tracer infusion, between 4 and 140 mg glucose/min were infused either at a single rate or at two different successive rates for equal periods (see Figures 4-1 and 4-4). The glucose infusion usually resulted in a gradual decrease in the SR of plasma glucose for 1 to 3 h before the glucose SR apparently plateaued. This plateau was maintained for intervals up to 5 h during the infusion of glucose (see Figure 4-1).

The increase in the irreversible loss of plasma glucose in response to a glucose infusion was less than the quantity of glucose infused, indicating that the irreversible loss of endogenous glucose was suppressed (Figure 4-2). The percentage of this endogenous glucose suppressed (Y_S) was linearly related ($P < 0.01$) with the glucose administered (X_G, mg/min) in sheep given lucerne (ration A) but this relationship was not significant ($P > 0.05$) when wheat (ration B) was fed (Figure 4-3a). However, these relationships were not significantly different

Figure 4-1. Effect of an intravenous infusion of glucose on the SR and concentration of plasma glucose in sheep 175, given 800 g lucerne daily and a constant infusion of [U-^{14}C]glucose. o, SR of glucose; • concentration of glucose. The infusion rate of glucose from the 310th to the 660th min (horizontal bar) of the tracer infusion was 99.3 mg/min.

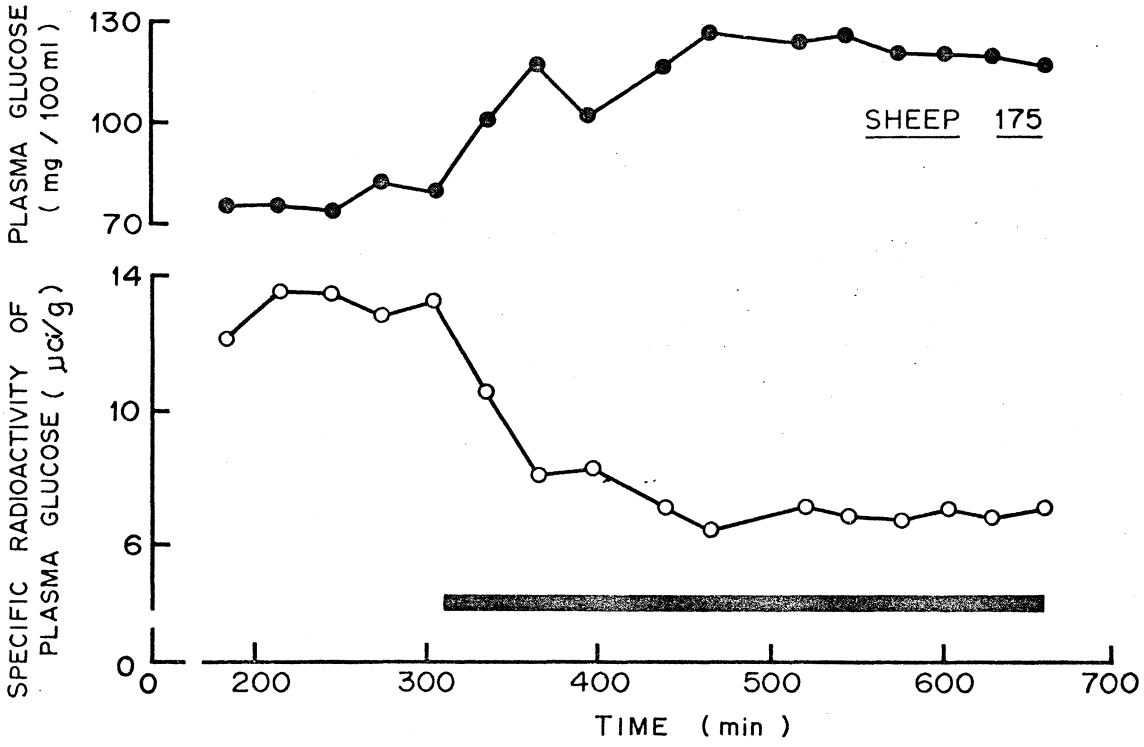
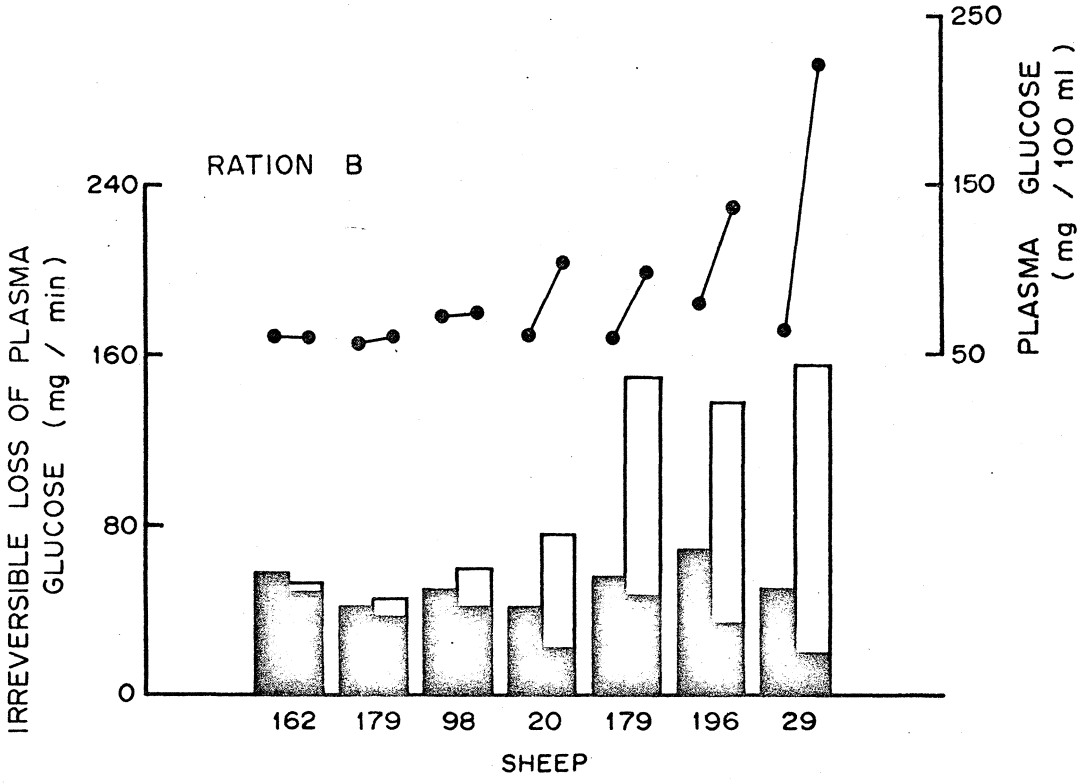

PLASMA GLUCOSE (mg / 100 ml)
130
100
70
SHEEP 175
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (µci/g)
14
10
6
0
0
200
300
400
500
600
700
TIME (min)

Figure 4-2. Effect of intravenous infusion of glucose on the irreversible loss of plasma glucose in sheep given wheat (B) or lucerne (A). Each set of two or three vertical columns represents one experiment with a sheep whose number is listed beneath the columns. Exogenous glucose was administered in the second (and third) period and the rate of infusion (mg/min) is represented by the unshaded column. The irreversible loss of plasma glucose is represented by the height of each column and was measured by using a constant infusion of [U-^{14}C]glucose or [6-^{3}H]glucose. Sheep whose number is marked with an asterisk received [6-^{3}H]glucose. The mean plasma glucose concentration (●) for each period is shown above the appropriate column.


RATION B
IRREVERSIBLE LOSS OF PLASMA GLUCOSE (mg / min)
240
160
80
0
250
150
50
PLASMA GLUCOSE (mg / 100 ml)
162
179
98
20
179
196
29
SHEEP

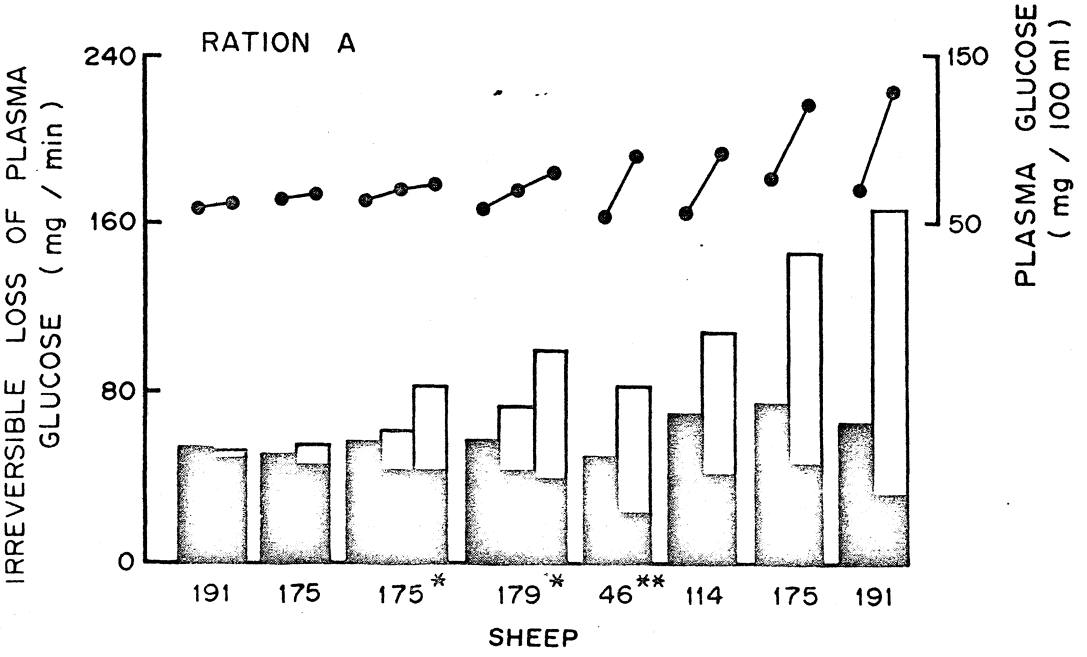
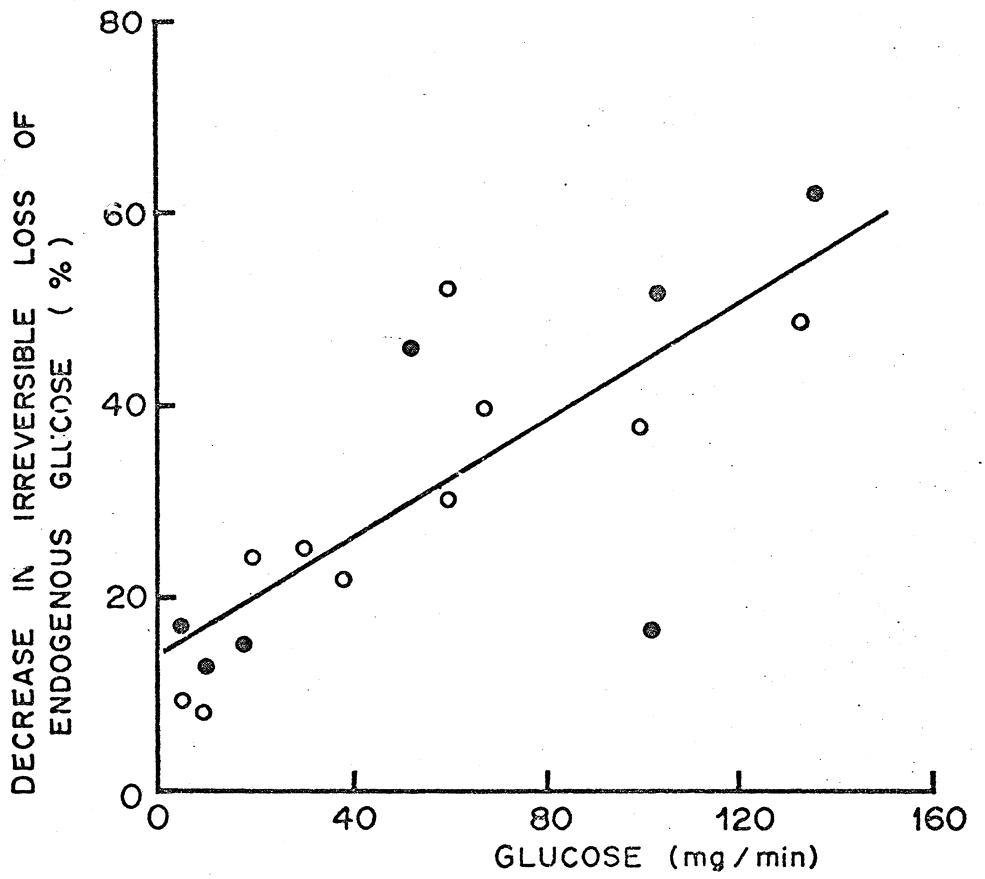
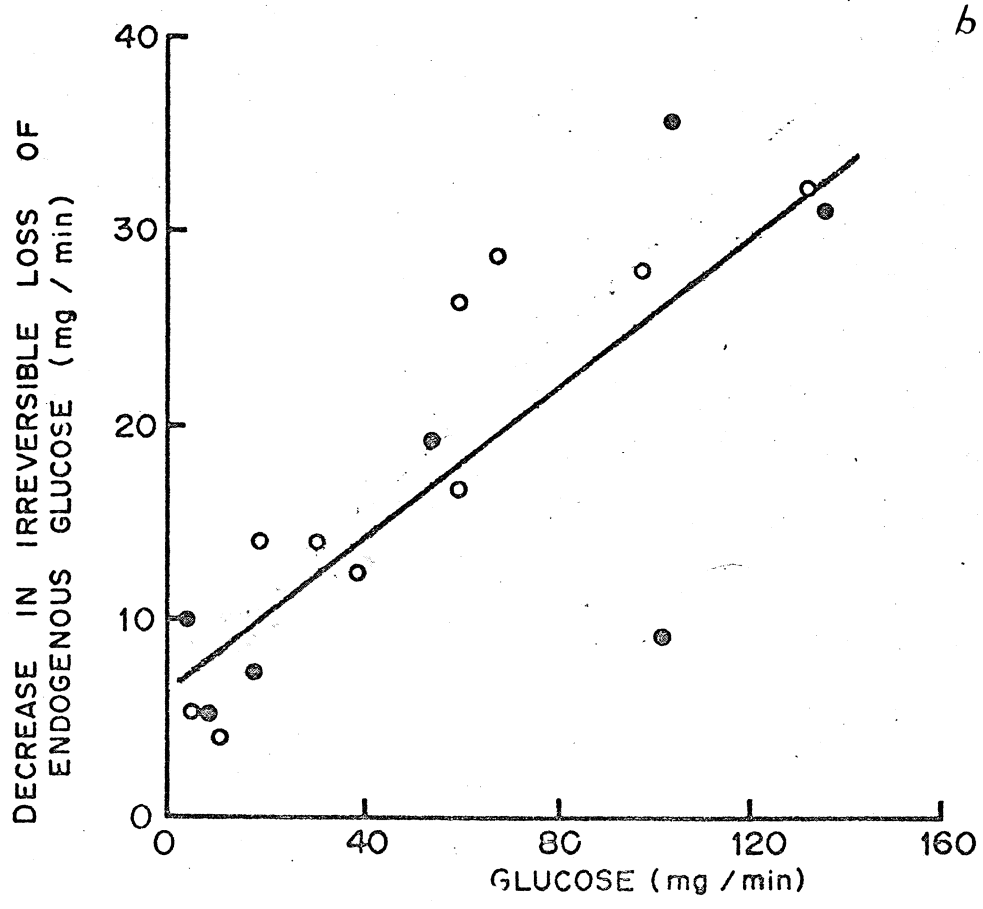

