Metabolic Interrelations of Glucose and Lactate in Sheep

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(Received 28 January 1963)

Entry rates of glucose in sheep have been estimated by Annison & White (1961, 1962) by using isotope-dilution techniques. The values obtained were surprisingly large in view of the small absorption of carbohydrate from the alimentary tract of sheep (Annison & Lewis, 1959) and the limited amount of propionate available for conversion into glucose (Annison, Hill & Lewis, 1957; Annison & Lindsay, 1962). When interpreting these results we have to consider to what extent a turnover rate, as measured by isotope dilution, may be equated with glucose requirement. Annison & White (1961) drew attention to the fact that an internal cycle such as that described by Cori & Cori (1928) would be included in such an estimate of turnover rate although it would have no significance in relation to carbohydrate requirements. The existence of the Cori cycle (muscle glycogen $\rightarrow$ lactate $\rightarrow$ liver glycogen $\rightarrow$ glucose $\rightarrow$ muscle glycogen) was inferred by Cori from experiments in animals under the action of adrenaline. Such texts as refer to it (Lovatt Evans, 1945) suggest that it is of importance only under the action of adrenaline or exercise. However, Andres, Cader & Zierler (1956), on the basis of arterio-venous difference measurements of blood glucose, lactate, oxygen and CO_2 in the resting human forearm, suggested that a major fraction of glucose utilized by muscle was dissimilated to lactate. Drury & Wick (1956) used $\text{L}(+)\text{-}^{14}\text{C}$ lactate to demonstrate that lactate oxidation at resting concentrations of blood lactate could account for up to 25% of the CO_2 output of rabbits. These results suggest that the Cori cycle could be more important than is commonly supposed, even under resting conditions. There appears to be no published evidence relating to ruminants.

A constant infusion of uniformly labelled $\text{L}(+)\text{-}^{14}\text{C}$ lactate has been used to determine the entry rate of lactate and the extent of its oxidation and conversion into glucose and glycogen in sheep. In addition, the rate of conversion of glucose into lactate has also been examined during the infusion of uniformly labelled ^{14}C glucose. Some measurements have been made in anaesthetized animals, where muscle activity might be expected to be minimal. The results obtained show that glucose and lactate are to a considerable extent interconvertible. This interconvertibility

does not appear to involve glycogen to any appreciable extent, and reasons are advanced for supposing that its existence does not substantially change previous estimates of carbohydrate requirements in ruminants.

MATERIALS AND METHODS

Experimental animals. Merino wethers (2–3 years of age) were maintained on a daily ration of 500 g. of lucerne and 100 g. of maize, which they consumed within 1–2 hr. This diet was sufficient to maintain their weight constant for several months.

Infusion procedure. The general procedure of Annison & White (1961) was used, except that for experiments with fed animals polythene catheters were inserted immediately after feeding, the infusion being carried out about 2 hr. later. A priming dose was used in glucose infusions (Annison & White, 1961) but not in lactate infusions.

For experiments with anaesthetized sheep, sodium pentobarbitone was used as anaesthetic, the level of anaesthesia being such as just to permit the pedal reflex.

When tissues for glycogen assay were required, the sheep was killed by exsanguination, and samples were removed as quickly as possible (1–2 min.) and immediately placed in 30% (w/v) KOH at 100° .

Insulin. This was obtained from the Commonwealth Serum Laboratories, Melbourne, Australia.

Organisms. *Lactobacillus delbrückii* was a culture (type no. 11 443) obtained from the American Type Culture Collection, 2029 M Street, N.W. Washington, D.C., U.S.A. *Leuconostoc mesenteroides* was a culture (strain 39) obtained from the Department of Bacteriology, Indiana University, Bloomington, Ind., U.S.A.

Radioactive materials. Uniformly labelled $\text{D-}^{14}\text{C}$ glucose and $\text{D-}^{14}\text{C}$ glucose were obtained from The Radiochemical Centre, Amersham, Bucks. Stepwise degradation of $\text{D-}^{14}\text{C}$ glucose by using the procedures described below indicated the virtual absence of radioactivity in C-1, C-2, C-3, C-4 and C-5, 97% of the total activity being recovered from C-6.

