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VOLATILE FATTY ACID METABOLISM IN SHEEP. 3. DIURNAL VARIATION IN THE CONTRIBUTION OF RUMINAL PROPIONIC ACID PRODUCTION TO THE WHOLE BODY GLUCOSE TURNOVER OF MERINO SHEEP FED LUCERNE HAY TWICE DAILY

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Abstract

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Three phases of the diurnal feeding cycle, namely, a pre-feeding phase (−2–0 h), a maximum fermentation (2–4 h) phase and a basal fermentation (8–10 h) phase, were chosen for investigation into the contribution of ruminal propionic acid production to the whole body glucose turnover, since near steady-state conditions in the ruminal volatile fatty acid (VFA) pool were obtained during these periods. Total VFA levels of 113,5–102,2; 156,1–151,1 and 90,8–89,8 meq/l respectively, were found during these 3 phases and parallel changes also occurred in the propionic acid pool (18,7–17,2; 31,5–29,5 and 16,1–15,6 meq/l). The acetic/propionic and propionic/butyric acid ratios revealed that propionic acid increased more than the other acids during the 2–4 h segment.

The diurnal cycle of the total VFA production rate followed the overall concentration pattern with the exception of the maximum fermentation segment (5,67–6,14 moles/12 h), in which an 89–105% increase over basal levels (3,00 moles/12 h) was significantly higher than the corresponding increase of 74–68% in total VFA concentration. The percentage production rate of propionic acid was statistically indistinguishable from its molar percentage concentration except for the pre-feeding segment in which the production rate (13,6±1,0%) was lower than the concentration (16,5±0,9 molar %) at −2 h.

The glucose entry rate remained almost constant over the course of the feeding cycle and averaged 4,07 mg/min/kg^{0,75} over the 3 periods. However, there was a significant difference in glucose pool (392,5±84,4–257,6±64,6 mg/kg^{0,75}) and rate constant (1,05±0,21–1,71±0,15% of pool/min) between the 1st and 2nd phases, respectively.

A constant fraction of the propionic acid production in the rumen (34±6%) served as precursor for gluconeogenesis. The equation describing this linear relationship between the total (x) and that fraction (y) of the propionic acid production converted to glucose was found to be $y=0,314\ x\ +0,02$ when both x and y were expressed in moles/12 h.

A direct relationship between plasma insulin levels and the respective glucose fractional entry rate (% of pool/min) was found, supporting the hypothesis that insulin acts through control of the rate of peripheral uptake.

Résumé

MÉTABOLISME DES ACIDES GRAS VOLATILS CHEZ LE MOUTON 3. VARIATION DIURNE DE L'APPORT D'ACIDE PROPIONIQUE PRODUIT DANS LE RUMEN AU TURNOVER DU GLUCOSE DANS TOUT L'ORGANISME CHEZ DES MÉRINOS NOURRIS DE FOURRAGE DE LUZERNE DEUX FOIS PAR JOUR

Pour examiner la contribution de l'acide propionique produit dans le rumen au turnover du glucose dans tout l'organisme, on a choisi trois phases du cycle d'alimentation diurne, à savoir une phase pré-alimentaire (−2–0 h), une phase de fermentation maximale (2–4 h) et une phase de fermentation basale (8–10 h): ces phases correspondent en effet à des états d'équilibre du pool des acides gras volatils (VFA) du rumen. Pendant ces 3 phases, on a trouvé, respectivement, des taux de VFA totalisant 113,5–102,2; 156,1–151,1 et 90,8–89,8 meq/l; des variations parallèles se sont aussi produites dans le pool d'acide propionique (18,7–17,2; 31,5–29,5 et 16,1–15,6 meq/l). Les rapports des acides acétiques/propioniques et propioniques/butyriques ont montré que l'acide propionique augmente plus que les autres acides pendant le segment 2–4 h.