RATION A
IRREVERSIBLE LOSS OF PLASMA GLUCOSE (mg / min)
240
160
80
0
150
50
PLASMA GLUCOSE (mg / 100 ml)
191
175
175*
179*
46**
114
175
191
SHEEP

Figure 4-3. Relationships between the percentage of the irreversible loss of endogenous glucose suppressed (a) or rate of suppression of the irreversible loss of endogenous glucose (b) and rate of infusion of glucose into sheep given lucerne (o) or wheat (●). Equations for the lines in 3(a) and 3(b) are given in the text.


a
DECREASE IN IRREVERSIBLE LOSS OF ENDOGENOUS GLUCOSE (%)
80
60
40
20
0
0
40
80
120
160
GLUCOSE (mg / min)


b
DECREASE IN IRREVERSIBLE LOSS OF ENDOGENOUS GLUCOSE (mg / min)
40
30
20
10
0
0
40
80
120
160
GLUCOSE (mg / min)

($P < 0.05$) in slope or intercept and the regression equation for the pooled results was:

$$Y_S = 14.2 + 0.287\ X_G \quad [RSD = \pm 11.1,\ r = 0.771,\ P < 0.001] \quad ...4\text{-}1$$

The quantitative suppression of the irreversible loss of endogenous glucose (Y_Q, mg/min) increased linearly at $P < 0.001$ and $P < 0.05$ with the rate of infusion of glucose (X_G, mg/min) in sheep given lucerne or wheat respectively (Figure 4-3b). These relationships were similar in slope and intercept ($P < 0.05$) and the combined regression equation relating these measurements was:

$$Y_Q = 6.87 + 0.192\ X_G \quad [RSD = \pm 6.32,\ r = 0.817,\ P < 0.001] \quad ...4\text{-}2$$

Plasma Glucose Concentrations

The response in plasma glucose concentration to a glucose infusion was similar for sheep on lucerne or wheat. In general the plasma glucose concentrations rose to a maximum value between 100 and 200 min after the start of the infusion and then was maintained or declined slowly for the remainder of the glucose infusion (see Figures 4-1 and 4-4). Glucose excreted in urine was negligible, a maximum value of 0.03 mg/min was measured when sheep 29 received 137 mg glucose/min (see Figure 4-2). Where the

plasma glucose concentration varied during infusions of glucose, the mean concentration was calculated from samples corresponding to the plateau SR of plasma glucose.

The relationships between the irreversible loss of plasma glucose (Y_P, mg/min) and plasma glucose concentration (X_C, mg/100 ml), the increase in plasma glucose concentration (Y_I, mg/100 ml) and the rate of infusion of glucose (X_G, mg/min), and the decrease in the irreversible loss of endogenous glucose (Y_D, mg/min) and the increase in plasma glucose concentration (X_I, mg/100 ml) for sheep given lucerne or wheat and intravenous infusions of glucose were:

$$Y_{PA} = -43.2 + 1.61\ X_{CA}\ (n = 10)\ [RSD = \pm 10.8,\ r = 0.694] \quad ...4\text{-}3$$

$$Y_{PB} = 22.6 + 0.687\ X_{CB}\ (n = 7)\ [RSD = \pm 33.0,\ r = 0.789] \quad ...4\text{-}4$$

$$Y_{IA} = -3.81 + 0.445\ X_{GA}\ (n = 19)\ [RSD = \pm 7.51,\ r = 0.921] \quad ...4\text{-}5$$

$$Y_{IB} = -13.5 + 0.891\ X_{GB}\ (n = 10)\ [RSD = \pm 37.1,\ r = 0.809] \quad ...4\text{-}6$$

$$Y_{DA} = 7.52 + 0.491\ X_{IA}\ (n = 10)\ [RSD = \pm 3.51,\ r = 0.945] \quad ...4\text{-}7$$

where n is the number of experiments, and subscripts A and B refer to rations A and B, respectively.

Equations 4-3, 4-5 and 4-7 were significant at $P < 0.001$ whereas equations 4-4 and 4-6 were significant at $P < 0.05$ and $P < 0.01$ respectively. The relationship between the decrease in

the irreversible loss of endogenous glucose and the increase in plasma glucose concentration for sheep given wheat was not significant ($P > 0.05$, $r = 0.753$). This relationship also differed significantly ($P < 0.05$) in slope but not intercept to the equation relating these measurements for sheep given lucerne (equation 4-7). Equations 4-3 and 4-4 were also only significantly different in slope ($P < 0.05$) but equations 4-5 and 4-6 were similar in slope and intercept ($P > 0.05$). With these relationships, the difference between diets was largely the result of the apparently exaggerated response in plasma glucose concentration of sheep 29 to a glucose infusion (see Figure 4-2).

Fixation of Blood Bicarbonate into Plasma Glucose

The SR of carbon of blood bicarbonate and plasma glucose in sheep plateaued from about 3 to 6 h respectively after the start of an intravenous infusion of $NaH^{14}CO_3$, lasting 9 or 14 h. The irreversible loss of blood bicarbonate and the percentage of glucose carbon derived from blood bicarbonate, calculated by comparing the plateau SR values of carbon were significantly less ($P < 0.01$) in sheep given wheat (ration B) than in sheep given lucerne (ration A) (Table 4-1).

Infusions of between 16 and 140 mg glucose/min during the final 6 h of a 14 h infusion of $NaH^{14}CO_3$ did not alter the plateau SR of blood bicarbonate, established before the administration of

Table 4-1. Percentage of carbon in plasma glucose derived from bicarbonate of jugular blood and the irreversible loss of bicarbonate in sheep given either lucerne or wheat and intravenous infusions of $NaH^{14}CO_3$

Mean values with their standard errors

No. of expt	Ration	Sheep wt (kg)	Glucose derived from bicarbonate (%)	Irreversible loss of blood bicarbonate (mg C/min)
12	Lucerne	35 ± 1.6	14 ± 0.3	144 ± 2.8
5	Wheat	32 ± 2.3	10 ± 0.6	104 ± 6.8

of glucose, but the glucose SR decreased to attain a new plateau some 1 to 3 h later (see Figure 4-4). The net rate of synthesis of glucose carbon from blood bicarbonate decreased with the quantity of glucose infused in sheep given lucerne or wheat but the percentage of endogenous glucose carbon apparently derived from bicarbonate was not markedly altered except for sheep given the greatest quantity of glucose, where the percentage of glucose carbon derived from bicarbonate apparently increased (see Table 4-2).

Contribution of Propionate to Glucose Synthesis

An intravenous infusion of [6-^{3}H]glucose and an intraruminal

Figure 4-4. Effect of an intravenous infusion of glucose on the SR values of plasma glucose and blood bicarbonate and the concentration of plasma glucose in sheep 191 given 800 g lucerne daily and an intravenous infusion of $NaH^{14}CO_3$. o, SR of glucose; □, SR of bicarbonate; ●, concentration of glucose. The infusion rate of glucose from the 480th to the 840th min (horizontal bar) of this tracer infusion was 133.0 mg/min.

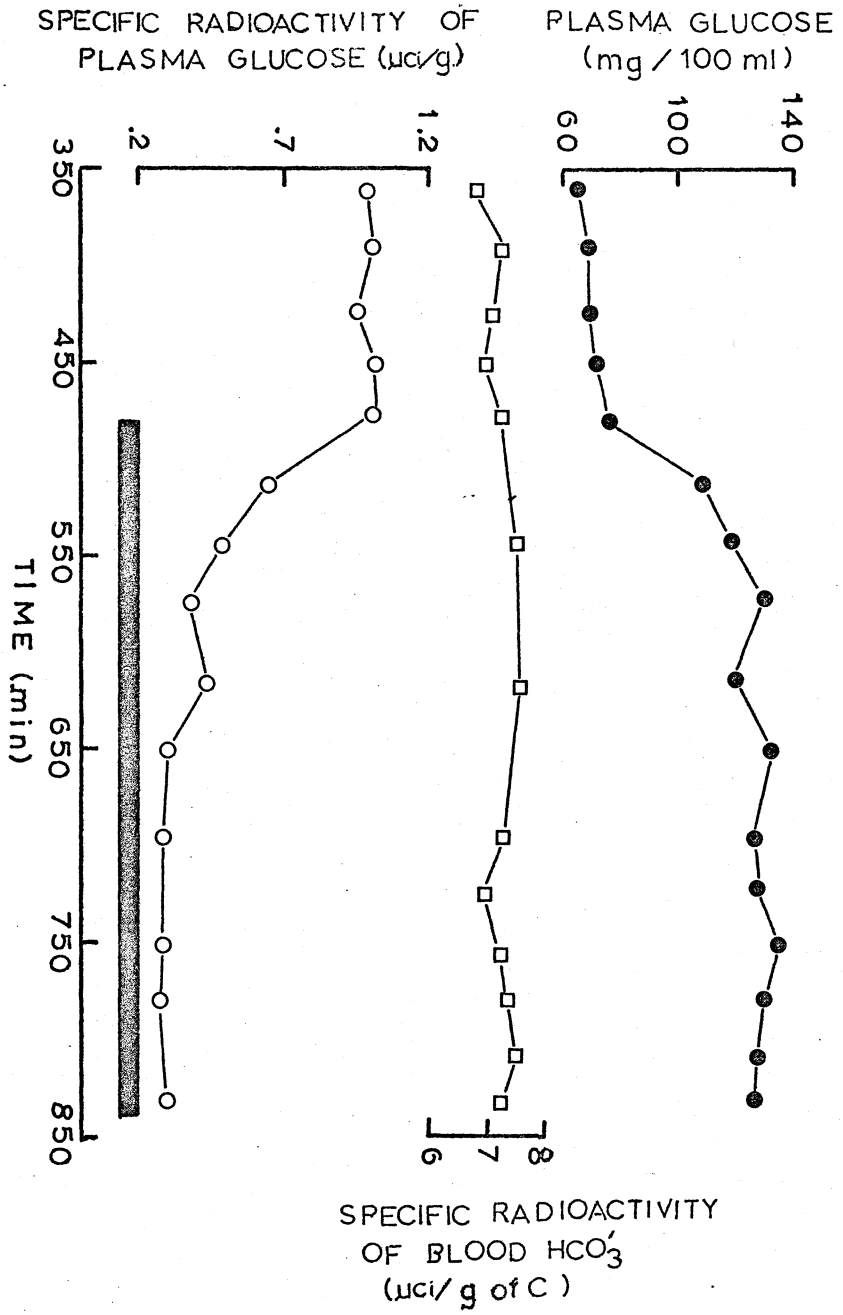

SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci/g)
.2
.7
1.2
PLASMA GLUCOSE (mg / 100 ml)
60
100
140
350
450
550
650
750
850
TIME (min)
6
7
8
SPECIFIC RADIOACTIVITY OF BLOOD HCO_3' (μci/g of C)

Table 4-2. Effect of an intravenous infusion of glucose on the net rate of synthesis of glucose carbon from blood bicarbonate and on plasma urea concentration in sheep given either lucerne or wheat

Each animal received intravenously a 12 h infusion of [U-^{14}C]glucose and a 15 h infusion of NaH$^{14}CO_3$ on separate occasions, 5 or 7 days apart. D-glucose was infused during the last 6 h of each tracer infusion. Mean plasma urea concentrations with their standard errors are given for four to six samples taken at 20 to 30 min intervals immediately before and during glucose infusion.

Sheep no.	Sheep wt (kg)	Ration	Glucose infusion (mg/min)	Glucose derived from bicarbonate* (%)	Irreversible loss of glucose** (mg C/min)	Rate of synthesis of glucose from bicarbonate (mg C/min)	Plasma urea[/] (mg/100 ml)
125	30.1	Lucerne	-	13.7	-	-	-
			16.4	11.3 (16)	22.2	2.5	-
114	33.6	Lucerne	-	14.2	28.6	4.1	52 ± .4
			67.3	5.2 (13)	44.0	2.3	49 ± .1
175	32.6	Lucerne	-	16.7	30.3	5.1	44 ± .3
			99.3	5.3 (16)	58.7	3.1	41 ± .2
191	38.6	Lucerne	-	14.1	26.7	3.8	30 ± .2
			133.0	3.9 (19)	67.0	2.6	28 ± .2
98	36.3	Wheat	-	8.9	19.9	1.8	39 ± .9[a]
			16.8	6.2 (8.8)	23.7	1.5	39 ± .2[a]
179	29.8	Wheat	-	8.8	22.6	2.0	34 ± .5
			102.3	2.9 (9.0)	59.9	1.7	29 ± .8
29	28.8	Wheat	-	10.3	20.2	2.1	51 ± .3
			137.0	3.0 (24)	62.6	1.9	48 ± .3

* Percentages of endogenous glucose produced that was derived from bicarbonate during glucose loads are given in parentheses. Calculated by comparing the synthesis rate of glucose carbon from blood bicarbonate with the difference between the irreversible loss of glucose carbon and the glucose carbon load.

** These values also appear in Figure 4-2.

/ For each sheep, mean values with the superscript a were not significantly different ($P < 0.05$) from each other.

infusion of [2-^{14}C]propionate were administered simultaneously for 11 or 14 h to sheep given lucerne to measure glucose synthesis from propionate before and during administration of glucose. The glucose was infused from approximately the 8th h of the infusion of these tracers and for about 3 h at a single rate or for about 6 h at two different successive rates of equal duration. The results from a typical experiment are given in Figure 4-5.

The total concentration of VFA and the SR of propionate in ruminal fluid from 6 h after the start of a [2-^{14}C]propionate infusion (Table 4-3) showed no significant change ($P > 0.05$) with time, indicating an approximately constant production of glucogenic precursors (propionate and microbial protein) during the experimental period. The net rate of glucose synthesis from propionate decreased with the administration of glucose, but the percentage of endogenous glucose apparently derived from ruminal propionate generally increased (Table 4-3) indicating a more effective suppression of glucose synthesis from substrates other than ruminal propionate.

Plasma lactate concentrations were not markedly altered with a glucose infusion (Table 4-3) but plasma urea concentrations usually decreased to attain an approximately constant level from about the 3rd h of the glucose infusion in sheep given lucerne or

Figure 4-5. Effect of successive intravenous infusions of glucose on the plasma concentrations of glucose and lactate and SR values of glucose in sheep 175 given 800 g lucerne daily and an intravenous infusion of [6-^{3}H]glucose, administered simultaneously with an intraruminal infusion of [2-^{14}C]propionate. o, SR of glucose carbon; Δ, SR of glucose (μCi ^{3}H/g); ○, concentration of glucose; ■, concentration of lactate; □, SR of ruminal propionate; ▲, concentration of total volatile fatty acids (VFA) in ruminal fluid. The infusion rates of glucose between the 480th and the 660th min and the 660th and the 840th min (horizontal bars) of the infusion of tracers were 19.2 and 38.5 mg/min respectively.