$\text{L}(+)\text{-}^{14}\text{C}$ Lactate was prepared by the fermentation of uniformly labelled $\text{D-}^{14}\text{C}$ glucose by *Lb. delbrückii* (Brin, 1953). The fermentation of ^{14}C glucose (75 mg.; 300 μC) was carried out in a 125 ml. manometric flask, with a bicarbonate medium, and was continued until acid production (CO_2 production) had ceased. After fermentation, the medium was centrifuged and the supernatant retained. The precipitate was washed once with water, and the combined supernatant and washings acidified to pH 2 with sulphuric acid, and dried overnight in a vacuum desiccator. Calcium sulphate was added to the dried material and the mixture transferred to a Soxhlet extraction thimble and extracted

for 4 hr. with ether. Longer periods of extraction were occasionally used, but it was found that prolonged periods at acid pH decreased the recovery of lactate, apparently owing to the formation of lactide. The ether extract was titrated with dilute alkali, and the dilute aqueous solution was deep-frozen until needed. On some samples, the aqueous solution was further purified by crystallizing the lactate as the zinc salt and removing the Zn^{2+} ions by passage through an ion-exchange column. The overall recovery of lactate was 75–80%.

Chemical methods

Methods used for (a) the measurement of plasma glucose, (b) the collection and assay of $Ba^{14}CO_3$, (c) the determination of the specific radioactivity of blood CO_2 , and (d) the isolation of plasma glucose as the osazone, were as described by Annison & White (1961). Glucosazones were plated and counted as described by Leng & Annison (1963). Glucose was isolated from large samples of plasma (100 ml.) as described by Annison & White (1962), and the distribution of ^{14}C determined by stepwise degradation by using procedures based on fermentation with *Ln. mesenteroides* (Bernstein & Wood, 1957), as modified by Leng & Annison (1963).

Determination of blood lactic acid. The method of Barker & Britton (1957) was used.

Isolation and assay of glycogen. Glycogen was isolated as described by Good, Kramer & Somogyi (1933), hydrolysed, assayed for glucose content and the specific radioactivity of the glucose measured as the osazone.

Measurement of the specific radioactivity of blood lactic acid. To whole blood (10 ml.) was added lithium lactate (52.4 mg.), sodium fluoride (25 mg.) and 4N-sulphuric acid (2 ml.) and, after mixing, the semi-solid material was concentrated *in vacuo*. Anhydrous calcium sulphate was mixed with the residue to make a free-running powder which was extracted with ethyl ether for 12 hr. The ether extract was evaporated to dryness and the residue dissolved in 2–3 ml. of water. Excess of zinc carbonate was added and the mixture was heated until no further effervescence occurred. The mixture and washings from the extraction flasks were filtered, the filtrate was concentrated to about 1 ml. and any precipitate was removed by centrifuging. Acetone was added to the clear supernatant and crystallization allowed to proceed after the first appearance of precipitate. This was a fairly rapid process. The precipitate was separated by centrifuging. The precipitate was redissolved in 1 ml. of water, and ethanol was added to the clear solution until the first appearance of precipitate. Crystallization was allowed to continue at 4° for 24 hr., when the mixture was centrifuged and the supernatant discarded. The precipitate was dissolved in the minimum amount of water and the zinc lactate was precipitated by the rapid addition of an excess of acetone.

The precipitate was collected on planchets, dried to constant weight, weighed and counted. The radioactivity was calculated by reference to a standard self-absorption curve for zinc lactate (Hendler, 1959).

Further recrystallization from aqueous ethanol of zinc lactate precipitates obtained from plasma after the infusion of $[^{14}C]$ glucose or $[^{14}C]$ lactate caused no change in the observed specific radioactivities. The determination of lactate in ten zinc lactate precipitates prepared by the standard techniques gave a mean recovery of $96.0 \pm 0.4\%$

(s.e.m.). Wet oxidation of the precipitates by the procedures of Van Slyke & Folch (1940) and assay of the $^{14}CO_2$ as $Ba^{14}CO_3$ confirmed the specific radioactivities obtained by direct counting.

RESULTS

Entry rates of lactate. Entry rates of lactate in fed sheep (2 hr. after feeding) and in starved (for 24 hr.) sheep were 1.7 ± 0.14 (four animals) and 1.2 ± 0.14 (five animals) mg./min./kg. respectively (Table 1). Fairly constant specific radioactivities of blood lactate were obtained after the infusion of $[^{14}C]$ lactate for 60–90 min., but random fluctuations in specific radioactivity of $\pm 10\%$ during periods of unchanging blood lactate concentrations were observed in most experiments.