Le cycle diurne de la production totale des VFA suivait le schéma général de la concentration, sauf pour le segment de fermentation maximale (5,67–6,14 moles/12 h) durant lequel une augmentation de 89–105% par rapport aux niveaux de base (3,00 moles/12 h) s'est avérée significativement supérieure à l'augmentation correspondante de la concentration totale en VFA, qui était de 74–68%. Le taux de production en pourcentage de l'acide propionique était statistiquement indiscernable d'avec son pourcentage molaire de concentration, sauf pour le segment pré-alimentaire, pendant lequel le taux de production (13,6±1,0%) a été inférieur au pourcentage molaire (16,5±0,9 molaire %), à −2 h.

Le taux d'entrée du glucose est resté pratiquement constant pendant le déroulement du cycle d'alimentation, à savoir une moyenne de 4,07 mg/min/kg^{0,75} pendant les 3 phases. Il y a eu toutefois une différence significative entre la 1re et la 2e phase en ce qui concerne le pool de glucose (respectivement 392,5±84,4 et 257,6±64,6 mg/kg^{0,75}) et la constante du taux (1,05±0,21 et 1,71±0,15% du pool/min).

Une fraction constante (34±6%) de la production d'acide propionique dans le rumen a servi de précurseur pour la gluconéogénèse. L'équation qui décrit cette relation linéaire entre le total (x) et la fraction (y) de la production d'acide propionique convertie en glucose peut s'écrire $y=0,314\ x+0,02$, avec x et y exprimés en moles/12 h.

On a trouvé une relation directe entre les concentrations d'insuline dans le plasma et les taux fractionnaires correspondants (en % de pool/min) d'entrée du glucose; ceci confirme l'hypothèse que l'insuline agit en contrôlant le taux d'assimilation périphérique.

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Introduction

The importance of gluconeogenesis in ovine metabolism was emphasized when ruminants were found to utilize almost as much glucose on a body-mass basis as monogastric mammals (Ballard, Hanson & Kronfeld, 1969), despite almost all of their dietary carbohydrate being fermented to volatile fatty acid (VFA) end-products in the rumen (Elsden & Phillipson, 1948). Propionic acid alone of the VFA has been implicated as a major glucogenic precursor (Armstrong, 1965), and Leng, Steel & Luick (1967) and Bergman, Roe & Kon (1966) have shown that 32% and 48% respectively of the propionate produced in the rumen is used for this purpose. Furthermore, these latter workers showed that a substantial but variable amount (19–62%) of the glucose entry was derived from propionic acid. The above results were obtained from sheep maintained under near steady-state conditions by continuous feeding techniques.

Previous work in this laboratory showed a marked diurnal variation in the rate of propionic acid production in the rumen of sheep fed lucerne hay twice daily, and the effect of this changing propionic acid production rate on the glucose turnover rate in sheep was the subject of an interesting study. During the long-term infusion of ^{14}C-labelled propionate into the rumen to establish the parameters of VFA turnover, D-glucose-2-^{3}H was injected to determine not only the parameters of glucose turnover, previously described by Van der Walt (1975), but also the relationship between both. This was repeated on 3 occasions to establish this relationship before feeding, at the height of fermentation in the rumen and during the post-feeding period. At the same time a study was made of the conversion of propionate to lactate and of the changes in blood insulin levels.

Methods

Animals and feeding

Three sheep, P53, P57 and P64, with body masses of 81, 79 and 85 kg respectively, were selected from the group of South African Mutton Merino wethers used in previous experiments (Van der Walt & Briel, 1976; Van der Walt, 1977a) and maintained on the same daily ration of 1 600 g lucerne hay with 15 g of a trace element and salt mixture*. The ration was divided into 2 equal portions of 800 g each and offered at 08h00 and 20h00. Free access to water was allowed at all times and 1 g of vitamin A** was given to each sheep once a week.

Experimental procedure

The diurnal feeding cycle (08h00–20h00) was subdivided into 3 segments (−2–0, 2–4 and 8–10 h after feeding), corresponding to a pre-feeding period and periods of high and basal fermentation rates in the rumen. The ruminal propionic and total VFA production rates were estimated at the beginning and end of each period, the long term infusion of sodium propionate 2-^{14}C*** into the rumen, as described previously (Van der Walt, 1977a) being used. Glucose turnovers were determined concurrently with the VFA production rates for each period by the intravenous injection technique using D-glucose-2-^{3}H*** (Van der Walt, 1975). Each period was investigated separately at 7-day-intervals in order to eliminate interference from any residual radio-activity.