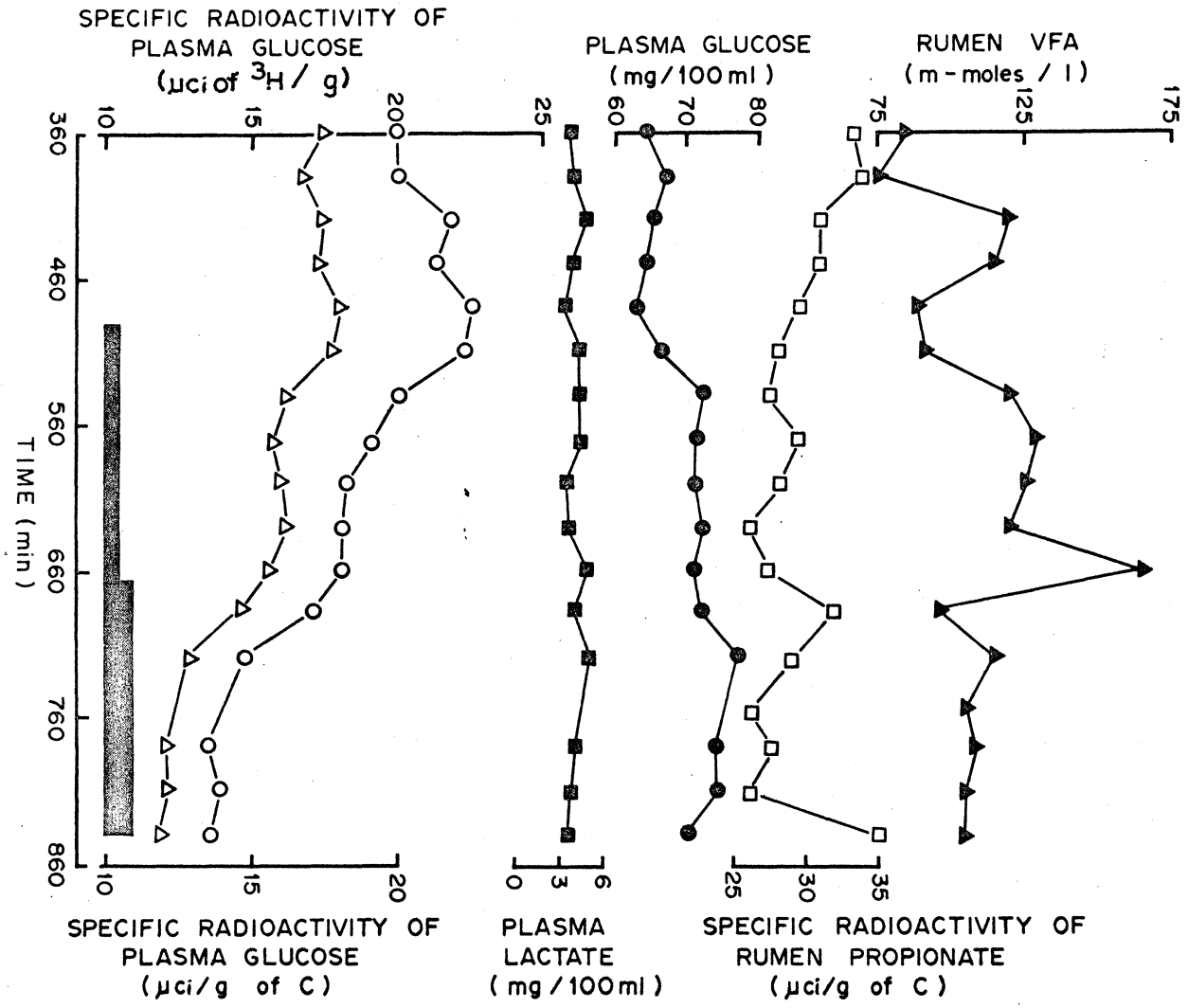

SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci of ^3H / g)
PLASMA GLUCOSE (mg/100 ml)
RUMEN VFA (m-moles / l)
TIME (min)
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci/g of C)
PLASMA LACTATE (mg/100ml)
SPECIFIC RADIOACTIVITY OF RUMEN PROPIONATE (μci/g of C)

Table 4-3. Plasma lactate concentration, irreversible loss of plasma glucose and net rate of synthesis of glucose from ruminal propionate in sheep given intravenous infusions of glucose

Animals were given infusions of D-glucose at a single rate for 3 h or for 6 h at two different successive rates of equal duration. Glucose synthesis rate from propionate immediately before and during the administration was estimated by using simultaneous intravenous infusion of [6-^{3}H]glucose and intraruminal infusion of [2-^{14}C]propionate, lasting either 14 h or only 11 h with a single infusion rate of glucose. Mean concentrations with their standard errors are given for four to six samples taken at 20 to 30 min intervals immediately before and during glucose infusion.

Sheep no.	Sheep wt (kg)	Glucose infusion (mg/min)	Plasma glucose* (mg/100 ml)	Plasma lactate* (mg/100 ml)	Total VFA in ruminal fluid** (mmol/l)	Total VFA in ruminal fluid** (molar % as propionate)	Propionate production rate (mmol/min)	Glucose synthesized from propionate≠ (%)	Irreversible loss of plasma glucose≠ (mg/min)	Rate of synthesis of glucose from propionate (mg/min)	Propionate produced and converted into glucose (%)
175	31.5	-	65 ± 0.8	4.4 ± .20[a]				74	57.1	42	50
		19.2	71 ± 0.3[a]	4.5 ± .20[a]	110	19.6	0.94	61 (88)	62.5	38	45
		38.5	72 ± 1.4[a]	4.2 ± .22[a]	± 5	± .8		46 (85)	83.3	38	45
179	35.2	-	59 ± 1.0	6.4 ± .17[a]				51	57.1	26	43
		30.0	68 ± 1.2	6.7 ± .23[a]	115	20.1	0.67	33 (56)	73.0	24	40
		60.1	80 ± 2.2	7.0 ± .65[a]	± 2	± .6		18 (45)	100.0	18	30
46	37.4	-	56 ± 0.8	-	92	16.8	0.50	36	50.5	18	40
		59.6	90 ± 1.6	-	± 2	± .2		13 (45)	84.0	11	24

* For each sheep, mean values with the same superscript a were not significantly different ($P > 0.05$) from each other.

** Mean concentrations and standard errors for twelve or seventeen samples. Molar proportions of propionate were determined with samples taken at 40 to 90 min intervals.

≠ These values also appear in Figure 4-2.

≠ Percentages of endogenous glucose derived from ruminal propionate during glucose infusions are given in parentheses. Calculated by comparing the synthesis rate of glucose from propionate with the difference between the irreversible loss of plasma glucose and infusion rate of glucose.

wheat (see Table 4-2).

Discussion

The present study indicates that the extent of suppression of endogenous glucose production in fed sheep is dependent upon the infusion rate of glucose (Figure 4-3). These observations support previous suggestions that a reduction in gluconeogenesis from propionate found in sheep given diets of different maize and roughage content may have been related to the quantity of glucose absorbed from the lower digestive tract (Section 3, Part A).

It is possible that the glucose administered to sheep given the wheat diet was in addition to glucose absorbed from the intestinal tract. Variation in the quantities of glucose absorbed between sheep may account for the more variable responses in endogenous glucose production to infused glucose found with sheep on this diet than with sheep on a lucerne diet. Absorption of glucose in sheep given wheat is also indicated by the lower proportion of glucose carbon derived from bicarbonate carbon-14 in these sheep when compared with sheep given lucerne.

In these studies, the fixation of carbon-14 from blood bicarbonate into plasma glucose was used as an index of gluconeogenesis. As carbonic anhydrase is usually low or absent in most extravascular tissue (see Roughton, 1935), this makes it possible for rapid diffusion of metabolically produced carbon dioxide to

extracellular fluid (see Bittar, 1964) and its replacement by circulating bicarbonate, 'tagged' with carbon-14. Two key reactions incorporate bicarbonate carbon into glucogenic intermediates. These reactions are (1) the conversion of pyruvate into oxaloacetate by pyruvate carboxylase (Utter and Keech, 1963; Cooper, Tchen, Wood and Benedict, 1968) and the subsequent equilibration of oxaloacetate through malate with a symmetrical molecule of fumarate, as proposed by Solomon, Vennesland, Klemperer, Buchanan and Hasting (1941), and (2) the conversion of propionate into succinate (Pennington and Sutherland, 1956), where propionyl-CoA is converted into methylmalonyl-CoA by propionyl-CoA carboxylase (see Kaziro and Ochoa, 1964). In the fed ruminant, most of the glucogenic substrates (i.e. propionate, lactate and certain amino acids) are converted into glucose by these reactions (Black et al., 1968; Leng, 1970a).

Since it is likely that little or no glucose is absorbed from the alimentary tract of sheep on the lucerne diet a relationship between the percentage of glucose carbon derived from blood bicarbonate (Y_P) and the percentage contributed by exogenous glucose to the irreversible loss of plasma glucose (X_P) in these sheep may be useful in predicting the magnitude of glucose absorption in sheep given other diets. The regression equation relating these

measurements for sheep given lucerne (see Table 4-2) was:

$$Y_P = 15.5 - 0.153\ X_P \quad [RSD = \pm 0.830,\ r = -0.979,\ P < 0.05] \quad \ldots 4\text{-}8$$

For the three sheep given the wheat diet (see Table 4-2), in which 8.8 to 10.3% of glucose carbon was apparently from bicarbonate, it may be predicted from equation 4-8 that about 34 to 44% of the irreversible loss of plasma glucose was derived from exogenous glucose. This suggests that glucose absorbed from the small intestine was equivalent to about 8% of the wheat starch ingested (see Kerr, 1950), which is only slightly greater than the estimate of 6% of MacRae and Armstrong (1969) for sheep on diets of whole barley, but is less than values of 12 to 29% for sheep given crushed maize (Tucker, Mitchell and Little, 1968; Ørskov et al., 1969; Beever et al., 1970). These differences are probably due to wheat and barley starch being less resistant than maize starch, to ruminal fermentation (Ørskov et al., 1969; Beever et al., 1970).

In the present study, only a small proportion (usually less than 10%) of the glucose administered to sheep was apparently retained in extracellular fluid, as indicated by the change in plasma glucose concentration. Reid (1958) reported that the inclusion of grain (900 g of crushed maize daily) with roughage in a ration accelerated the rate of removal of injected glucose in

sheep. Enhanced rates of removal of injected glucose were also apparent in hyperphagic goats given concentrates ad libitum (Baile et al., 1969). Glucose was probably absorbed on these high-starch diets and may have increased the animals' 'tolerance' to a glucose infusion. Improved rates of glucose disappearance following repeated glucose infusion have been reported for humans (see Szabo, Maier, Szabo and Camerini-Cavalos, 1969). An explanation for the similarity in the rate of removal of exogenous glucose from plasma of sheep given either lucerne or wheat (compare equations 4-5 and 4-6) is in the feeding regimen adopted in the present study. The ration was given in equal amounts hourly throughout the day, thus minimising any adaptation process in sheep given grain once daily as was done by Reid (1958). The decline in the peak concentration of plasma glucose which occasionally occurred during a 6 h infusion of glucose to sheep given wheat or lucerne may indicate some adaptation to glucose intake.

Bergman (1964) suggested that the rate of utilization of glucose in sheep is a function of plasma glucose concentration. This is only indicated in the present study with sheep given glucose infusions (equations 4-3 and 4-4). From an examination of data recorded in this thesis with 44 sheep on different diets, including the present results, no significant relationship ($P > 0.05$) was found between plasma glucose concentration and the

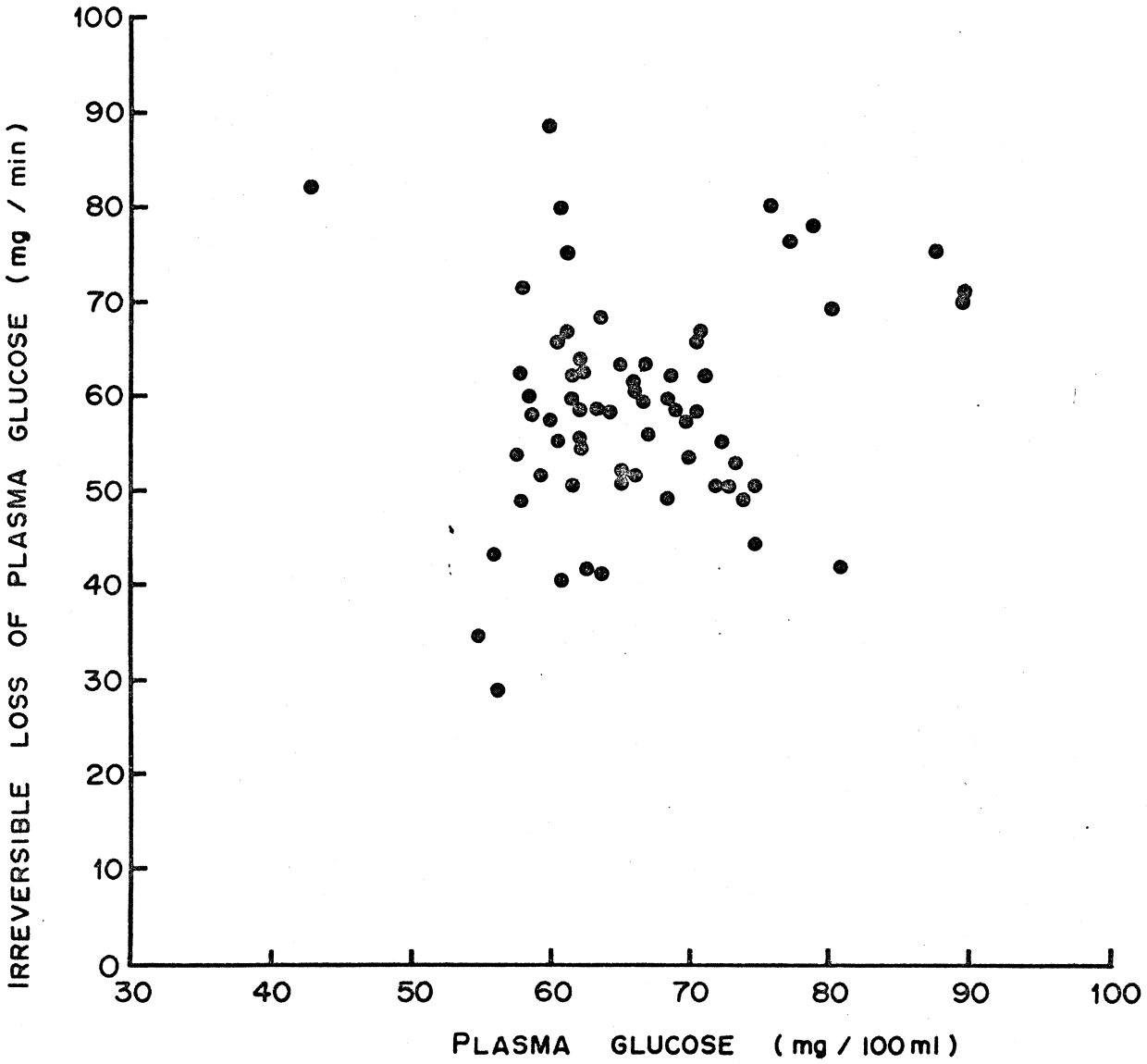
irreversible loss of (^{14}C)glucose from plasma (Y_G) ($r = 0.159$, $n = 66$) or $Y/W^{0.75}$ ($r = 0.107$, $n = 66$) where W is the live weight of the animal (see Figure 4-6). It is possible that if individual animals were examined a relationship may exist, and this would explain the relationship when fed and starved sheep are grouped, since the same animal is generally used as is evident in the relationship found by Bergman (1964). That is, individual sheep may control their plasma glucose concentration within narrow limits.