When the entry rates of lactate obtained in all experiments on fed and starved sheep were plotted against the corresponding blood lactate concentrations (Fig. 1), some dependence of entry rate on lactate concentration was observed. A regression of entry rate (y) on lactate concentration (x) was found to be significant ($P = 0.01$). The best estimate of the regression was:

$$y = 0.1x + 0.574$$

Interconversion of glucose and lactate. Values for the mean specific radioactivity of blood lactate during the infusion of $[^{14}C]$ lactate, and the corresponding final specific radioactivities of plasma glucose after 2 hr. of infusion, are shown in Table 1. The ratio of these values (Table 1) is a measure of the fraction of the total glucose pool derived from lactate. The values of this fraction ranged from 7 to 19% and were unrelated to the nutritional state. The specific radioactivity of glucose increased steadily during infusions of $[^{14}C]$ lactate, constant values being reached in only two experiments. Specific radioactivities of glucose shown in Table 1 were, therefore, minimal, and indicated a substantial conversion of lactate into glucose.

Comparison of the specific radioactivities of glucose and lactate during the infusion of $[^{14}C]$ glucose showed a substantial production of lactate from glucose. In two experiments 39 and 43% of the lactate was derived from glucose after infusion for 3 hr. At this time specific radioactivities of lactate were still slowly rising, indicating that the values for the conversion of glucose into lactate were minimal.

The effects of raised concentrations of blood glucose on glucose–lactate interrelations were studied by infusing $[^{14}C]$ glucose (after a priming dose) for 140 min. to achieve a constant specific radioactivity of blood glucose, and then continuing the infusion of $[^{14}C]$ glucose together with unlabelled glucose at a rate of 67 mg./min. In a

Table 1. *Entry rates of lactate in fed and starved sheep*

Entry rates were measured by infusing uniformly labelled $1-(+)-[^{14}\text{C}]\text{lactate}$ at a rate of $1\ \mu\text{mole/min.}$ ($0.35\ \mu\text{C/min.}$). The incorporation of ^{14}C into plasma glucose and blood CO_2 was also measured. Experimental details are given in the text.

Expt. no.	Time after feeding (hr.)	Time of infusion (min.)	Mean concn. of blood lactate (mg./100 ml.)	Mean concn. of plasma glucose (mg./100 ml.)	Entry rate of lactate (mg./min./kg.)	Mean sp. radioactivity of blood lactate ($\mu\text{C/g. of C}$)	Final sp. radioactivity of plasma glucose ($\mu\text{C/g. of C}$)	Final sp. radioactivity of blood CO_2 ($\mu\text{C/g. of C}$)	Percentage of glucose derived from lactate	Percentage of CO_2 derived from lactate
L4	2	130	6.6	86	1.4	16.8	2.8	—	17	—
L5	2	120	10.5	107	1.7	7.8	1.3	0.5	17	6
L6	2	120	9.2	63	1.6	8.0	1.3	0.6	16	7
L12	2	180	13.1	63	2.0	12.2	1.5	1.1	13	9
L2	24	120	6.9	50	0.8	32.2	3.5	1.7	11	5
L8	24	208	4.8	52	1.3	19.4	2.5	1.4	13	7
L9	24	240	6.6	40	1.6	9.8	1.4	1.1	14	10
L10	24	240	8.9	71	1.3	22.5	2.0	1.4	9	8
L11	24	180	7.8	67	1.0	18.0	3.5	1.4	19	6
L3	48	150	5.1	54	1.1	26.8	2.0	2.4	7	9

typical experiment (Table 2) plasma glucose and blood lactate concentrations were considerably increased by the infusion of carrier glucose, and the specific radioactivity of glucose, but not that of lactate, was substantially decreased. The close correspondence of the specific radioactivities of glucose and lactate implied that under glucose load lactate production from sources other than blood glucose was abolished. Calculations of the entry rates of glucose under glucose load confirmed previous work indicating almost complete inhibition of glucose production at raised concentrations of blood glucose (Annisson & White, 1961).

