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.

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

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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]acetate, but with [^{14}C]propionate, [^{14}C]propionate, [^{14}C]butyrate, [^{14}C]butyrate and [^{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.

¹⁴C-labelled fatty acids. The ¹⁴C-labelled and unlabelled fatty acids used in the present studies were as described by Leng & Annison (1963).

Organism. A culture of *Leuconostoc mesenteroides* (strain 39) was obtained from the Department of Bacteriology, Indiana University, Bloomington, Ind., U.S.A.

Chemical methods. The methods used for the measurement of plasma glucose concentration and specific radioactivity were as described by Annison & White (1961). Glucose was isolated from blood as described by Annison & White (1962*a*), and the distribution of ^{14}C determined by stepwise degradation with *L. mesenteroides* (Leng & Annison, 1963). The measurement of blood ketone bodies, the isolation of β -hydroxybutyrate by column chromatography and its stepwise degradation were as described by Leng & Annison (1963). Formic acid was measured by gas chromatography, and assayed for radioactivity as described by Annison & White (1962*a*). Acetic acid, propionic acid and butyric acid were isolated from blood by gas-liquid chromatography and their specific radioactivities determined by the methods described by Annison & Lindsay (1962). Acetic acid was degraded by a modification of the method of Phares (1951) as used in the degradation of glucose (Leng & Annison, 1963). Lactic acid concentrations and specific radioactivities were determined as described by Annison, Lindsay & White (1963), the acid being isolated from blood as the zinc salt, and on some occasions purified by column chromatography (Leng & Annison, 1963). The distribution of ^{14}C in lactic acid was determined as described by Leng & Annison (1963).

RESULTS

Incorporation of fatty acids into glycogen, glucose and lactate. The incorporation of ^{14}C into glucose and glycogen from [^{14}C]propionate was greater

than that from [^{14}C]acetate or [^{14}C]butyrate, but the degree of labelling of glycogen in each case was low relative to that of plasma glucose (Table 1). The specific radioactivity of lactate was lower than that of glucose after the infusion of all ^{14}C -labelled fatty acids, except [2- ^{14}C]butyrate when it slightly exceeded that of glucose.

The distribution of ^{14}C in plasma glucose and blood lactate after the infusion of ^{14}C -labelled fatty acids is shown in Table 2. The results confirm the glucogenicity of propionate, but the labelling patterns in glucose and lactate from $[^{14}\text{C}]$ acetate and $[^{14}\text{C}]$ butyrate were consistent with entry through acetyl-CoA and the tricarboxylic acid cycle (Weinman, Strisower & Chaikoff, 1957). The labelling patterns in glucose obtained from $[1\text{-}^{14}\text{C}]$ acetate, $[1\text{-}^{14}\text{C}]$ butyrate or $[3\text{-}^{14}\text{C}]$ butyrate were closely similar. The pattern from $[2\text{-}^{14}\text{C}]$ acetate was similar to that from $[2\text{-}^{14}\text{C}]$ butyrate.

Incorporation of fatty acids into β -hydroxybutyrate, acetate and formate. Infusions of [^{14}C]butyrate and [^{14}C]acetate gave rise to ^{14}C -labelled blood β -hydroxybutyrate, the raised concentrations of β -hydroxybutyrate observed during infusions of butyrate probably occurring in response to the infusion of carrier butyrate (Table 1). Partial degradation of ^{14}C -labelled β -hydroxybutyrate isolated from blood after the infusion of [1- ^{14}C]-, [2- ^{14}C]- or [3- ^{14}C]-butyrate indicated that 86, 0.1 and 8% respectively of the total ^{14}C was located at C-1. The production of ^{14}C -labelled β -hydroxybutyrate from [1- ^{14}C]- or [2- ^{14}C]-propionate was unexpected, but consistent with the appearance of

Table 1. *Incorporation of ^{14}C from ^{14}C acetate, ^{14}C propionate and ^{14}C butyrate into glycogen and blood constituents in sheep*

The specific radioactivities and concentrations of blood acetate, propionate, butyrate, lactate and β -hydroxybutyrate, and of plasma glucose, were measured 60 min. after the start of infusions of labelled fatty acids. Experimental details are given in the text.