The disposal of infused glucose in ruminants has not been fully elucidated. The liver (and possibly the kidney) only partly 'adapt' to a glucose infusion in the present study since the rate of suppression of plasma glucose synthesis was apparently less than the infused rate of glucose. Similar observations were recorded with dogs given glucose intravenously (Steele et al., 1965; Hetenyi and Wrenshall, 1968). In ruminants, the conversion of glucose into fatty acids is restricted by the virtual absence or negligible activity of ATP citrate lyase and NADP-malate dehydrogenase of the citrate cleavage pathway in adipose and liver tissue (Hardwick, 1966; Hanson and Ballard, 1967). The activities of these enzymes in cattle do not appear to be markedly altered when diets of high grain content are fed (Baldwin and Ronning, 1966; Baldwin, Ronning, Radanovics and Plange, 1966; Young et al., 1969). Large increases in the activities of these

enzymes have been reported for sheep given prolonged intravenous infusions of glucose but the responses were variable in sheep fed high carbohydrate diets or given abomasal infusions of glucose (Ballard, Filsell and Jarrett, 1972).

The studies of Annison and White (1961) and Bartley and Black (1966) indicate that much of the exogenous glucose administered to ruminants is promptly oxidized. In the intact rat, Depocas (1964) has also shown that oxidation of glucose is closely regulated by its availability. Since the irreversible loss of blood bicarbonate was not altered in the present studies, and hence energy expenditure probably remained constant during infusions of exogenous glucose, any increase in glucose oxidation must have displaced the oxidation of other substrates. The significantly greater estimates of the irreversible loss of blood bicarbonate in sheep maintained on the lucerne diet than in sheep maintained on the wheat diet (see Table 4-1) agree with the findings of Corbett, Farrell, Leng, McClymont and Young (1971) that the irreversible loss of blood bicarbonate provides a sensitive index of energy expenditure in sheep. Acetate is a major source of energy in ruminants (Annison et al., 1967), and it is possible that an increased rate of oxidation of glucose spared acetate for lipogenesis in extrahepatic tissue (Skarda and Bartos, 1969). Alternatively, the endogenous production of acetate may have been suppressed by the glucose infusions (Annison and Lindsay, 1961).

Figure 4-6. Relationship between the irreversible loss of plasma glucose and plasma glucose concentration in sheep fed on a wide variety of diets (see text).


IRREVERSIBLE LOSS OF PLASMA GLUCOSE (mg / min)
100
90
80
70
60
50
40
30
20
10
0
30
40
50
60
70
80
90
100
PLASMA GLUCOSE (mg / 100 ml)

Free fatty acid metabolism is low in the fed sheep (West and Annison, 1964) but glucose infusions could also have decreased the mobilization or oxidation of these substrates (Annison, 1960).

In the present study, alterations in the irreversible loss of endogenous glucose with glucose infusions probably reflected changes in the rate of gluconeogenesis since it is unlikely that the liver of ruminants has a great potential to store large amounts of glycogen (see Ballard et al., 1969), particularly when in addition to levels of 3 to 5 g/100 g liver present in the fed sheep (Ford, 1962). Bartley et al. (1966) reported that liver glycogen increased slightly, from about 1.7 to 2.6 g/100 g liver in cows given a duodenal infusion of 1 g glucose/min for 4 h. However, these cows were probably not fed on the experimental day and the glycogen synthesized may have replenished that which was mobilized.

Krebs (1964a) has proposed that the rate of gluconeogenesis (and glycolysis) can be controlled at certain 'pacemaker' reactions. One of these enzymes appears responsive, for in cow liver the activity of glucose 6-phosphatase is halved 4 h after the start of an intraduodenal infusion of glucose (Bartley et al., 1966). Filsell et al. (1969) have shown that the concentration of another of these enzymes, pyruvate carboxylase, in the liver of sheep was very responsive to changes in hormonal and dietary perturbations.

If pyruvate carboxylase activity was suppressed when glucose was infused this may have been effective in raising the availability of propionate relative to other substrates for glucose synthesis (see Table 4-3), concomitant with a reduction in the synthesis of glucose carbon from bicarbonate (see Table 4-2).

The liver cell is considered to be freely permeable to glucose (Cahill, Ashmore, Renold and Hasting, 1959), so the concentrations of glucose in plasma and hepatic cells were probably similar. However, it is unlikely in the present study that increased plasma glucose concentrations *per se* suppressed glucose release through alterations in metabolite levels in the hepatic cell since sheep liver apparently has a poor ability to utilize glucose, probably because of the absence of glucokinase (Ballard and Oliver, 1964; Ballard, 1965). Further, Ruderman and Herrera (1968) could show only a small suppression of gluconeogenesis from alanine by 17 mmol glucose in the isolated perfused rat liver, and Exton and Park (1967) found no inhibition of gluconeogenesis from lactate by 19 mmol glucose with similar preparations of rat liver.

In the dog, suppression of endogenous glucose production by infused glucose is thought to be primarily the result of an increased secretion of insulin (Steele *et al.*, 1965). Furthermore, in the depancreatomized dog, endogenous glucose production is not

suppressed by glucose infusions (Ishiwata, Hetenyi and Vranic, 1969). These dogs were given constant infusions of insulin at a rate sufficient to maintain a normal endogenous production rate of glucose but were unable to respond to the glucose infusion by producing insulin. Insulin has been shown to suppress glucose synthesis from pyruvate or lactate in perfused rat livers and in intact rats (see Friedmann, Goodman and Weinhouse, 1970; Exton et al., 1970) possibly by suppressing pyruvate carboxylase activity (Williamson, 1967; Scrutton and Utter, 1968). It is possible that the effect of exogenous glucose on endogenous glucose production in sheep might also be largely mediated through an increased secretion of insulin, in response to raised concentrations of plasma glucose or increased flux rates of glucose through plasma (Boda, 1964; Manns and Boda, 1967; Horino et al., 1968), since intraportal infusions of insulin rapidly inhibit glucose release in sheep (West and Passey, 1967).

Glucose-mediated effects or insulin, or both, may have also diminished the availability of glycerol or gluconeogenic amino acids for glucose synthesis. Bergman (1968) reported that the administration of glucose or insulin to ketotic sheep decreased the production rate of plasma glycerol probably largely through inhibition of lipolysis since glycerol cannot be reutilized for

triglyceride synthesis in adipose tissue (Vaughan, 1961; Khachadurian et al., 1967). The decreased plasma urea concentration indicates a decreased amino acid catabolism during infusions of glucose and is in accord with the report of Potter, Purser and Cline (1968) that intra-arterial infusions of glucose in sheep, 6 and 24 h after feeding depressed plasma amino acid concentrations.

These possible extrahepatic effects of exogenous glucose are probably more effective in suppressing gluconeogenesis in the starved animal which relies in part, or wholly, on the mobilization of endogenous precursors for glucose synthesis. This may, in part, account for the almost complete suppression of glucose synthesis in sheep 24 h after feeding (Annison and White, 1961; West and Passey, 1967) but given infusions of glucose similar to those used in the present study, which were shown to only partly suppress endogenous glucose production (about 40%) in the fed sheep. The linear relationship between the suppression of endogenous glucose production and glucose infused indicates, however, that further suppression of glucose synthesis in fed sheep may have been obtained with glucose infusions in excess of those used in the present study.

PART B: SHORT-TERM EFFECTS OF PROPIONATE, AMINO ACIDS AND BUTYRATE INFUSIONS

Introduction

The strong correlation observed between digestible energy intake and the irreversible loss of plasma glucose in sheep given different roughage diets (Section 3, Part B) suggests that the gluconeogenic rate may be dependent upon the availability of fermentation products absorbed from the digestive tract. Of the major products formed in the rumen, only acetate appears to have little or no immediate effect on glucose metabolism (see literature survey). The possible significance of the other products, propionate, amino acids and butyrate in the control of gluconeogenesis in sheep was examined in this study.

Experimental

Eleven sheep were fitted with ruminal cannulas and a further two with abomasal cannulas. Five of the animals with ruminal cannulas were also prepared with mesenteric-vein catheters.

Sheep given their daily ration in 24 equal portions at hourly intervals were given intravenous infusions of [U-^{14}C]glucose, [6-^{3}H]glucose and NaH$^{14}CO_3$ and intraruminal infusions of [2-^{14}C]-propionate as described in Part A of this section.

Sodium propionate, sodium butyrate or enzymic-hydrolysed casein (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) were administered to sheep following a period in which pre-infusion values were obtained for the concentration and SR of metabolites isolated from blood and ruminal fluid as described for glucose infusions (Section 4, Part A). Sodium propionate and butyrate were of analytical reagent grade and only traces of other VFA were detected by gas-liquid chromatography. These salts were dissolved in distilled water and infused intravenously via the mesenteric vein catheter or intraruminally (for propionate solutions) at rates of 0.2 to 0.6 ml/min. Physiological saline was infused at 0.2 ml/min into the mesenteric vein catheter during the 'control' period of the experiment. When sodium propionate was infused intraruminally during infusions of [2-^{14}C]propionate, the separate leads carrying these substrates to the animal were joined by a Y-piece to the infusion probe within the rumen so that the infused propionate and tracer were mixed before their distribution within the rumen.

Two blood samples of equal volume, taken at 20 to 30 min intervals immediately before or during the completion of the propionate and butyrate infusions were bulked and the VFA isolated for chromatography of individual VFA.

Results

Effect of Propionate Infusions on the Irreversible Loss of Plasma Glucose

Propionate varying between 0.35 and 6.35 mmol/min was infused into the rumen of five sheep at a single rate or at two different successive rates of equal duration. A further three sheep were given intravenous infusions of propionate via the mesenteric vein at 0.99 or 1.92 mmol/min. The irreversible loss of plasma glucose before and during propionate infusions was measured by using a constant infusion of [U-^{14}C]glucose or [6-^{3}H]glucose, or both, lasting 13 to 14 h. Propionate was infused over the last 5 or 6 h of the tracer infusion. Results from typical experiments in which [6-^{3}H]glucose was infused simultaneously with $NaH^{14}CO_3$ or with intraruminal infusions of [2-^{14}C]propionate are given in Figures 4-7 and 4-8 respectively.

In general, the approximately constant SR of plasma glucose obtained with an infusion of labelled glucose decreased in response to a propionate infusion, to plateau 1.5 to 3 h later. The changes in the SR of glucose, which were more rapid with an intravenous than with an intraruminal infusion of propionate (compare Figures 4-7 and 4-8), indicate an increased rate of glucose synthesis. The increment in the irreversible loss of plasma glucose (Y_I, mg/min) was linearly related ($P < 0.001$) to the quantity of propionate infused intra-

Figure 4-7. Effect of an intravenous infusion of propionate on the SR values of plasma glucose and blood bicarbonate and the plasma concentrations of glucose and lactate in sheep 140 during a constant infusion of a mixture of [6-^{3}H]glucose and NaH$^{14}CO_3$. o, SR of glucose (μCi/g C); Δ, SR of glucose (μCi ^{3}H/g); □, SR of blood bicarbonate; •, concentration of glucose; ■, concentration of lactate. Sodium propionate was infused into a mesenteric vein at 0.99 mmol/min between the 480th and 770th min (horizontal bar) of the infusion of tracers.