There was very little incorporation of ^{14}C into glycogen during the infusion of $[^{14}\text{C}]\text{lactate}$ or $[^{14}\text{C}]\text{glucose}$ (Table 3). The specific radioactivity of liver glycogen never exceeded 0.4% of that of plasma glucose or blood lactate (Table 3). In one experiment in which $[^{14}\text{C}]\text{glucose}$ was infused at a rate of $133\ \text{mg./min.}$ ($0.4\ \mu\text{C/min.}$), together with insulin at a rate of $0.02\ \text{i.u./kg./min.}$, the final specific radioactivities of plasma glucose, liver glycogen and skeletal-muscle glycogen were 6.6, 2.4 and $0.5\ \mu\text{C/g. of C}$ respectively; the corresponding plasma glucose concentration was $213\ \text{mg./100 ml.}$

Glucose recycling via lactate was examined by infusing $[6\text{-}^{14}\text{C}]\text{glucose}$ for 2 hr. (after a priming dose) and measuring the distribution of ^{14}C in glucose isolated at the end of the infusion. The extent of relocation of ^{14}C from C-6 to C-1 provided a measure of recycling. The radioactivity in C-1, C-2, C-3, C-4, C-5 and C-6 was 5, 2, 1, 0, 2 and 90% of the total radioactivity respectively, indicating about 10% recycling.

Specific radioactivity of blood carbon dioxide. Estimates of the contribution of lactate oxidation

Fig. 1. Relationship between the entry rate of lactate and the blood lactate concentration in sheep.

Table 2. *Effect of raised concentrations of blood glucose on glucose-lactate interconversions in sheep*

Uniformly labelled [^{14}C]glucose was infused at a rate of 0.5 mg./min. (0.40 $\mu\text{C}/\text{min.}$) for 140 min. after injection of a priming dose of [^{14}C]glucose (25 μC). The concentration of glucose in the infusion solution was then increased to give an infusion rate of 66.7 mg./min. (0.40 $\mu\text{C}/\text{min.}$) during the period 140–290 min. Experimental details are given in the text.

Time after start of infusion (min.)	Concn. of plasma glucose (mg./100 ml.)	Concn. of blood lactate (mg./100 ml.)	Sp. radio-activity of plasma glucose ($\mu\text{C}/\text{g. of C}$)	Sp. radio-activity of plasma lactate ($\mu\text{C}/\text{g. of C}$)	Sp. radio-activity of blood CO_2 ($\mu\text{C}/\text{g. of C}$)
100	63	10.7	33	10	—
110	64	9.2	29	12	2.2
121	63	8.5	27	12	2.3
133	63	8.7	27	13	2.3
139	64	9.3	27	12	2.6
243	117	13.6	13	12	—
251	116	13.1	14	9	3.5
275	107	12.0	13	13	—
289	115	11.3	13	13	3.8

Table 3. *Final specific radioactivities of glycogen in various tissues in sheep resulting from the infusion of uniformly labelled [^{14}C]lactate and uniformly labelled [^{14}C]glucose*

Details of the lactate infusion (Expts. L10 and L11) are shown in Table 1. Uniformly labelled [^{14}C]glucose was infused at the rate of 0.5 mg./min. (0.35 $\mu\text{C}/\text{min.}$) for 160 min. after a priming dose of [^{14}C]glucose (25 μC). Experimental details are given in the text. Mean specific radioactivities of plasma glucose in Expts. C1 and C2 were 10.4 and 26.0 $\mu\text{C}/\text{g. of C}$ respectively; the corresponding plasma glucose concentrations were 65 and 73 mg./100 ml.

Expt. no.	Substrate infused	Sp. radioactivity of glycogen ($\mu\text{C}/\text{g. of C}$)				
		Liver	Skeletal muscle	Diaphragm	Heart	Kidney
L10	[^{14}C]Lactate	0.04	0.01	—	—	—
L11	[^{14}C]Lactate	0.005	0.009	—	—	—
C1	[^{14}C]Glucose	0.04	0.04	0.04	0.04	0.13
C2	[^{14}C]Glucose	0.02	0.01	—	—	—

to total CO_2 output were made by comparing the specific radioactivity of CO_2 of jugular blood with that of blood lactate. The results shown in Table 1 suggested that about 7–8 % of the CO_2 arose from the oxidation of lactate. The values for the specific radioactivity of CO_2 after 2 hr. of infusion of [^{14}C]lactate showed only a slow approach to a constant value, and the estimate of 7–8 % of CO_2 arising from the oxidation of lactate can be considered as indicating only very roughly the contribution of lactate to oxidative metabolism.