Concentration (μ moles/ml.)							
Fatty acid infused	Fatty acid infused	Acetate	β -Hydroxy-butyrate	Glucose	Lactate		
[1- 14 C]Acetate	0.59	0.59	0.20	4.20	0.90		
[2- 14 C]Acetate	0.52	0.52	0.13	4.10	1.31		
[1- 14 C]Propionate	0.40	0.21	0.08	4.35	0.99		
[2- 14 C]Propionate	0.35	0.25	0.23	3.55	0.80		
[1- 14 C]Butyrate	0.12	1.02	0.38	4.35	1.07		
[2- 14 C]Butyrate	0.15	0.83	0.82	7.20	1.63		
[3- 14 C]Butyrate	0.13	0.58	0.84	2.70	0.99		
Specific radioactivity (μ c/g. of C)							
Fatty acid infused	Fatty acid infused	Acetate	β -Hydroxy-butyrate	Glucose	Lactate	Liver glycogen	Muscle glycogen
[1- 14 C]Acetate	72	72	4.7	2.0	1.0	0.008	0.006
[2- 14 C]Acetate	92	92	14.0	5.1	0.6	0.006	0.001
[1- 14 C]Propionate	—	0.4	1.8	13.6	3.5	0.04	0.001
[2- 14 C]Propionate	123	0.4	2.6	37.9	5.3	0.22	—
[1- 14 C]Butyrate	103	6.4	45.5	3.4	1.8	0.05	0.005
[2- 14 C]Butyrate	166	13.2	51.2	4.2	4.8	0.01	0.002
[3- 14 C]Butyrate	174	17.2	59.2	2.8	2.1	0.01	0.008

Table 2. *Distribution of ^{14}C in glucose and lactate isolated from plasma in sheep after the infusion of specifically labelled [^{14}C]acetate, [^{14}C]propionate or [^{14}C]butyrate*

The rates of infusion were: [^{14}C]acetate, 0.5 $\mu\text{mole/min./kg. body wt.}$ (0.1 $\mu\text{C/min./kg.}$); [^{14}C]propionate and [^{14}C]butyrate, 5 m-moles/min./kg. body wt. (0.1 $\mu\text{C/min./kg.}$). The period of infusion was 60 min. in all cases. Experimental details are given in the text.

Fatty acid infused	Distribution of ^{14}C in glucose and lactate carbon atoms (%)								
	Glucose						Lactate		
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3
[1- ^{14}C]Acetate	3	7	40	46	3	1	88	8	6
[2- ^{14}C]Acetate	23	22	9	9	26	11	26	48	26
[1- ^{14}C]Propionate	1	2	47	45	4	1	84	11	5
[2- ^{14}C]Propionate	25	20	4	4	22	25	10	50	40
[1- ^{14}C]Butyrate	2	4	38	45	8	3	79	8	13
[2- ^{14}C]Butyrate	26	16	8	10	22	18	25	37	38
[3- ^{14}C]Butyrate	5	6	41	39	4	5	85	6	9

^{14}C -labelled blood acetate during the infusion of [1- ^{14}C]- or [2- ^{14}C]-propionate. Stepwise degradation of the ^{14}C -labelled acetate arising during the infusion of [1- ^{14}C]- or [2- ^{14}C]-propionate revealed that in each case the ^{14}C was distributed approximately equally between C-1 and C-2.

^{14}C -labelled formate was detected in blood after the infusion of [^{14}C]acetate, [^{14}C]propionate or [^{14}C]butyrate. Considerable variation between experiments was observed, the specific radioactivity of formate isolated from blood ranging from 1 to 5 % of that of the ^{14}C -labelled fatty acid.

The infusion of sodium propionate (5 m-moles/min./kg.) invariably depressed blood acetate concentrations (Table 1).

DISCUSSION

The evidence for the possible glucogenicity of butyrate and acetate was reviewed by Leng & Annison (1963). The results of the present studies confirmed the previous findings, with liver slices, that the incorporation of acetate C and butyrate C into glucose in sheep tissues involves no net synthesis of carbohydrate (Leng & Annison, 1963). Kronfeld, Campbell, Hooper & Galligan (1959) have suggested that butyrate may be incorporated into glucose as a C_3 unit (acetone) via the propanediol pathway. Conversion of butyrate into acetoacetate undoubtedly gives rise to acetone which may be directly converted into a glycolytic intermediate in some animal tissue (Mourkides, Hobbs & Koeppe, 1959; Shull & Miller, 1960). However, the high specific radioactivity of blood β -hydroxybutyrate, and the relatively little ^{14}C at C-2 and C-5 of glucose isolated after the infusion of [3- ^{14}C]butyrate, indicate that pathways involving acetone are not quantitatively important in butyrate metabolism in sheep tissues. Similar con-

clusions were reached by Black, Kleiber & Brown (1961), in studies with the lactating cow.