Analytical methods

VFA production data: The methods described in previous studies for the extraction and separation of the ruminal VFA (Van der Walt & Briel, 1976; Van der Walt, 1977a) were used. The samples were analysed for total as well as individual VFA concentrations and specific activities (Van der Walt, 1977b). The values obtained from this analysis were used to calculate the diurnal variation in the ruminal fermentation pattern of the VFA ratios as well as the changes occurring in the production rates of propionic acid and of the total VFA. The gross propionic acid production rates thus obtained were used without further correction, since the interchange of ^{14}C between this and any other acid produced in the rumen proved to be negligible (Leng, Corbett & Brett, 1968).

Glucose turnover data: The techniques described previously by Van der Walt (1975) were used with one modification. The glucose-containing fraction of the effluent from the Sephadex G-10 column was allowed to drop directly onto a 7,0 cm diameter Whatman 40* filter paper disc mounted above a stream of hot air sufficient to maintain a rate of evaporation greater than the elution rate. The dried filter paper was then combusted in a Packard 306** Oxidiser and the ^{3}H and ^{14}C content determined separately in a Packard Tricarb Series 3 000** liquid scintillation spectrometer. All count rates were converted to absolute activities, using the channels-ratio quench correction technique for both isotopes.

Lactic acid determination: The Lactate U-V Method*** test kit was used to measure the lactic acid levels and the specific activity was determined on the lactate-containing fraction of the effluent from the Sephadex G-10 column (Van der Walt, 1975).

Insulin determination: Plasma insulin levels were estimated by means of a radio-immuno-assay technique utilizing dextran-coated charcoal for the separation of the bound and unbound insulin-antigen complex. The method was first described by Yalow & Berson (1960) and subsequently adapted in our laboratory (Procos & Labuschagne, personal communication, 1975).

Results

VFA production data

Analysis of the rumen fluid samples yielded the total and individual VFA concentrations listed in Table 1.

Despite the 70% variation in total VFA levels found over the course of the diurnal feeding cycle, both the composition and the concentration of the VFA pool remained fairly constant for each segment, namely, 113,5–102,2 meq/l for the −2–0 h pre-feeding period, 156,1–151,1 meq/l for the 2–4 h maximum fermentation period and 90,8–89,8 meq/l for the 8–10 h afternoon period. The pre-feeding values were slightly higher than those for the 8–10 h period although this was not statistically significant (Student's t test, $P<0,9$), similar to results that were obtained previously (Van der Walt & Briel, 1976).

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TABLE 1 The total and individual VFA concentrations found in the rumen over the course of a 12-hour feeding cycle, all values listed being the average of 3 sheep ± standard deviation

Time after feeding (hours)	Total VFA concentration meq/l	Relative molar VFA concentration (% of total)					
		*A	P	IB	B	2 MB+IV	V
−2	113,5±13,8	72,2±5,2	16,5±0,9	1,2±0,6	8,1±2,9	1,5±0,8	0,5±0,3
0	102,2±22,8	71,6±2,6	16,7±1,4	1,3±0,5	8,3±2,3	1,7±0,7	0,4±0,1
2	156,1± 9,3	69,3±1,9	20,2±1,2	0,9±0,3	7,7±0,7	1,3±0,1	0,6±0,1
4	151,1±21,7	69,1±2,9	19,5±1,8	0,9±0,1	8,5±1,1	1,0±0,1	1,0±0,1
8	90,8±12,2	69,8±0,2	17,8±0,9	1,1±0,1	9,7±1,2	1,0±0,2	0,6±0,2
10	89,8± 7,2	71,3±0,1	17,5±0,1	1,0±0,2	8,5±1,1	1,1±0,6	0,6±0,2

*A=acetic acid
P=propionic acid
IB=isobutyric acid
B=butyric acid
2 MB+IV=2-methyl butyric plus isovaleric acid
V=valeric acid

Of the individual acids which also increased in concentration from the pre-feeding to the maximum fermentation period, propionic acid increased considerably more than the others, as changes in the acetic/propionic ($\frac{A}{P}$) and propionic/butyric ($\frac{P}{B}$) ratios presented graphically in Fig. 1 indicate.