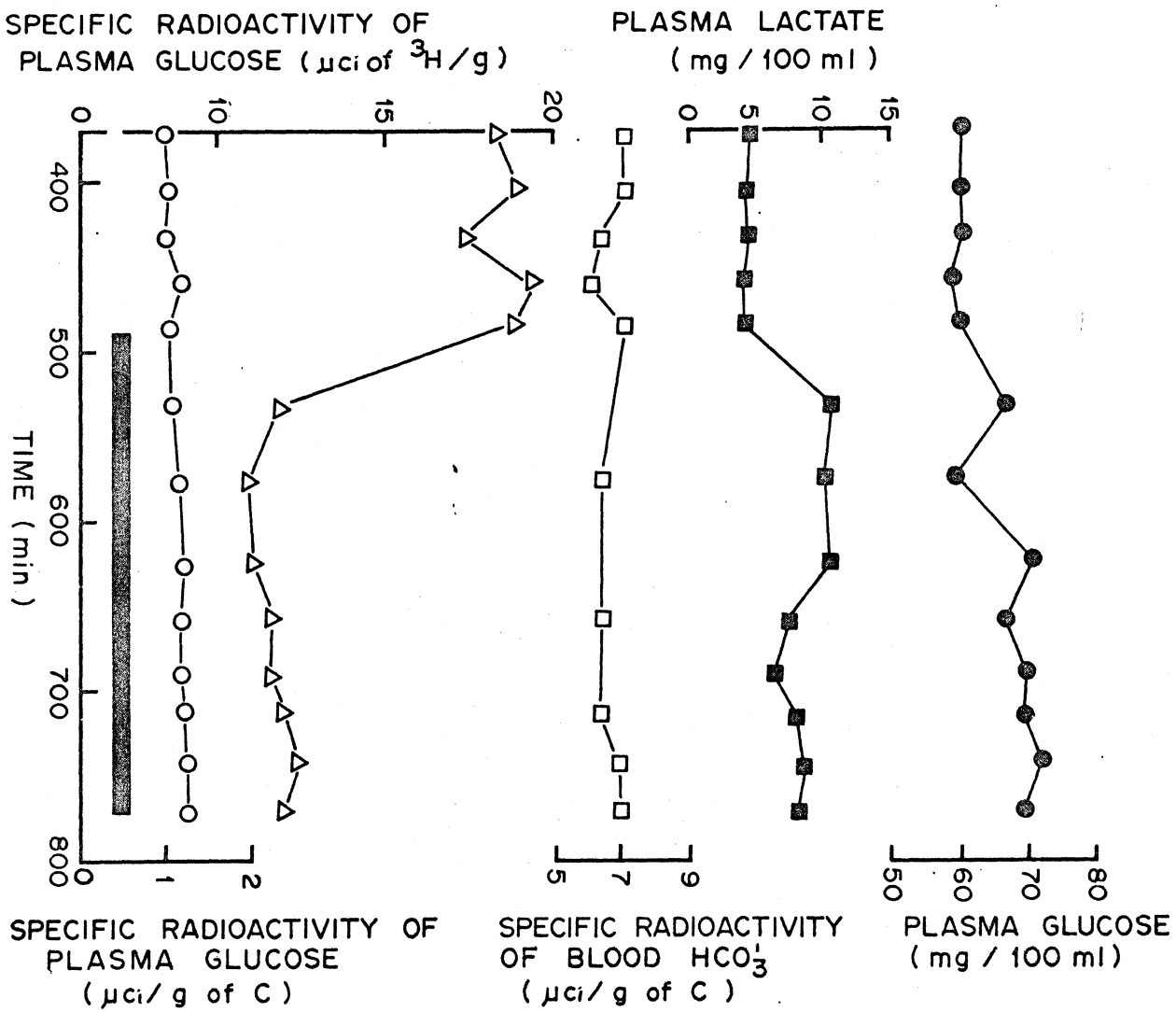
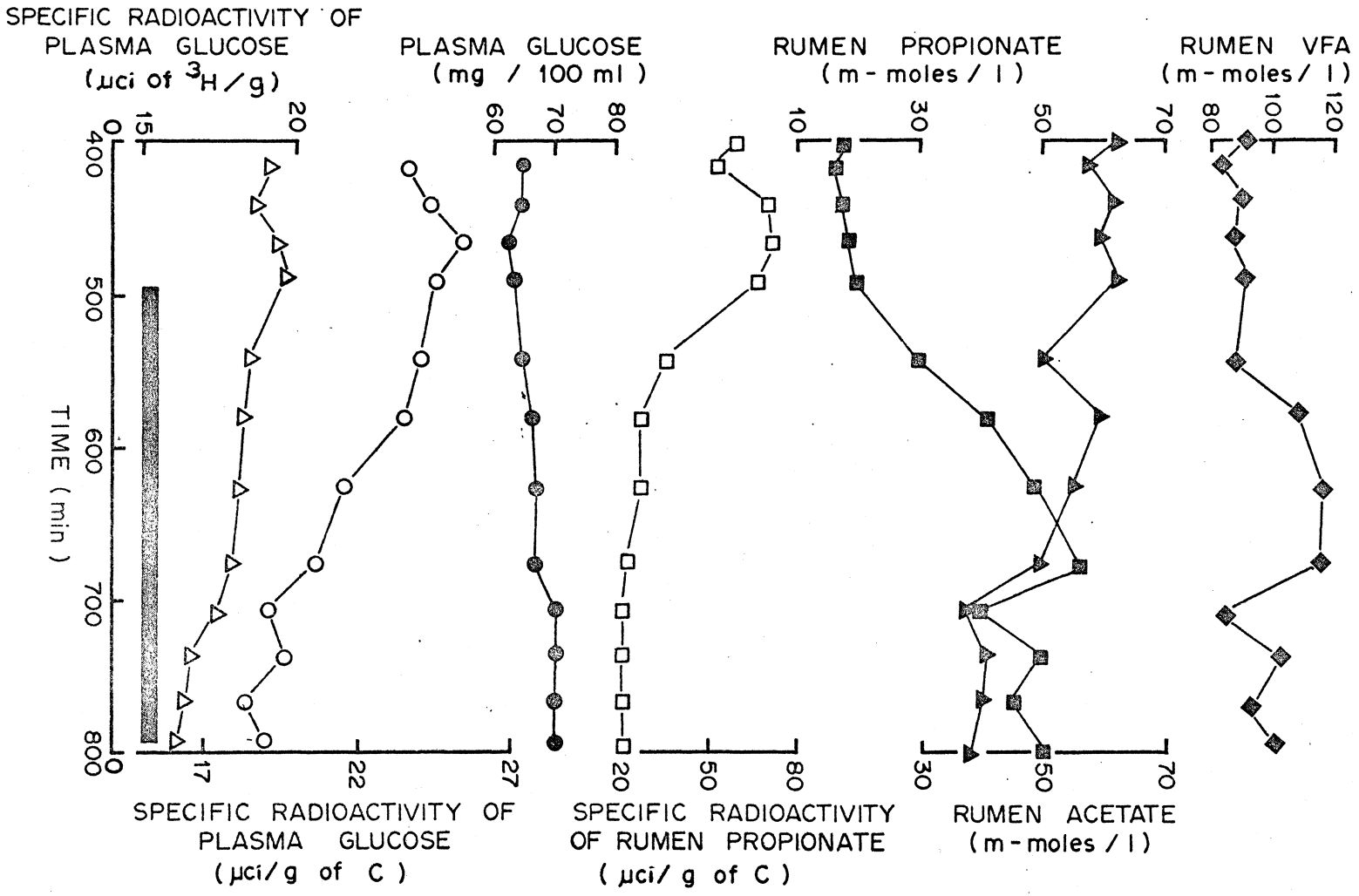

SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (µci of ^3H / g)
PLASMA LACTATE (mg / 100 ml)
TIME (min)
0
400
500
600
700
800
0
10
15
20
0
5
10
15
0
1
2
5
7
9
50
60
70
80
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (µci / g of C)
SPECIFIC RADIOACTIVITY OF BLOOD HCO_3^- (µci / g of C)
PLASMA GLUCOSE (mg / 100 ml)

Figure 4-8. Effect of an intraruminal infusion of propionate on the SR of plasma glucose and ruminal propionate, and the concentrations of plasma glucose and of acetate, propionate and total VFA in ruminal fluid of sheep 175 during an intravenous infusion of [6-^{3}H]glucose and an intraruminal infusion of [2-^{14}C]propionate, administered simultaneously. o, SR of glucose (μCi/g c); Δ , SR of glucose (μCi ^{3}H/g); □ , SR of propionate; ●, concentration of glucose; ■ , concentration of propionate; ▲, concentration of acetate; ◆, concentration of total VFA. Sodium propionate was infused between the 490th and 790th min (horizontal bar) of the tracer infusions.


SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci of ^3H / g)
PLASMA GLUCOSE (mg / 100 ml)
RUMEN PROPIONATE (m-moles / l)
RUMEN VFA (m-moles / l)
TIME (min)
0
400
500
600
700
800
15
20
17
22
27
60
70
80
10
30
50
70
80
100
120
20
50
80
30
50
70
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci/g of C)
SPECIFIC RADIOACTIVITY OF RUMEN PROPIONATE (μci/g of C)
RUMEN ACETATE (m-moles / l)

ruminally (X_P, mmol/min) and was described by the equation:

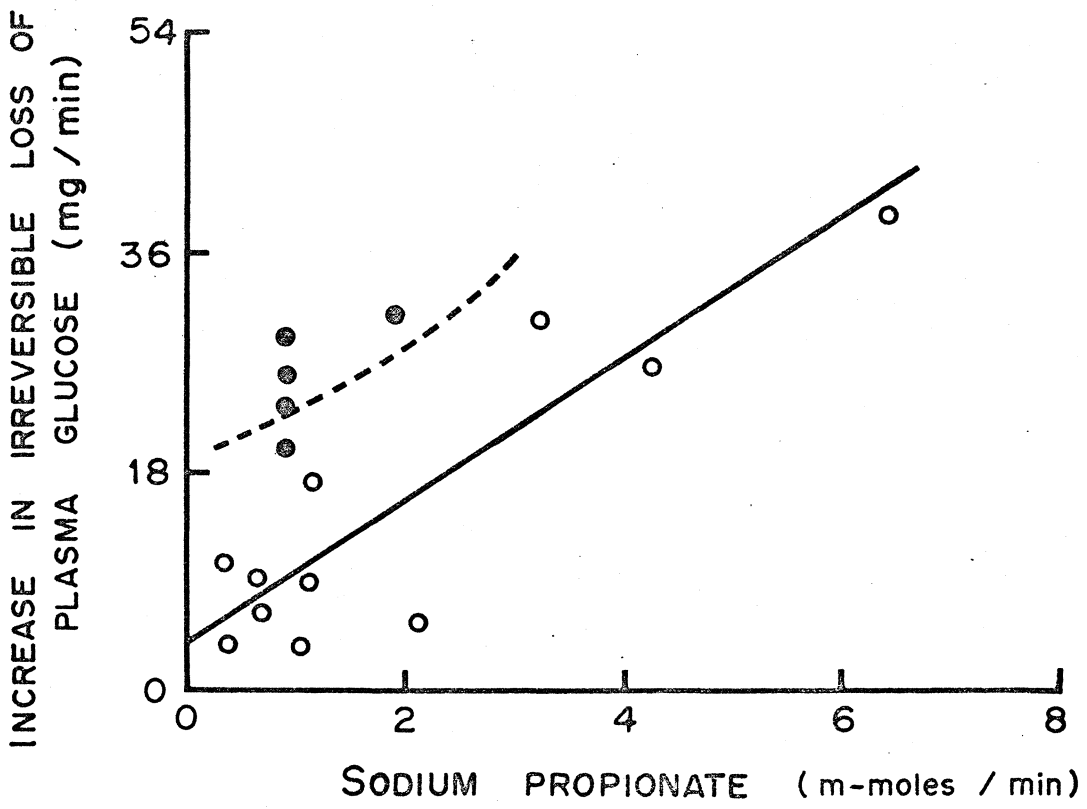
$$Y_I = 3.82 + 5.97\ X_P\ [RSD = \pm 5.13,\ r = 0.914] \quad \ldots 4\text{-}9$$

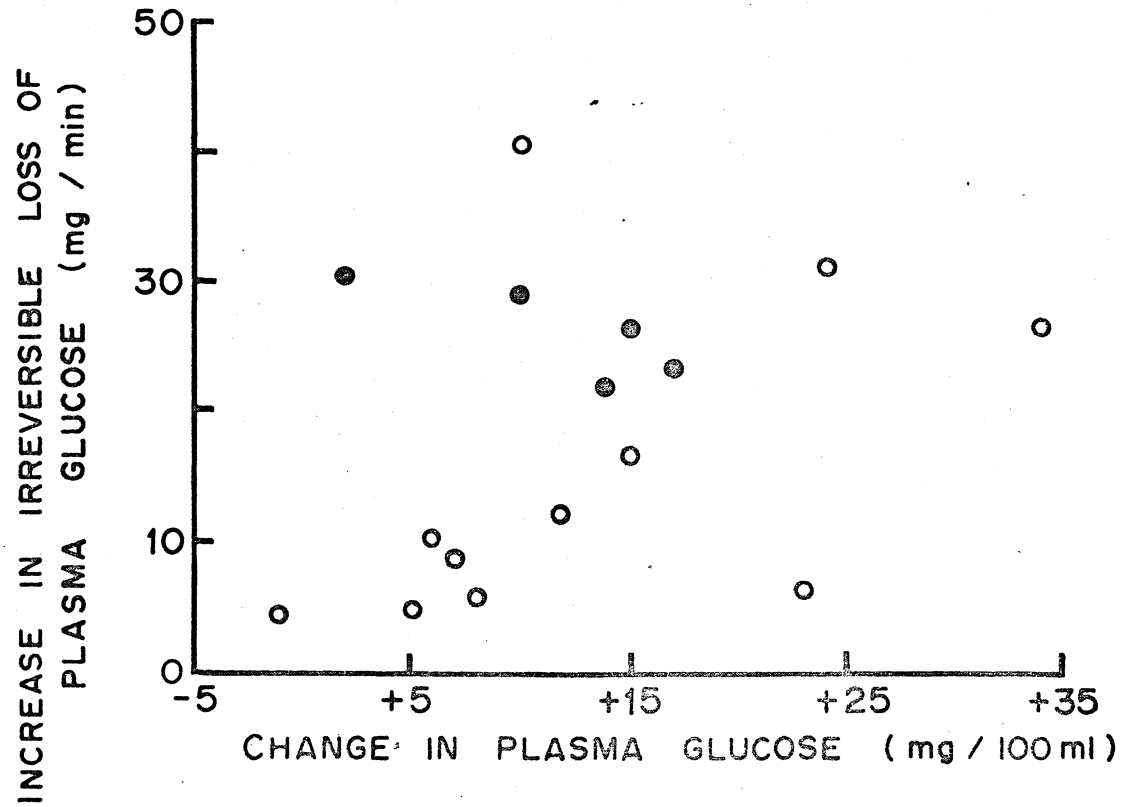
These results are given in Figure 4-9. The broken line on Figure 4-9 is a confidence interval beneath which 95% of observations would be expected to lie. Four out of five results for intravenous infusions were above the line, which suggests that intravenous infusions of propionate elicit a greater response in the irreversible loss of glucose than intraruminal infusions of propionate. Estimates of the irreversible loss of glucose immediately before and during successive 3 h infusions of sodium chloride into the mesenteric vein of a sheep at 0.99 and 1.99 mmol/min were 86, 83 and 80 mg/min respectively; corresponding mean values for plasma glucose concentrations were not significantly different ($P > 0.05$) from each other.

Plasma glucose concentration usually increased in response to an infusion of propionate to attain an approximately constant level some 1 to 3 h later. The change in the mean concentration of plasma glucose was not correlated ($P > 0.05$) with the marked increase in the irreversible loss of plasma glucose ($r = 0.438$) or with the rate of infusion of propionate ($r = 0.324$) (see Figures 4-10 and 4-11a). Plasma lactate concentrations also generally increased during an infusion of propionate to attain approximately constant

Figure 4-9. Relationship between the increment in the irreversible loss of plasma glucose and rate of infusions of sodium propionate into the rumen (o) or into a mesenteric vein (•) in sheep. The regression equation and portion of the 95% confidence limit for individual observations relating these measurements in sheep given intraruminal infusions of propionate are indicated by unbroken and broken lines respectively.

Figure 4-10. Relationship between the increment in the irreversible loss of plasma glucose and the change in plasma glucose concentrations in sheep in response to sodium propionate, infused into the rumen (o) or into a mesenteric vein (•).


INCREASE IN IRREVERSIBLE LOSS OF PLASMA GLUCOSE (mg / min)
54
36
18
0
0
2
4
6
8
SODIUM PROPIONATE (m-moles / min)


INCREASE IN IRREVERSIBLE LOSS OF PLASMA GLUCOSE (mg / min)
50
30
10
0
-5
+5
+15
+25
+35
CHANGE IN PLASMA GLUCOSE (mg / 100 ml)

values about 2 h after the start of the infusion (see Figure 4-7). Figure 4-11b shows the non-significant ($P > 0.05$, $r = 0.449$) relationship between the changes in the mean concentration of plasma lactate and propionate infusions.

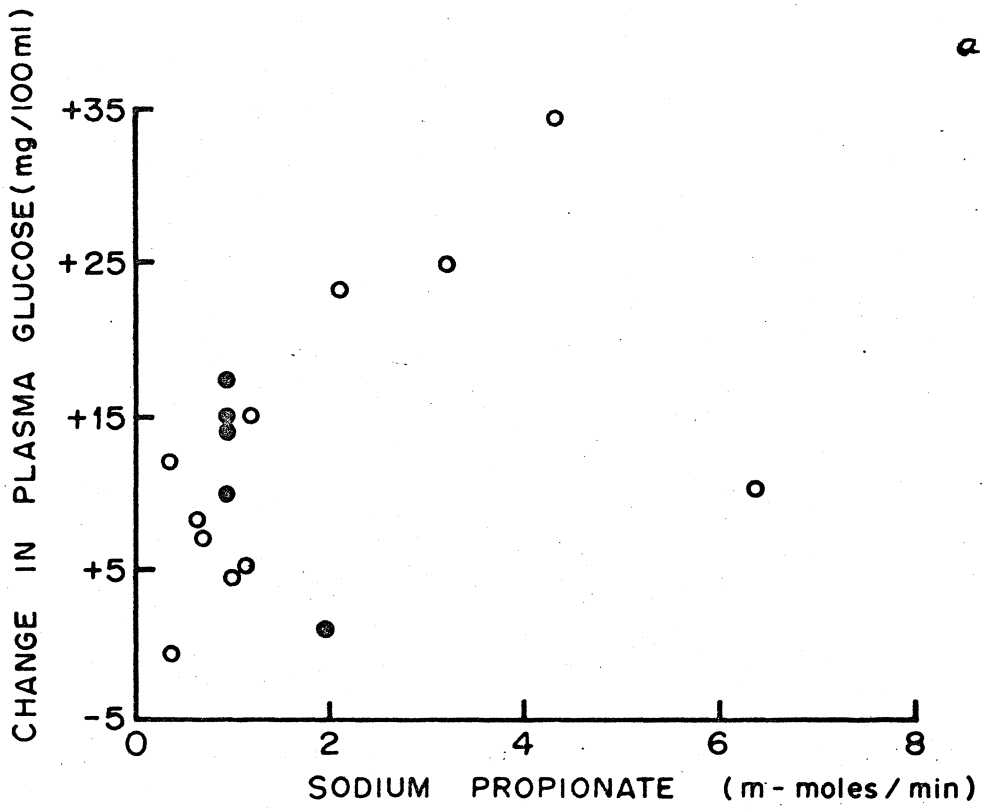
The molar proportion of propionate in VFA isolated from peripheral blood also increased as the propionate increased (Table 4-4). Urea concentrations in plasma were significantly depressed ($P < 0.01$) with intravenous infusions of propionate at 0.99 mmol/min. Mean concentrations (with their standard errors) for three samples taken immediately before and between the 3rd and the 6th h of a propionate infusion were respectively 52 $\pm$ 1.1 and 47 $\pm$ 0.8 for sheep 140 and 51 $\pm$ 0.4 and 42 $\pm$ 0.6 for sheep 143.