DISCUSSION

Measurements of the entry rates of L(+)-lactate by isotope dilution have indicated an extensive production of lactate in resting sheep. Entry rates in fed and starved (24 hr.) animals were 1.7 ± 0.14 (four animals) mg./min./kg. and 1.2 ± 0.14 (five animals) mg./min./kg. respectively, or about 60 and 80 % respectively of the corresponding values for glucose (Annison & White, 1962). There is other evidence supporting the concept of extensive production of lactate in resting animals. Huckabee & Judson (1958) showed that 'excess' of lactate

accumulated in human subjects performing mild muscular work comparable with that of normal daily activity, and suggested that about 5 % of the total energy was derived from the anaerobic production of lactate. Andres *et al.* (1956) demonstrated that a substantial fraction of the glucose utilized by resting human-forearm muscle was converted into lactate. Direct measurements of the entry rates of L(+)-lactate have not been previously reported, but from the results of Drury & Wick (1956) who infused [^{14}C]lactate into rabbits it can be calculated that entry rates were 4–5 mg./min./kg.

Comparison of the specific radioactivities of lactate and glucose during infusions of [^{14}C]lactate indicated that about 14 % of the glucose pool was derived from lactate. This corresponds to about 0.3 mg. of glucose/min./kg., since the entry rate of glucose in sheep is about 2.0 mg./min./kg.

The values for the percentage of CO_2 derived from lactate (Table 1) were low since constant specific radioactivities of blood CO_2 had not been attained and a value of at least 10 % may be assumed. Since the total CO_2 output of a 40 kg. sheep is about 7 m-moles/min., about 0.5 mg. of

lactate/min./kg. is oxidized to CO_2 . Conversion into glucose and oxidation could, therefore, account for most of the lactate turnover.

Comparison of specific radioactivities of blood lactate and glucose during the infusion of [^{14}C]-glucose suggested that at least 40% of lactate entering the body pool was derived from glucose. This value must be regarded as minimal, since constant specific radioactivities of blood lactate were not attained. When the concentration of blood glucose was raised by infusing unlabelled glucose with [^{14}C]glucose, the specific radioactivities of infused glucose, plasma glucose and blood lactate became closely similar (Table 2), indicating substantial inhibition of both glucose production and lactate production from sources other than plasma glucose.

Investigation of recycling of the glucose-lactate interconversion by using [$6\text{-}^{14}\text{C}$]glucose confirmed conclusions drawn from the relative specific radioactivities of glucose and lactate during infusions of uniformly ^{14}C -labelled substrates. [$6\text{-}^{14}\text{C}$]Glucose gives rise to [$3\text{-}^{14}\text{C}$]lactate which is resynthesized to [$1,6\text{-}^{14}\text{C}_2$]glucose or, if the lactate is in equilibrium with a C_4 symmetrical intermediate, to [$1,2,5,6\text{-}^{14}\text{C}_4$]glucose (Reichard, Moury, Hochella & Weinhouse, 1961). Operation of the pentose phosphate cycle (Katz & Wood, 1960) would result in preferential loss of ^{14}C from C-1 and some relocation of ^{14}C to C-2 and C-3. Since during the infusion of uniformly labelled [^{14}C]glucose the specific radioactivity of blood lactate was about 40% of that of glucose, and during the infusion of [^{14}C]lactate the corresponding glucose value was about 15%, we should expect about 15% of 40% (i.e. 6%) of recycling. The actual values obtained with [$6\text{-}^{14}\text{C}$]glucose indicated the relocation of 5% of ^{14}C to C-1, or 10% of recycling, in fair agreement with values obtained indirectly.

The glucose-lactate interrelation established in the present studies on resting sheep differs from the cycle described by Cori & Cori (1928) in that glycogen is not involved. This finding is important when assessing the influence of glucose-lactate interconversions on the entry rates of glucose (Annison & White, 1961), which involve the use of a balanced priming dose and infusion rate of [^{14}C]glucose. An ideal result, as suggested by Steele, Wall, de Bodo & Altzuler (1956), is that the specific radioactivity of plasma glucose should fall slowly to an asymptotic value which should be that generated by the infusion alone, in the absence of both priming dose and recycling. In an actual experiment the specific radioactivity of plasma glucose at any time between 90 and 180 min. is the resultant of three components: a constant component due to the infusion, a declining component due to the fading primary dose and an augmenting

component due to any recycling. If an ideal result is obtained the effect of any recycling has been eliminated by correct balance of primary dose and infusion rate. Considerable departures from ideality (Steele *et al.* 1956) do not result in major inaccuracies in the determination of entry rates.

As the type of recycling concerned in this argument occurs within the experimental period it does not affect the interpretation of entry rates in relation to carbohydrate requirements, but this would not be so if a prolonged and substantial hold-up occurred in any intermediary of the Cori cycle other than glucose. We may exclude this second possibility as we have demonstrated only a rapid recycling between glucose and lactate, with negligible incorporation of either metabolite into glycogen.