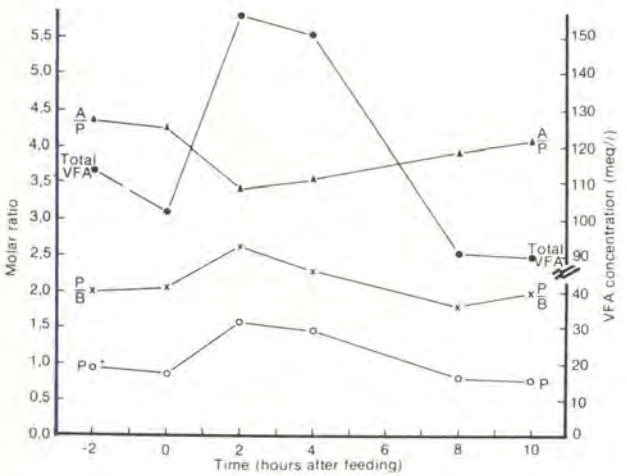

FIG. 1 Diurnal variation in both total VFA and propionic acid concentrations in the rumen together with the acetic/propionic ($\frac{A}{P}$) and propionic/butyric ($\frac{P}{B}$) acid ratios

Estimations of the total net VFA and propionic acid production rates at the beginning and end of each period are listed in Table 2 and are compared with the changes taking place in the VFA pool.

When the non-peak fermentation VFA production rates were plotted against the corresponding ruminal VFA levels for each sheep (see Fig. 2), a relationship, similar to that previously reported (Van der Walt, 1977a), was found where regression analysis gave the equation:

$y=0,029x+0,37$ with a correlation coefficient $r=0,85$

where y = VFA production in moles/12 h

and x = VFA concentration in meq/l

Note that the 2–4 h data from sheep P64 have been included in this regression analysis.

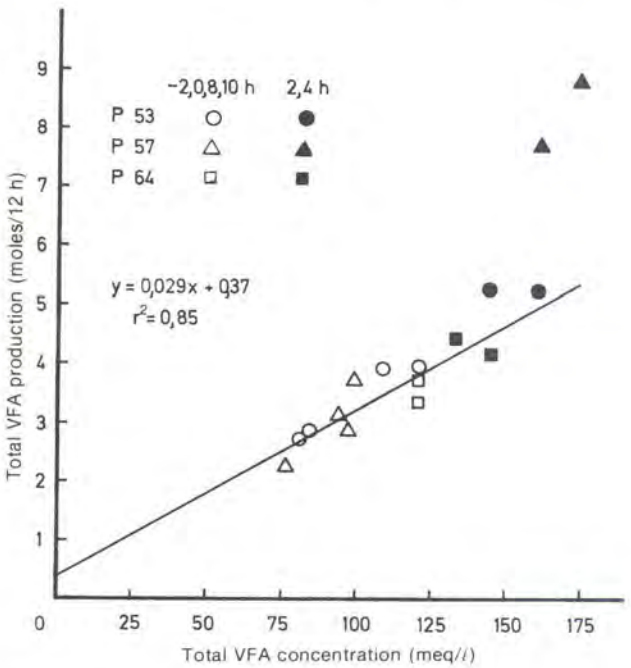

FIG. 2 Correlation between the total net VFA production rate in the rumen of each sheep (P53, P57 and P64) and the corresponding VFA concentration at various stages of the feeding cycle (−2, 0, 2, 4, 8 and 10 h after feeding. Closed symbols indicate the period of peak fermentation)

The propionic acid production rates, expressed as a percentage of the total VFA production, corresponded closely to their respective molar percentage concentrations, with the exception of the −2 h data which differed significantly ($0,95>P>0,90$) between the $13,6\pm1,9\%$ propionate production and the $16,5\pm0,9\%$ molar concentration.