Effect of Propionate Infusions on the Fixation of Blood Bicarbonate into Glucose

Since only two reactions, catalysed by propionyl CoA carboxylase and pyruvate carboxylase fix bicarbonate carbon into glucose precursors the total incorporation of carbon-14 into glucose during constant infusions of $NaH^{14}CO_3$ is an indication of the extent of gluconeogenesis through these two pathways.

Mixtures of $NaH^{14}CO_3$ and [6-^{3}H]glucose were infused intravenously and the carbon-14 fixed into glucose and the irreversible loss of glucose were measured before and during intravenous infusions of propionate (see Figure 4-7). The plateau SR of blood bicarbonate

Figure 4-11. Relationships between the changes in plasma concentration of glucose (a) or lactate (b) and rate of infusion of sodium propionate into the rumen (o) or into a mesenteric vein (●) of sheep.


a
CHANGE IN PLASMA GLUCOSE (mg/100ml)
+35
+25
+15
+5
-5
0
2
4
6
8
SODIUM PROPIONATE (m-moles/min)

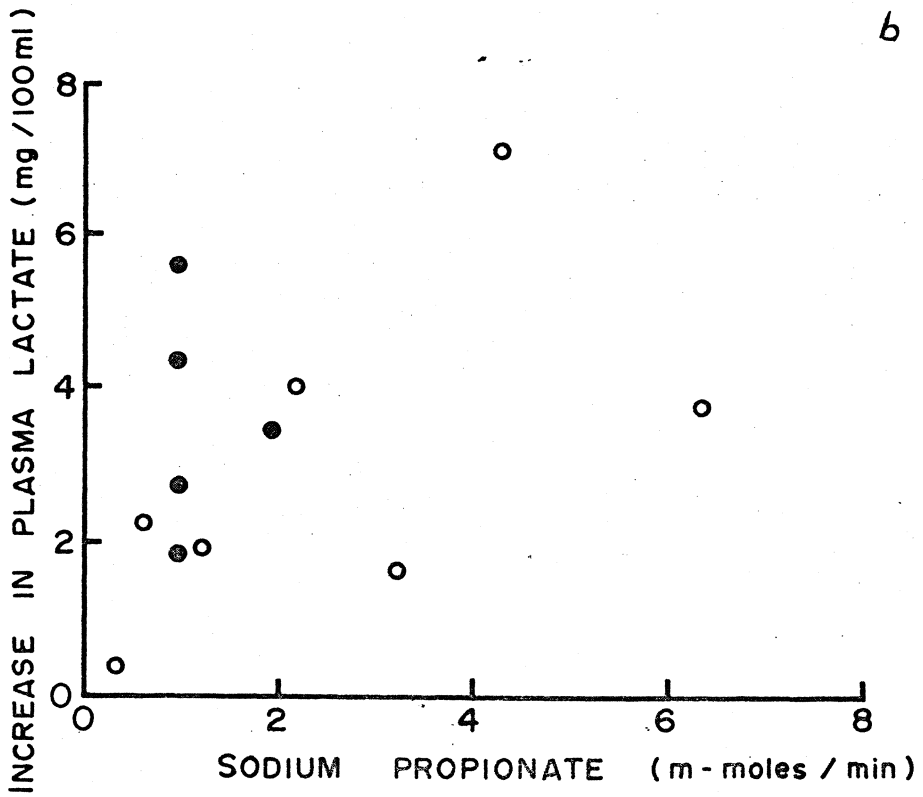

b
INCREASE IN PLASMA LACTATE (mg/100ml)
8
6
4
2
0
0
2
4
6
8
SODIUM PROPIONATE (m-moles/min)

Table 4-4. Molar proportions of individual volatile fatty acids (VFA) in jugular blood of sheep before and during intraruminal or intravenous (via a mesenteric vein) infusions of sodium propionate

Sheep no.	Sheep wt (kg)	Propionate infusion (mmol/min)	Molar percentage of VFA as: Acetic	Propionic	Butyric	Other
140	32.7	-	90.4	2.6	1.7	5.3
		0.99*	72.9	21.4	2.3	3.3
130	32.4	-	94.2	5.9	nd	nd
		1.92*	56.1	43.9	nd	nd
114	34.1	-	97.8	2.2	nd	nd
		0.62	96.6	3.4	nd	nd
175	31.1	-	82.2	6.7	4.8	6.4
		1.12	80.9	13.5	2.1	3.5

nd not determined.

* Intravenous infusions.

≠ Intraruminal infusions.

≠ Isobutyric, isovaleric and valeric acids in the approximate molar proportions of 2:4:1.

attained during the pre-infusion period was maintained during infusions of propionate but the SR of glucose carbon increased to plateau 2 to 3 h later. The mean values for the plateau SR of glucose before and during the propionate infusion were significantly different ($P < 0.05$) indicating a small increase in the proportion of glucose carbon entering through bicarbonate fixation reactions (Table 4-5).

Table 4-5 also includes estimates of glucose oxidized before and during propionate infusions. These estimates were calculated according to the procedure of Depocas and De Freitus (1970) for a two-pool model: in this study, the plasma glucose and blood bicarbonate pools. The proportion of blood bicarbonate originating from glucose was determined by comparing the plateau SR values of carbon of bicarbonate and glucose obtained with infusions of [U-^{14}C]glucose. The SR of blood bicarbonate, which plateaued from about 7 h after the start of an infusion of [U-^{14}C]glucose, decreased for about the first 3 h of the propionate infusion before attaining a plateau value. The proportion of plasma glucose oxidized apparently decreased during infusions of sodium propionate but the apparent rate of glucose oxidation was not altered ($P > 0.05$) since there was an increase in the irreversible loss of plasma glucose.

Table 4-5. Response in percentage of carbon in jugular-blood bicarbonate derived from plasma glucose, and apparent rate of glucose oxidation in sheep to infusions of propionate

Animals received an intravenous infusion of sodium propionate via a mesenteric vein over 5 h. Interconversion rates for carbon between glucose and bicarbonate immediately before and during the infusion were estimated by using intravenous infusions of [U-^{14}C]glucose and NaH$^{14}CO_3$, each lasting about 15 h and administered separately, 5 or 7 days apart.

Sheep no.	Sheep wt (kg)	Propionate infusion (mmol/min)	Irreversible loss of (mg C/min): Plasma glucose	Blood bicarbonate	Glucose derived from bicarbonate (%)	Bicarbonate derived from glucose (%)	Glucose oxidised (%)	Glucose oxidised (mg C/min)
140	32.7	-	23.5	149	16	14	91	21
		0.99	33.1		18	15	70	23
143	30.2	-	21.8	143	13	12	82	18
		0.99	32.0		17	13	60	19

Effect of Propionate on Glucose Synthesis from Ruminal Propionate

Intraruminal infusions of sodium propionate resulted in an increase in the measured production rate of propionate in the rumen (see example in Figure 4-8) but the increment was less than the rate of addition of propionate (see Table 4-7). This increment in propionate production rate, expressed as a percentage of the rate of infusion of propionate was uncorrelated with the rate of infusion ($P > 0.05$, $r = 0.392$) and its mean value (with standard error) was $65 \pm 6.4\%$.

The proportion of propionate in total VFA and the concentration of propionate in ruminal fluid also increased in response to propionate infusions (Table 4-6). The concentrations of the VFA usually became approximately constant about 2 h after the start of the propionate infusion. The decreased proportion of other VFA was usually associated with a decrease in the concentration of these acids in ruminal fluid (see also Figure 4-8). The production rate of propionate (Y_P, mmol/min) during intraruminal infusions of sodium propionate was linearly related ($P < 0.001$) to the concentration of ruminal propionate (X_C, mmol/l). The relationship between Y_P and X_C was:

$$Y_P = -0.242 + 0.0321\ X_C \quad (n = 8)\ [RSD = \pm 0.854,\ r = 0.904] \qquad ...4\text{-}10$$

The regression coefficient for propionate concentration was similar

Table 4-6. Responses in concentrations and molar proportions of volatile fatty acids (VFA) in ruminal fluid of sheep to intraruminal infusions of sodium propionate

Sodium propionate was infused into the rumen for 5 to 6 h, at a single rate or at two different successive rates of equal duration. Mean concentrations and molar proportions of VFA immediately before and during each infusion are given with their standard errors for three to six samples taken at 20 to 30 min intervals.

Sheep no.	Sheep wt (kg)	Propionate infusion (mmol/min)	Total VFA (mmol/l)	Molar percentage of VFA as: Acetic	Propionic	Butyric	Isobutyric	Isovaleric	Valeric
191	40.1	-	78 ± 1	73.6 ± 0.8	14.8 ± 0.3	6.9 ± 0.4	1.5 ± 0.1	2.0 ± 0.1	1.2 ± 0.2
		0.35	90 ± 4	65.1 ± 1.3	23.8 ± 0.7	6.5 ± 0.4	1.7 ± 0.1	1.7 ± 0.1	1.2 ± 0.0
114	34.1	-	99 ± 4	74.1 ± 1.3	17.0 ± 0.8	5.3 ± 0.6	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.2
		0.62	114 ± 2	61.1 ± 1.1	28.0 ± 0.9	6.2 ± 0.2	1.5 ± 0.1	1.7 ± 0.1	1.5 ± 0.0
175	31.1	-	90 ± 2	67.7 ± 0.2	19.7 ± 0.4	7.6 ± 0.1	2.0 ± 0.1	1.7 ± 0.0	1.4 ± 0.1
		1.12	98 ± 6	41.8 ± 1.1	48.3 ± 0.9	5.2 ± 0.1	1.9 ± 0.3	1.5 ± 0.1	1.3 ± 0.1
179	37.4	-	129 ± 4	68.3 ± 0.2	19.4 ± 0.1	7.3 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	1.1 ± 0.1
		0.98	201 ± 9	43.0 ± 1.2	48.2 ± 1.6	4.7 ± 0.4	1.6 ± 0.2	1.4 ± 0.1	1.1 ± 0.0
172	38.0	-	133 ± 3	69.5 ± 1.2	17.4 ± 0.6	8.6 ± 0.2	1.1 ± 0.2	1.9 ± 0.1	1.6 ± 0.4
		2.12	158 ± 9	58.6 ± 0.6	29.1 ± 0.9	7.3 ± 0.1	1.5 ± 0.1	1.8 ± 0.0	1.7 ± 0.1
		4.23	205 ± 1	32.8 ± 0.3	59.7 ± 0.1	4.2 ± 0.1	1.0 ± 0.4	1.3 ± 0.1	1.2 ± 0.1
57	36.7	-	107 ± 6	68.5 ± 0.4	18.1 ± 0.3	8.2 ± 1.0	1.7 ± 0.2	1.5 ± 0.0	2.0 ± 0.1
		3.20	174 ± 4	36.2 ± 0.7	55.9 ± 0.9	4.1 ± 0.1	1.6 ± 0.3	0.8 ± 0.1	1.3 ± 0.1
		6.35	243 ± 8	23.6 ± 0.8	71.0 ± 1.2	2.6 ± 0.2	1.3 ± 0.1	0.6 ± 0.1	1.0 ± 0.1

to the value of 0.0344 derived by Leng (1970b) for the regression coefficient relating these variables in sheep on a wide variety of diets.

The effect of infusion of propionate into the rumen on the net rate of glucose synthesis from ruminal propionate was studied by using, simultaneously, an intravenous infusion of [6-^{3}H]glucose and an intraruminal infusion of [2-^{14}C]propionate (Figure 4-8). Results obtained with this technique were not significantly different ($P > 0.05$) from those values determined with the experimental procedures reported previously (see Section 3) where [2-^{14}C]propionate and [U-^{14}C]glucose were administered to sheep on separate occasions (Table 4-7). The relatively constant SR of carbon of ruminal propionate and plasma glucose decreased in response to infusions of propionate to approximate a constant value about 2 h later; occasionally the SR of plasma glucose carbon decreased slowly throughout the infusion of propionate and these data were not considered for estimation of glucose synthesis from propionate. Comparison of the plateau SR values of carbon of ruminal propionate and plasma glucose shows that the proportion of glucose synthesized from propionate increased in response to propionate infusions (Table 4-7).

Table 4-7. Response in production rate of propionate in the rumen, irreversible loss of plasma glucose and net rate of synthesis of glucose from propionate in sheep to infusions of propionate

Animals were given intraruminal infusions of sodium propionate for 5 to 6 h at a single rate or at two different successive rates of equal duration. Glucose synthesis rate from propionate immediately before and during the administration was estimated by using, simultaneously, an intraruminal infusion of [2-^{14}C]propionate and an intravenous infusion of [6-^{3}H]glucose, each lasting for 13 to 14 h.

Sheep no.	Sheep wt (kg)	Propionate infusion (mmol/min)	Rumen propionate (mmol/l)	Propionate production rate (mmol/min)	Glucose synthesized from propionate (%)	Irreversible loss of plasma glucose* (mg/min)	Rate of synthesis of glucose from propionate* (mg/min)	Propionate produced and converted into glucose* (%)
191	40.1	-	12	0.43	25	60.6 (62.1)	15 (16)	39 (41)
		0.35	21	0.70	46	64.9 (69.4)	30 (32)	48 (51)
114	34.1	-	17	0.89	58	61.4 (53.2)	37 (31)	46 (39)
		0.62	32	1.20	73	69.4 (62.1)	51 (45)	47 (42)
175	31.1	-	18	0.42	38	51.6 (62.1)	20 (24)	53 (63)
		1.12	47	1.21	83	61.4 (76.9)	51 (64)	47 (59)
179	37.4	-	25	0.45	31	56.8	18	44
		0.98	37	1.08	60	61.4	37	38
172	38.0	-	23	0.87	55	48.5	27	34
		2.12	46	1.54	-	55.0	-	-
		4.23	122	3.47	-	75.2	-	-
57	36.7	-	19	0.54	36	65.8	24	49
		3.20	97	3.08	87	97.1	85	31
		6.35	173	6.17	-	106.4	-	-

* Values in parentheses were calculated by using estimates of the irreversible loss of plasma glucose obtained from separate intravenous infusions of [U-^{14}C]glucose. [2-^{14}C]propionate and [U-^{14}C]glucose were administered 5 or 7 days apart.