A further implication is that the 'glucose space' (Steele *et al.* 1956), as measured by isotope dilution, must include that part of the lactate space which has equilibrated with infused [^{14}C]glucose within the time of the experiment. These considerations indicate that the entry rate of lactate is a parameter of limited interest. Much of the entry rate reflects merely the rate at which part of the body carbohydrate pool (lactate) reaches equilibrium with plasma glucose; a substantial proportion of the remainder indicates the extent of glucose conversion into lactate before oxidation to CO_2 .

SUMMARY

1. Measurement of the entry rates of lactate in sheep by the constant infusion of L(+)-lactate gave values of 1.7 ± 0.14 (four animals) mg./min./kg. for fed animals and 1.2 ± 0.14 (five animals) mg./min./kg. for starved (24 hr.) animals.

2. A roughly linear relationship between the entry rate of lactate and blood lactate concentration was observed.

3. Comparison of the specific radioactivities of blood lactate and plasma glucose during the infusion of [^{14}C]lactate indicated that a minimum of about 15% of the glucose pool was derived from lactate.

4. Estimation of the specific radioactivities of blood CO_2 during the infusion of [^{14}C]lactate indicated that a minimum of about 7% of CO_2 arose from lactate.

5. The relative specific radioactivities of blood lactate and plasma glucose during the infusion of [^{14}C]glucose indicated that at least 40% of the lactate pool is derived from glucose.

6. Evidence for the inhibition of lactate entry from sources other than glucose was obtained when the blood sugar concentration was raised by the infusion of unlabelled glucose with [^{14}C]glucose.

7. Comparison of specific radioactivities of blood lactate and plasma glucose during the infusion of [^{14}C]glucose or [^{14}C]lactate indicated that about 6% of glucose was recycled through the glucose-lactate interconversion. Evidence from the relocation of ^{14}C in glucose during the infusion of [6- ^{14}C]glucose indicated about 10% of recycling.

8. Negligible incorporation of glucose or lactate into liver or muscle glycogen was observed.

This investigation was made possible by the generous support of the Australian Wool Research Committee. Mr J. Roberts and Mr J. Wilcher gave valuable technical assistance.

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Biochem. J. (1963) **88**, 248

The Metabolism of Acetic Acid, Propionic Acid and Butyric Acid in Sheep

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(Received 28 January 1963)

The production in the rumen of large amounts of acetic acid, propionic acid and butyric acid, which are absorbed into the portal system, is well established. Propionate and butyrate are largely removed by the liver under normal feeding conditions, but substantial quantities of acetate appear in peripheral blood (Annison, Hill & Lewis, 1957). The possible glucogenicity of acetate and butyrate in ruminants was studied previously with liver slices (Leng & Annison, 1963), when no evidence was obtained of net synthesis of carbohydrate from these substrates. These results have been confirmed in the present studies with intact animals. Solutions of [^{14}C]acetate, [^{14}C]propionate or [^{14}C]butyrate were infused into the portal vein of anaesthetized sheep, and the incorporation of ^{14}C into blood glucose, lactate and ketone bodies, and into liver and muscle glycogen, was examined. Some acetate production from propionate and butyrate was demonstrated.

MATERIALS AND METHODS

Experimental animals. Merino wethers (2-3 years of age) were housed indoors and fed once daily (early morning) on a standard ration of 800 g. of lucerne chaff. Animals were not fed on the day of an experiment.

Experimental procedure. Polyethylene catheters were inserted into both jugular veins on the day before an experiment. Solutions of the sodium salts of [^{14}C]acetic acid, [^{14}C]propionic acid or [^{14}C]butyric acid were infused at the rate of 0.1 $\mu\text{C}/\text{min.}/\text{kg. body wt.}$ for 60 min. into the portal vein of anaesthetized animals by the procedure described by Annison & Lindsay (1962). Negligible amounts of unlabelled sodium acetate (0.5 $\mu\text{mole}/\text{min.}/\text{kg.}$) were infused with [^{14}C]acetate and [^{14}C]propionate, but with [^{14}C]propionate, [^{14}C]butyrate, [^{14}C]butyrate, [^{14}C]butyrate, unlabelled substrate was infused at a rate of 5 m-moles/min./kg. Blood samples were withdrawn from the right jugular vein 60 min. after the start of the infusion. At the end of the infusion samples of liver and muscle were removed as quickly as possible for assay of glycogen.