Glucose turnover parameters

The values obtained for each period are listed in Table 3.

TABLE 2 The correlation between propionic and total volatile fatty acid production in the rumen and their respective concentrations are given for the beginning and end of each period. All values represent the average of 3 experiments±standard deviation

Time after feeding (hours)	Total VFA				Propionic acid			
	Concentration		Production rate		Concentration		Production rate	
	meq/l	% increase over basal	moles/12 h	% increase over basal	meq/l	molar % of total	moles/12 h	% of total
−2	113,5±13,8	19	3,51±0,56	7	18,7±1,0	16,5±0,9	0,47±0,08	13,6± 1,9
0	102,2±22,8		3,17±0,85		17,2±1,2	16,7±1,4	0,45±0,17	14,1± 4,6
2	156,1± 9,3	70	5,67±1,81	90	31,5±1,9	20,2±1,2	0,97±0,41	17,0± 6,7
4	151,1±21,7		6,14±2,29		29,5±2,7	19,5±1,8	1,20±0,82	19,4±12,5
8	90,8±12,1	0	3,22±0,67	0	16,1±1,4	17,8±0,9	0,56±0,13	17,6± 2,0
10	89,8± 7,2		3,00±0,20		15,6±1,3	17,5±0,1	0,56±0,01	18,7± 1,8

TABLE 3 Some of the relevant parameters of glucose metabolism in wethers found during the periods −2 to 0, 2 to 4 and 8 to 10 h after feeding. Averages are given±standard deviation

Time after feeding (hours)	Sheep	†Plasma glucose (mg/100 ml)	Glucose pool (mg/kg^{0,75})	*Glucose space (% body mass)	Glucose entry rate		†Plasma insulin (μU/ml)
					% of pool/ min	mg/min/ kg^{0,75}	
−2–0	P53	57,5	299,5	17,4	1,23	3,68	—
	P57	77,8	413,8	17,9	0,82	3,99	35,4± 2,3
	P64	74,8	464,2	20,5	1,11	5,13	39,7± 1,7
	Average	70,0±11,0	392,5±84,4	18,6±1,7	1,05±0,21	4,07±0,93	37,5± 3,0
2–4	P53	60,85	278,9	15,3	1,55	4,32	71,6± 3,6
	P57	64,9	185,0	9,6	1,83	3,99	45,9± 2,2
	P64	61,8	308,8	16,5	1,76	5,44	47,3± 5,1
	Average	62,5±2,1	257,6±64,6	13,8±3,7	1,71±0,15	4,38±1,03	54,9±12,7
8–10	P53	51,4	259,6	16,8	1,30	3,37	59,3± 4,5
	P57	75,9	259,7	11,5	1,59	4,13	42,2± 7,9
	Average	63,7±17,3	259,7±0,1	14,2±3,7	1,45±0,21	3,75±0,54	51,7±10,7

* Metabolic mass of P53=26,95 kg^{0,75}
P57=26,37 kg^{0,75}
P64=27,94 kg^{0,75}

† Average of 6 samples taken at regular intervals throughout the period

TABLE 4 The contribution of the propionic acid pool in the rumen to the whole body glucose turnover is derived from the glucose and propionic acid parameters summarized in Tables 2 and 3

Period after feeding (hours)	Sheep	Plasma lactate specific activity (μCi/Atom C)	Plasma glucose		Ruminal propionic acid*		Glucose turnover derived from propionic acid		Propionic acid production going to glucose	
			Specific activity (μCi/Atom C)	Turnover rate (mg/ min/kg^{0,75})	Specific activity (μCi/Atom C)	Production rate (moles/12 h)	% of turnover	mg/min/ kg^{0,75}	% of production	moles/12 h
−2–0	P53	27,00	9,01	3,68	40,84	0,59	22	0,81	29	0,17
	P57	24,46	11,08	3,39	64,14	0,36	17	0,59	33	0,12
	P64	28,77	9,13	5,13	59,88	0,57	15	0,77	28	0,16
2–4	P53	17,61	13,02	4,32	32,35	0,82	40	1,74	45	0,37
	P57	10,94	11,15	3,99	14,17	1,79	79	2,67	31	0,56
	P64	13,03	7,00	5,44	33,66	0,66	21	1,13	36	0,24
8–10	P53	24,77	9,50	3,37	43,66	0,51	22	0,74	31	0,16
	P57	21,90	12,13	4,13	42,40	0,61	29	1,18	41	0,25