Effect of Infusions of a Mixture of Amino Acids on Glucose Synthesis

Responses in the irreversible loss of plasma glucose and in the fixation of carbon into glucose from blood bicarbonate to abomasal infusions of enzymic-hydrolysed casein were measured in two sheep by using intravenous infusions of a mixture of [6-^{3}H]-glucose and NaH$^{14}CO_3$, lasting about 15 h. The casein, varying between 57 and 159 mg of total amino acids/min, was administered during the last 7 h of this infusion of isotopes at two different successive rates of equal duration. An example of the results obtained is given in Figure 4-12.

In general, the concentrations of glucose, urea and α-amino nitrogen in plasma increased but varied only slightly after each infusion of casein hydrolysate had been in progress for about 1.5 to 2 h. The approximately constant SR of plasma glucose, labelled with [6-^{3}H]glucose, decreased in response to infusions of casein. However, the SR of glucose was again steady about 2 h after the start of each infusion except for sheep 722 in which the SR of glucose apparently continued to decline during infusions of 158.6 mg casein/min. It was assumed for this infusion rate of casein, that a plateau SR was attained after the 3rd h of the infusion (see Figure 4-12). The approximately constant SR values of carbon of plasma glucose and blood bicarbonate, labelled with

Figure 4-12. Effect of successive abomasal infusions of casein hydrolysate on the SR values of plasma glucose and blood bicarbonate, and plasma concentrations of glucose, urea and α-amino nitrogen in sheep 722 during a constant infusion of a mixture of [6-^{3}H]-glucose and NaH$^{14}CO_3$. o, SR of glucose (μCi/g C); Δ, SR of glucose (μCi ^{3}H/g); □, SR of blood bicarbonate; ●, concentration of glucose; ■, concentration of α-amino N; ▲, concentration of urea. The infusion rates of casein hydrolysate between the 530th and 730th min and the 730th and 910th min (horizontal bars) of the infusion of tracers were 77.7 and 158.6 mg/min respectively.

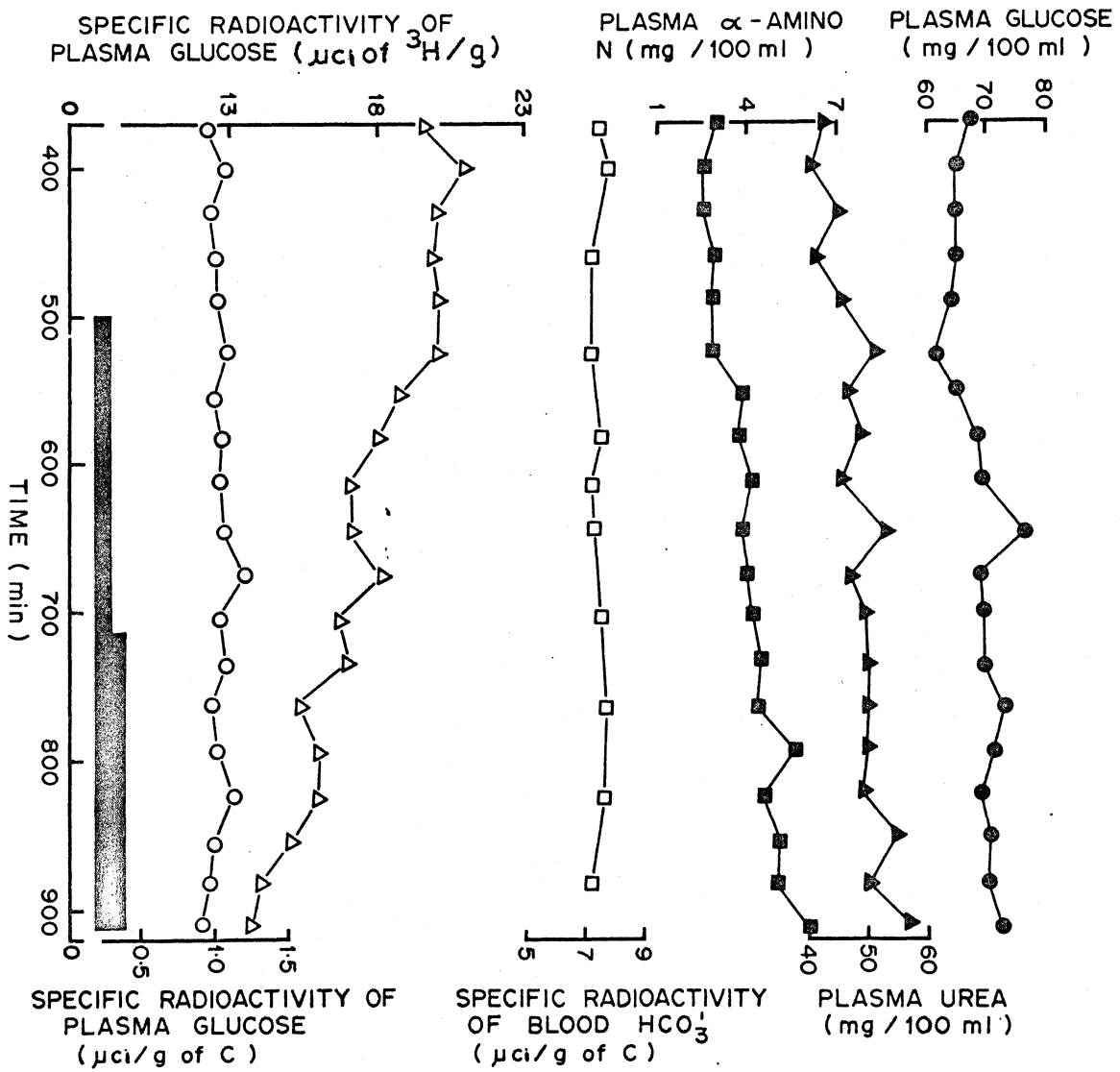

SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci of ^3H/g)
PLASMA α-AMINO N (mg /100 ml)
PLASMA GLUCOSE (mg / 100 ml)
TIME (min)
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci/g of C)
SPECIFIC RADIOACTIVITY OF BLOOD HCO_3^- (μci/g of C)
PLASMA UREA (mg / 100 ml)

Table 4-8. Response in plasma glucose, urea and α-amino nitrogen concentrations, irreversible loss of plasma glucose and net rate of synthesis of glucose carbon from blood bicarbonate in sheep to infusions of casein hydrolysate

Animals received intra-abomasal infusions of casein hydrolysate for 7 h at two different successive rates of equal duration. Glucose synthesis rate and fixation of bicarbonate immediately before and during the infusions were estimated by using an intravenous infusion of a mixture of [6-^{3}H]glucose and NaH$^{14}CO_3$, lasting for about 15 h. Mean concentrations of plasma substrates are given with their standard errors for three to seven samples taken at 20 to 30 min intervals.

Sheep no.	Sheep wt (kg)	Casein hydrolysate infusion (mg/min)	Plasma concentration* (mg/100 ml) of: Glucose	Urea	α-Amino nitrogen	Irreversible loss of: Plasma glucose (mg/min)	Blood bicarbonate** (mg C/min)	Glucose derived from bicarbonate** (%)	Synthesis of glucose from bicarbonate (mg C/min)
256	44.2	-	65 ± 0.7	45 ± 0.7^{a}	3.2 ± .06	53.8			3.0
		57.5	70 ± 1.1	50 ± 0.4^{b}	3.7 ± .15	59.9	145	14	3.4
		112.2	75 ± 0.2	49 ± 1.1^{ab}	4.7 ± .15	70.4			3.9
722	43.2	-	66 ± 0.6^{a}	44 ± 1.7	2.7 ± .09	49.8			2.8
		77.7	70 ± 1.7^{ab}	49 ± 1.1	3.9 ± .08	57.8	134	14	3.2
		158.6	71 ± 0.9^{b}	56 ± 4.1	5.2 ± .31	73.0			4.1

* For each sheep values with the same superscript a or b were not significantly different ($P > 0.05$) from each other.

** These values were not altered with intra-abomasal infusions of casein hydrolysate (see text).

$NaH^{14}CO_3$, were not altered by infusing casein. A summary of the results appears in Table 4-8.

The increment in the irreversible loss of plasma glucose (Y_I, mg/min) was linearly related ($P < 0.001$) to the rate of infusion of casein hydrolysate (X_A, mg/min). The relationship between Y_I and X_A was:

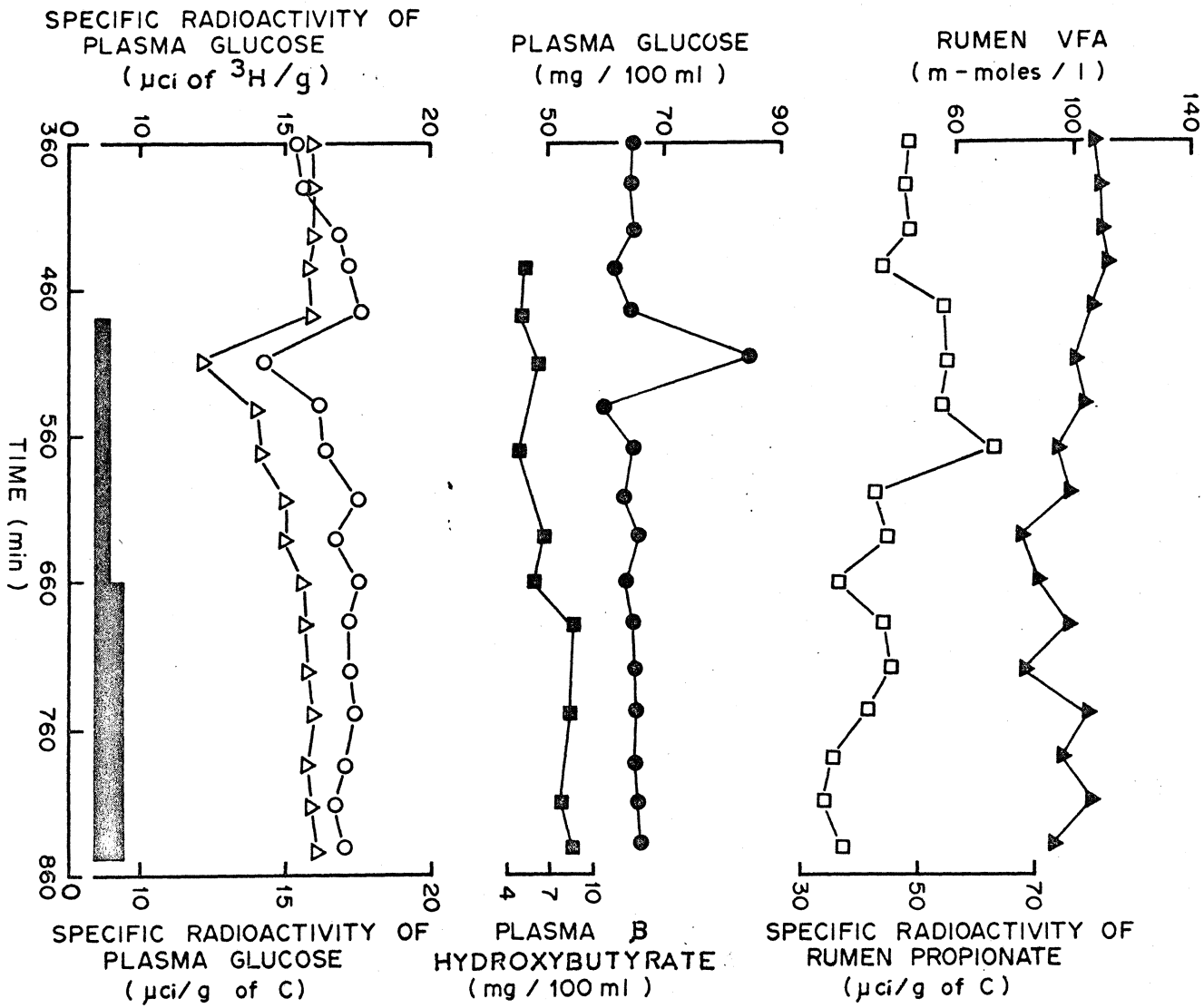
$$Y_I = -4.55 + 0.178\ X_A \quad (n = 4)\ [RSD = \pm 1.31,\ r = 0.991] \qquad ...4\text{-}11$$

Effect of Infusions of Butyrate on Glucose Synthesis from Propionate

Two sheep were given, simultaneously, an intravenous infusion of [6-^{3}H]glucose and an intraruminal infusion of [2-^{14}C]propionate, lasting about 14 h. Sodium butyrate was infused into a mesenteric vein during the last 6 h of the tracer infusions at 0.25 mmol/min for 3 h and increased to 0.50 mmol/min for a further 3 h. Responses in the SR and concentration of plasma glucose to butyrate in each sheep were similar and the mean results are shown in Figure 4-13. A transient increase in plasma glucose concentration concomitant with a decrease in the SR of glucose occurred immediately after the start of the butyrate infusion. These values returned to approximate pre-infusion values within 2 h and were not altered by the additional infusion of butyrate.

The irreversible loss of plasma glucose and the net rate

Figure 4-13. Effect of successive intravenous infusions of sodium butyrate on the SR values of plasma glucose, and plasma concentrations of glucose and β-hydroxybutyrate during an intravenous infusion of [6-^{3}H]glucose and an intraruminal infusion of [2-^{14}C]propionate, administered simultaneously. The results are the means for sheep 130 and 183. o, SR of glucose (μCi/g C); Δ, SR of glucose (μCi ^{3}H/g); □ , SR of ruminal propionate; ●, concentration of glucose; ■ , concentration of β-hydroxybutyrate; ▲, concentration of total VFA in ruminal fluid. The infusion rates of butyrate from the 480th to 660th min and the 660th to 840th min (horizontal bars) of the tracer infusions were 0.25 and 0.50 mmol/min respectively.


SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci of ^3H/g)
PLASMA GLUCOSE (mg / 100 ml)
RUMEN VFA (m-moles / l)
TIME (min)
0
360
460
560
660
760
860
10
15
20
50
70
90
60
100
140
4
7
10
30
50
70
SPECIFIC RADIOACTIVITY OF PLASMA GLUCOSE (μci/g of C)
PLASMA β HYDROXYBUTYRATE (mg / 100 ml)
SPECIFIC RADIOACTIVITY OF RUMEN PROPIONATE (μci/g of C)

of synthesis of glucose from propionate are given in Table 4-9 and were calculated from a consideration of the plateau SR values of glucose immediately before and during the butyrate infusion. Approximate estimates of glucose synthesis from propionate during the transient increase in plasma glucose concentrations are included in Table 4-9 and were calculated by using the SR values associated with the maximum response recorded in glucose concentration. These calculations indicate that although the proportion of plasma glucose derived from ruminal propionate decreased markedly during the transient change in glucose production, the net rate of glucose synthesis from propionate was altered only slightly.