* Mean of the beginning and end of each period (see Table 2)

Despite a wide variation in the availability of such a major gluconeogenic precursor as propionic acid (0,45–1,20 moles/12 h on average, Table 2), the glucose entry rate remained almost constant throughout the feeding cycle (4,07–4,38 mg/min/$kg^{0,75}$). Despite this, the apparent drop in the size of the glucose pool (392,5±84,4–257,6±64,6 mg/$kg^{0,75}$) was found to be statistically significant ($0,95>P>0,90$ Student's t test) and was balanced by a rise in the fractional glucose entry rate (1,05±0,21–1,71±0,15% of the glucose pool/min) during the period of maximum propionic acid production. Although not statistically different ($P<0,90$) from those of the 2–4 h period, the values obtained for the afternoon period (8–10 h after feeding) showed a tendency to return to the pre-feeding levels.

Plasma insulin concentration

Parallel changes occurred in the plasma insulin levels. The significant increase ($P>0,99$) from the pre-feeding (37,5±3,0μU/ml) to the maximum fermentation period (54,9±12,7 μU/ml) was followed by a slight but insignificant drop later in the day to 51,7±10,7 μU/ml.

Regression analysis of the apparent relationship between the glucose turnover rate (y=mg/min/$kg^{0,75}$) and the plasma insulin level (x=μU/ml) failed to reveal a significant correlation (r=0,35) even when the high insulin levels of P53 were excluded. However, Fig. 3 clearly indicates that a highly significant correlation (r=0,96) was obtained when the glucose turnover rate was expressed as the equivalent rate constant, as described by the straight line found for sheep P57 and P64:

$$y=0,088x-2,28$$

where y=turnover rate (% of pool/min)

and x=insulin level (μU/ml).

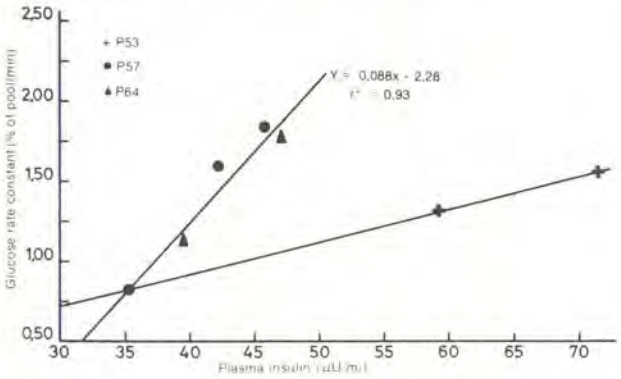

FIG. 3 Correlation between the plasma insulin levels and the corresponding fractional turnover rates obtained for glucose in 3 sheep

Contribution of propionic acid to the glucose turnover

The rate of conversion of propionic acid into glucose was calculated from the entry rate of glucose, the production of propionic acid and the ratios of the respective specific activities as described by Leng *et al.*, (1967), the results of which are given in Table 4. Despite a wide variation (15–79%) in the amount of glucose production derived from propionate, this fraction of the glucose production was proportional to the total ruminal production rate of that acid. In other words, a constant fraction of the propionic acid production went towards gluconeogenesis (34±6%). This relationship was clearly demonstrated in Fig. 4 where the total ruminal propionic acid production rate was plotted against that portion destined for gluconeogenesis. When similarly analysed to the glucose data, the specific activity results obtained for plasma lactate (see Table 4) indicated that a constant fraction (mean=54±13%) of the lactate entering the blood stream was derived from the ruminal propionic acid production. This is in contrast to the glucose specific activity data which indicated that a highly variable fraction (15–79%) of the glucose production was derived from propionate.

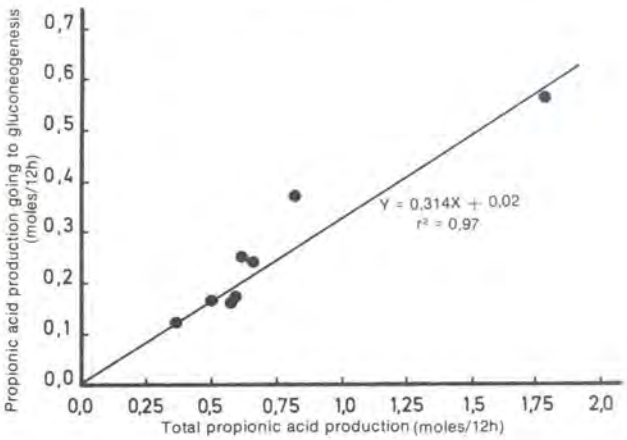

FIG. 4 The straight line relationship between the total propionic acid production in the rumen and that fraction utilized for gluconeogenesis

Discussion

The cyclic diurnal pattern found previously by Van der Walt (1977a) in the ruminal production of VFA in sheep fed lucerne hay twice daily was confirmed, although the present findings differed in magnitude and between individual sheep. Despite this variation, 3 periods of this cycle, corresponding to a pre-feeding (−2–0 h), a maximum (2–4 h) and a basal fermentation (8–10 h) phase, were each shown to have near steady-state conditions over the selected 2 h periods, where total VFA production rates and levels and individual VFA ratios and their absolute quantities remained nearly constant. This enabled whole body glucose turnover parameters to be obtained concurrently with the VFA production data, allowing direct calculation of their interactions.

As shown previously (Van der Walt, 1977a), the propionic acid concentration in the rumen increased more than the other VFA's during the period of maximum fermentation. This generally corresponded to the changes taking place in the ruminal propionic acid production, expressed as a percentage of the total VFA production, and pointed to a change in fermentation pattern rather than a change in absorption rate through the rumen epithelium (Leng, 1970). However, the −2 h pre-feeding propionic acid production rate (13,6±1,9%) was significantly ($0,95>P>0,90$) lower than its corresponding concentration (16,5±0,9%), and probably resulted from a change in relative absorption rates. Probably as a result of increased saliva flow, the rumen pH of lucerne-fed animals was found to rise 1–2 h before feeding (Gilchrist & Mackie, unpublished observation), and this could alter propionic acid transport across the rumen wall.

The direct relationship between ruminal total VFA production and concentration was essentially similar to that found previously (Van der Walt, 1977a), as was the dilution of the VFA concentration during the period of maximum fermentation (2–4 h after feeding). The 2 and 4 h data obtained from sheep P64 showed no such dilution effect, however, and, when included in the regression analysis, did not materially affect the equation given above (Fig. 2).

The homeostatic control of the overall glucose metabolism as measured by the turnover estimates was remarkable in the light of the 2,4 fold variation in average ruminal propionate production, since Katz & Bergman (1969) found that an increase in gluconeogenesis during the immediate post-prandial period was due to the greater availability of suitable substrates. A slight but statistically insignificant ($P<0,9$, paired t test) increase in the glucose turnover rate was found during the period of maximum propionic acid production in the rumen. Furthermore, none of the average values calculated for the 3 periods examined were significantly different from the results ($3,91\pm 0,78$ mg/min/kg^{0,75}) obtained in a previous study (Van der Walt, 1975).

Despite this overall homeostasis, significant changes did occur after feeding in both the glucose pool ($392,5\pm84,4$–$257,6\pm64,6$ mg/kg^{0,75}, with $0,95>P>0,90$) and the fractional entry rate ($1,05\pm0,21$–$1,71\pm0,15$% of pool/min, with $0,99>P>0,95$), thereby implicating the existence of 2 separate control sites.