Mean concentrations of β-hydroxybutyrate and urea in plasma before and during infusions of butyrate are given in Table 4-9. In general, the concentrations of β-hydroxybutyrate and urea increased and decreased respectively in response to butyrate but these changes were usually not significant ($P > 0.05$). The molar proportion of butyrate in VFA in jugular blood increased with infusions of butyrate. These changes were similar for the two sheep and the mean molar percentages immediately before and during butyrate infusions at 0.25 and 0.50 mmol/min were respectively 96.0, 94.5 and 92.8 for acetate, 3.3, 3.3 and 3.4 for propionate and 0.7, 2.2 and 3.8 for butyrate.

Table 4-9. Response in glucose, β-hydroxybutyrate and urea concentrations, irreversible loss of plasma glucose and net rate of synthesis of glucose from ruminal propionate in sheep to infusions of butyrate

Animals received infusions of sodium butyrate via a mesenteric vein for 6 h at two different successive rates of equal duration. Glucose synthesis rate from propionate immediately before and during the butyrate infusions was estimated by using, simultaneously, an intravenous infusion of [6-^{3}H]glucose and an intraruminal infusion of [2-^{14}C]propionate, each lasting for about 14 h. Mean concentrations of plasma substrates are given with their standard errors for two to six samples, taken at 20 to 30 min intervals.

Sheep no.	Sheep wt (kg)	Butyrate infusion (mmol/min)	Plasma concentration* (mg/100 ml) of: Glucose	β-hydroxy-butyrate	Urea	Total VFA in ruminal fluid** (mmol/l)	(molar % as propionate)	Propionate production rate (mmol/min)	Glucose synthesized from propionate (%)	Irreversible loss of plasma glucose (mg/min)	Rate of synthesis of glucose from propionate (mg/min)	Propionate produced and converted into glucose (%)
130	31.8	-	67±0.5	5.6±.00^{a}	65±0.8^{a}				42	64.5	27	45
		0.25	79			101±3	23.1±.8	0.67	34′	81.3′	28′	46′
		0.25	64±0.5^{a}	6.3±.63^{a}	61±0.5^{a}				42″	64.5″	27″	45″
		0.50	65±0.2^{a}	7.2±.46^{a}	62±0.6^{a}							
183	31.8	-	62±0.5^{a}	5.3±.05^{a}	61±1.3^{ab}				34	62.5	21	42
		0.25	89			97±3	21.2±.4	0.55	28′	85.5′	25′	51′
		0.25	64±1.0^{ab}	5.3±.36^{a}	60±0.1^{a}				33″	63.3″	21″	42″
		0.50	64±0.5^{b}	9.2±.40^{a}	57±0.7^{b}							

* For each sheep, values with the same superscript a or b were not significantly different ($P > 0.05$) from each other.

** Mean concentrations of total volatile fatty acids (VFA) are given for seventeen samples taken at 20 min intervals and the mean proportions of total VFA as propionate are given for six or seven samples taken at 40 to 90 min intervals.

′ Approximate values corresponding to the initial hyperglycaemic response to the butyrate infusion (see text).

″ These values were approximately constant during infusion of butyrate at 0.25 and 0.5 mmol/min.

Discussion

Effect of Propionate on Gluconeogenesis

The present experiments demonstrate that an increased availability of propionate in sheep given a lucerne diet stimulated an increase in the irreversible loss of plasma glucose and that this increase appeared to be dependent on the quantity of propionate infused. The response in glucose production to intraruminal infusions of propionate was due to an increase in synthesis of glucose from ruminal propionate. Similarly, the marked increase in the rate of fixation of carbon from blood bicarbonate into plasma glucose when propionate was infused into the mesenteric vein suggests an enhanced rate of gluconeogenesis. The extra glucose was probably synthesized from infused propionate since intraportal infusions of sodium propionate have been shown to increase the proportion of plasma glucose derived from propionate in fed sheep (Bergman, Roe and Kon, 1966). The apparently greater response in gluconeogenesis to intravenous than to intraruminal infusions of propionate (see Figure 4-9) may have been due to the accumulation of sodium propionate in the rumen inhibiting microbial production of propionate. The procedure used in this study for measuring the propionate produced in the rumen is unlikely to result in appreciable error (see Leng, 1970b) and it seems likely that the consistently lower increment in rate of

production of propionate compared with the rate of addition of propionate to the rumen was real. There is evidence that rate of growth of ruminal micro-organisms increases upon dilution (El-Shazly and Hungate, 1965; Hobson, 1965; Hobson and Summers, 1967), which may indicate a possible inhibitory effect of end-products of microbial metabolism (Walker and Forrest - quoted by Walker, 1965).

Propionate absorbed from the rumen of the sheep is largely removed from portal blood in its passage through the liver. The capacity of the liver or other tissues to remove propionate, however, was apparently exceeded in the present study, when propionate was infused into either the rumen or mesenteric vein. Smith and Marston (1971) calculated from in vitro studies that the maximum rate of utilization of propionate by liver of a 30 kg sheep was about 0.5 mmol/min, equivalent to the synthesis of approximately 45 mg glucose/min if all the propionate was converted into glucose. This estimate of glucose synthesis from propionate is only about half the recorded maximal production of glucose from ruminal propionate in the present study (see Table 4-7). In the intact animal, the conversion of propionate into glucose or into other glucogenic precursors may have occurred also in tissues other than liver.

Krebs and Yoshida (1963) and Weidemann and Krebs (1969) have shown that kidney cortex slices from sheep readily form glucose from propionate. However, the contribution of this organ to total glucose synthesis in the intact sheep appears to be quantitatively insignificant (Kaufman and Bergman, 1971), but this may not be so when circulating levels of propionate or lactate are raised by infusions of propionate.

Rates of production of lactate may have increased with propionate infusions, as indicated by the increased concentrations of plasma lactate (Annison et al., 1963b). In support of this suggestion Leng et al. (1967) reported that as much as 70% of the ruminal propionate converted into glucose in fed sheep first formed lactate and Annison et al. (1963a) showed that intraportal infusions of substantial quantities of [^{14}C]propionate into anaesthetized sheep also led to the labelling of lactate. It is possible that much of this lactate was produced from propionate by extrahepatic tissue such as ruminal epithelium (see literature survey), and that its subsequent conversion into glucose by liver or kidney tissue provided a significant proportion of the glucose synthesized during propionate infusions.

Lactate, however, may also have been produced from propionate in liver (Leng and Annison, 1963) and from the

resulting increase in glucose utilization during propionate infusions, although the latter pathway was probably not of importance since little or no increase in plasma lactate was observed in the previous study with fed sheep given intravenous infusions of glucose. Unlike ruminal epithelium (Young et al., 1969), sheep liver contains little or no detectable NADP-malate dehydrogenase activity (Hardwick, 1966; Ballard and Hanson, 1967). Any synthesis of lactate from propionate in liver probably occurs by the oxidation of succinate to oxaloacetate and the conversion of the latter into phosphoenolpyruvate before the synthesis of pyruvate and lactate. Hence, extensive conversion of propionate into lactate in liver during propionate infusions could indicate that the activity of pyruvate kinase or the activity of enzyme(s) distal to phosphoenolpyruvate in the gluconeogenic pathway were limiting glucose synthesis.

The majority of glucogenic intermediates in ruminants enter the tricarboxylic acid cycle before they are oxidized or converted into glucose (see Black et al., 1968; Leng, 1970a). The simplest explanation for the apparent suppression of the contribution of substrates other than propionate to glucose synthesis during intraruminal infusions of propionate (Table 4-7) is the dilution of their carbon by propionate in the glucogenic pathway, particularly as the percentage of propionate absorbed

from the rumen and converted into glucose was not markedly altered but provided a greater proportion of the glucose synthesized. Lactate or other glucogenic precursors arising from propionate metabolism (e.g. amino acids, see Black and Kleiber, 1958) may also have the same effect as propionate. A possible increase in protein synthesis or decrease in protein catabolism with VFA infusions, as indicated by a diminution in the plasma concentration of urea (present study) and amino acids (Potter et al., 1968; Halfpenny, Rook and Smith, 1969) is unlikely to result in any marked reduction in the availability of amino acids for gluconeogenesis since with butyrate infusions glucose synthesis from propionate or other precursors was not altered.

Energy expenditure of sheep was apparently not altered by intravenous infusions of sodium propionate (see Table 4-5) although a greater proportion of this energy was probably derived from propionate catabolism (Bergman et al., 1966). Similarly, an increased catabolism of amino acids (see below) did not alter the irreversible loss of blood bicarbonate (Table 4-8). It is possible that an increased activation of propionate or an increased oxidation of glucose arising from propionate or amino acids spared the oxidation of other substrates. The small increase in the oxidation rate of glucose observed here (Table 4-5) may not reflect

the true extent of this change since the SR of bicarbonate of jugular blood and not mixed venous blood was used to calculate the contribution of glucose carbon to total bicarbonate production. Mixed venous blood is required since the brain of sheep catabolizes glucose but not acetate (McClymont and Setchell, 1956) and acetate is a major precursor of respired CO_2 of fed sheep (Annison et al., 1967). The severe inhibitory effect of propionate on acetate oxidation in isolated tissues from ruminants (Pennington, 1957; Pennington and Appleton, 1958; Pritchard and Tove, 1960; Leng and Annison, 1963) was not supported by Davis, Brown and Staubus (1960) who obtained a much smaller inhibition of propionate on acetate metabolism (about 25%) in intact cattle. The low levels of ATP citrate lyase and NADP-malate dehydrogenase normally present in ruminant tissues implies that propionate can make little contribution to fatty acid synthesis or supply reducing equivalents in the form of NADPH although it may contribute significantly to glycerol formation.

Effect of Amino Acids on Gluconeogenesis

Amino acid carbon is believed to be a major source of glucose carbon in ruminants. Because of the large number of glucogenic amino acids the measurement of the proportion of plasma glucose derived from amino acids in the intact animal is difficult,

particularly as the SR of the circulating amino acids may not be representative of those amino acids converted into glucose (see Reilly and Ford, 1971). In the present study a constant infusion of $NaH^{14}CO_3$ was administered intravenously with [6-^{3}H]glucose to determine the rate and indicate the source of substrate for glucose synthesis in sheep given abomasal infusions of enzymic-hydrolysed casein.

The quantity of hydrolysate infused into the abomasum was positively correlated with the increase in irreversible loss of plasma glucose. The contribution of amino acids to glucose synthesis may have increased as indicated by the raised levels of α-amino nitrogen and urea in plasma. It is unlikely that any amino acids from the casein hydrolysate reached the large intestine since casein placed into the abomasum of sheep at comparable rates is completely digested and absorbed (Reis and Schinckel, 1961; Blaxter and Martin, 1962). Reilly and Ford (1971) reported that the apparent rate of conversion of amino acids into glucose was linearly related to the production rate of plasma amino acids.

Although only two animals have been examined it appears from a consideration of equation 3 that for every 100 g casein hydrolysate administered to sheep approximately 13 g of extra plasma glucose were synthesized. This is considerably less than the theoretical maximum of about 57 g glucose if all the glucogenic amino acids

were deaminated and converted into glucose (Krebs, 1964b). Lindsay and Williams (1971) reported an increased production of glucose of 27 to 60 g/day in fed sheep when given an abomasal infusion of 100 g casein daily for 3 days. The increase in glucose production, however, need not indicate the quantity of glucose derived from the infused substrate as discussed earlier for intraruminal infusion of [^{14}C]propionate. If infused amino acids did contribute substantially to glucose synthesis in this study, it is of interest to note that the proportion of glucose carbon derived from blood bicarbonate was not altered. Casein is composed of a high proportion of glucogenic amino acids which do not require a carboxylation step for their conversion into glucose. One possible explanation for this anomaly is that infused amino acids, such as glutamate, might have been partly degraded in extrahepatic tissue and transported to the liver in the form of alanine (see Krebs, 1969; Felig, Pozefsky, Marliss and Cahill, 1970). Alanine readily forms glucose (see Black et al., 1968) and its deamination probably occurs almost exclusively by transamination to pyruvate (Krebs, 1964b).

Effect of Butyrate on Gluconeogenesis

Black et al. (1966) and Leng (1970a) have proposed that butyrate may be an important modulator of gluconeogenesis in

ruminants since butyrate absorbed from the rumen is metabolized preferentially by ruminal epithelium and liver and provides a source of acetyl CoA or butyryl CoA which can activate pyruvate carboxylase (Keech and Utter, 1963; Wallace and Utter, quoted by Ballard et al., 1969).

Many studies, including those of Jarrett, Potter and Filsell (1952), Ash, Pennington and Reid (1964) and Phillips et al. (1965) have shown that injections of butyrate produce a hyperglycaemic response in ruminants. In the present study the transient increase in plasma glucose concentration initiated by intramesenteric vein infusions of small quantities of sodium butyrate (0.25 mmol/min) was not associated with any change in the net rate of conversion of ruminal propionate to plasma glucose. Further it is unlikely that this transient increase in glucose production was due to the stimulation of pyruvate carboxylase activity since plasma lactate is labelled extensively during intraruminal infusions of [2-^{14}C]propionate (Leng et al., 1967).

The most probable explanation for the transient increase in the rate of glucose production in sheep was the mobilization of liver glycogen. Phillips et al. (1965) have reported that an increased phosphorylase activity in the liver of lambs given injections of sodium butyrate was associated with the hyperglycaemia.

This effect of butyrate is believed to be mediated by increased secretions of glucagon (Phillips, House, Miller, Mott and Sooby, 1969). In the present study infused butyrate entered the general circulation and could have stimulated glucagon secretion by the pancrease (Phillips and Black, 1966; Manns, 1969).

The increases in the concentration and production of plasma glucose obtained with infusions of propionate, amino acids or butyrate were usually of sufficient magnitude to suppress glucose synthesis in fed sheep (see Part A). It was suggested that this inhibitory effect of plasma glucose on gluconeogenesis was probably largely mediated through an increase in insulin secretion. Propionate and butyrate also stimulate insulin secretion in ruminants and there is some evidence that the digestion of protein in the alimentary tract of sheep was associated with an increased secretion of insulin (see literature survey). However, associated increases in glucagon secretion were also possible with infusions of propionate or amino acids which may have antagonized glucose-mediated or insulin effects on glucose synthesis.

The maximal rates of glucose synthesis recorded in this study with infusions of propionate or casein hydrolysate were considerably less than those reported for pregnant or lactating sheep (see Leng, 1970a). However, the increased response

in glucose synthesis to these infusions were linear, indicating that the maximal capacity of the animal for gluconeogenesis was not attained, especially if these two substrates were additive in stimulating gluconeogenesis. The availability of these substrates may in part account for the differences in the irreversible loss of plasma glucose in sheep given roughage diets of different energy and protein content (see Section 3). In accord with this suggestion Katz and Bergman (1969) reported that hepatic glucose production in sheep was greatest 2 to 4 h after feeding, which probably coincided with maximal production of the glucogenic precursors propionate and microbial protein in the rumen (see Gray, Weller, Pilgrim and Jones, 1966).

In conclusion, whenever extra glucogenic substrates were given to sheep its rate of synthesis of glucose increased, indicating that the animal has at all times a capacity to synthesize more glucose than it apparently does. Approximately one-third of the substrate added was channelled into glucose. The control of glucose metabolism in the fed ruminant appears to be centred around the availability of glucogenic precursors and the effect these have on hormonal secretions, particularly those of the pancreas.