The results showed that a constant fraction of 34% (range 28–45%) of the ruminal propionic acid production rate contributed to the whole body glucose turnover. This was similar to the average of 32% (range 25–38%) found by Leng *et al.*, (1967) for a lucerne hay diet and 37% (range 21–47%) derived by Judson, Anderson, Luick & Leng (1968) on a variety of grain-supplemented lucerne hay diets. Since glucose entry rate remained relatively constant while propionic acid production rose by a factor of 2,4, the propionic acid production contributing to the glucose entry rate covered a wide range (15–79%), corresponding to the results (18–62%), obtained by others such as Leng *et al.*, (1967), Judson *et al.*, (1968) and Bergman *et al.*, (1966) under various dietary steady-state conditions.

All these results pointed to the conclusion that the availability of propionic acid is not a rate-limiting or controlling step in the process of gluconeogenesis. Rather, it would appear from the work of Smith & Russell (1967), Smith, Osborne-White & Russell (1967), Smith (1971) and Smith & Osborne-White (1971) on sheep liver homogenates that propionate is metabolized only in the mitochondria via the methylmalonyl route and consequently enters the pool of common metabolic intermediates as succinyl-CoA. They further demonstrated that the rate of propionate consumption was specifically determined by the rate of oxidation of succinate even when an alternative source of energy was apparently available. This was linked to the intra-mitochondrial production of guanosine triphosphate by the deacylation of succinyl-CoA. These authors proposed that this guanosine triphosphate was used preferentially for the transphosphorylation of the adenosine monophosphate that resulted from the activation of propionate to propionyl-CoA. In this way the generation of phospho-enol-pyruvate from propionate was limited to a fixed proportion of the propionate available for oxidation.

In contrast to glucose, a fixed proportion of the lactic acid turnover rate was derived from the ruminal propionic acid production rate, the magnitude (54%) agreeing with that quoted by Leng *et al.*, (1967). Although little is known about lactic acid turnover in the sheep, Weigand, Young & McGilliard (1972) have shown that only about 5% of the propionic acid transported across the rumen wall *in vivo* is converted into lactic acid. This is in agreement with the findings of Ash & Baird (1973) who showed that propionic acid was chiefly metabolized in the liver and not in the rumen epithelium. The resultant labelling pattern found in the glucose molecule would then be further complicated by the contribution of the Cori cycle (approximately 5% of the total glucose entry rate, Brockman, Bergman, Pollak & Brondum, 1975), whereby lactic acid produced endogenously from glucose at peripheral sites would be reconverted into glucose in the liver. The contribution of lactic acid to the glucose pool of the sheep, other than that derived from the Cori cycle, deserves closer investigation.

The rate of gluconeogenesis in sheep is closely linked to the insulin/glucagon ratio (Bassett, 1974, 1975) where glucagon promotes gluconeogenesis, particularly from amino acids (Lindsay, 1971; Brockman & Bergman, 1974), and insulin inhibits this process while enhancing peripheral utilization. Although Bassett, Weston & Hogan (1971) related insulin levels to glucose entry rates, no such relationship could be demonstrated with the limited data obtained from these 3 experimental sheep. However, a close inspection of the glucose and insulin data did reveal an apparent relationship between the average insulin level for each period and the corresponding glucose fractional entry rate. This was true also for sheep P53 which nevertheless showed a decreased sensitivity requiring higher levels of insulin to promote normal peripheral uptake of glucose. This finding also indicates 2 separate control points for pool size and fractional entry rate.

Bassett (1973) found that the insulin/glucagon ratio changed little during a feeding cycle (1,9–3,3) despite an overall 2–3-fold increase in both levels 2 h after feeding (800 g of a 1:1 lucerne hay:oat grain diet once daily). This reflected a dominance of glucagon effects on the liver (Park & Exton, 1972), and is characteristic of a low carbohydrate diet in monogastric mammals. That twice-daily feeding of a lucerne hay diet would tend to make this ratio even smaller is confirmed by the relatively steady overall glucose entry rate found throughout the feeding cycle and the comparatively small increase in insulin levels (50%) 2–4 hours after feeding.

In conclusion, the submission is that propionate serves as a non-rate-limiting source of substrate for gluconeogenesis at the level of production normally encountered in the rumen when sheep are fed a roughage diet. Furthermore, the control of gluconeogenesis resides in both the availability of absorbed amino acids and the hormonal response elicited by this absorption.

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