The incorporation of ^{14}C from [1- ^{14}C]- or [2- ^{14}C]-propionate into glucose was consistent with the conversion of propionate into a symmetrical C_4 compound before conversion into phosphoenolpyruvate, and was in agreement with the established pathway, namely propionate $\rightarrow$ methylmalonate $\rightarrow$ succinate $\rightarrow$ phosphoenolpyruvate $\rightarrow$ glucose. The equal distribution of ^{14}C between C-2 and C-3 of lactate arising from [2- ^{14}C]propionate was evidence against metabolic pathways involving the direct conversion of propionate into lactate (or pyruvate), e.g. propionate $\rightarrow$ acrylate $\rightarrow$ lactate $\rightarrow$ pyruvate (Mahler & Huennekens, 1953). The slight incorporation of ^{14}C into acetate and β -hydroxybutyrate from [1- ^{14}C]propionate implies the production of acetyl-CoA from propionate by a pathway other than the well-established route of propionate $\rightarrow$ succinate $\rightarrow$ pyruvate $\rightarrow$ acetyl-CoA, which would result in loss of ^{14}C as $^{14}\text{CO}_2$. Comparison of the specific radioactivities of blood propionate, acetate and β -hydroxybutyrate (Table 1) indicated that this pathway was not quantitatively significant. The high specific radioactivity of β -hydroxybutyrate relative to that of acetate probably reflected the greater rate of acetate entry into body pools. James, Peeters & Laurysens (1956) reported the production of ^{14}C -labelled acetate from [1- ^{14}C]propionate by the perfused goat udder.

Comparison of the specific radioactivities of blood β -hydroxybutyrate and blood fatty acid after the infusion of [^{14}C]acetate and [^{14}C]butyrate (Table 1) indicated the high incorporation of butyrate C relative to that of acetate C into β -hydroxybutyrate. In non-ruminants the production of ketone bodies is largely based on condensation of acetyl-CoA (Krebs, 1961), but the labelling

patterns in β -hydroxybutyrate produced from [^{14}C]butyrate indicated the conversion of butyrate into β -hydroxybutyrate largely as a C_4 unit, emphasizing the special role of butyrate in ketone body metabolism in ruminants. The low contribution of acetate to β -hydroxybutyrate production probably reflects low utilization of acetate by liver tissues, a major site of ketone body production.

The isolation of lactate of slightly higher specific radioactivity than that of glucose after the infusion of [$2\text{-}^{14}\text{C}$]-but not of [$1\text{-}^{14}\text{C}$]- or of [$3\text{-}^{14}\text{C}$]-butyrate was unexpected, and inconsistent with the evidence from glucose and lactate labelling patterns that butyrate was metabolized as two C_2 units. The possible contamination of lactate with β -hydroxybutyrate cannot be excluded, although control experiments showed that inactive zinc lactate could be recovered from blood (10 ml.) containing added lithium lactate (50 mg.) and β -hydroxybutyrate (10 mg., 10 μmc).

The infusion of [$2\text{-}^{14}\text{C}$]acetate or [$2\text{-}^{14}\text{C}$]butyrate resulted in appreciable labelling of C-3 and C-4 of glucose. In experiments in which these ^{14}C -labelled fatty acids were incubated with liver slices (Leng & Annison, 1963) much less ^{14}C was found in C-3 and C-4 of glucose. Propionate metabolism was shown by Leng & Annison (1963) to lead to increased fixation of CO_2 arising from butyrate oxidation. Therefore, the increased labelling of C-3 and C-4 of glucose in the experiments with the whole animal may reflect greater availability of propionate.

The relatively low specific radioactivity of formate produced from [^{14}C]acetate, [^{14}C]propionate and [^{14}C]butyrate suggested that these short-chain fatty acids are not major precursors of formate.

The concentration of acetate in blood was markedly depressed by the infusion of propionate (Table 1). Concentrations in starved sheep with rumen contents removed are rarely lower than 0.3 $\mu\text{mole/ml}$. (Annison & White, 1962*b*), and the present results suggest that propionate either inhibits endogenous acetate production or increases rates of removal of acetate from blood.

The results of the present studies suggest that, in spite of the dependence of ruminants on large amounts of short-chain fatty acids produced by fermentation in the rumen, the metabolic pathways involved in their utilization are similar to those known to occur in non-ruminants (cf. Lifson, Lorber, Sakami & Wood, 1948).

SUMMARY

1. Portal infusions of [$1\text{-}^{14}\text{C}$]acetate, [$2\text{-}^{14}\text{C}$]acetate, [$1\text{-}^{14}\text{C}$]propionate, [$2\text{-}^{14}\text{C}$]propionate, [$1\text{-}^{14}\text{C}$]-

butyrate, [$2\text{-}^{14}\text{C}$]butyrate or [$3\text{-}^{14}\text{C}$]butyrate were made into anaesthetized sheep.

2. The specific radioactivity of liver and muscle glycogen was low after all infusions. The highest value was less than 0.2 % of the specific radioactivity of the fatty acid infused ([$2\text{-}^{14}\text{C}$]propionate).

3. The specific radioactivity of plasma glucose was 2–5 % that of the fatty acid infused after the infusion of acetate or butyrate, but 30 % after the infusion of [$2\text{-}^{14}\text{C}$]propionate.

4. The specific radioactivity of blood lactate was less than that of blood glucose except after the infusion of [$2\text{-}^{14}\text{C}$]butyrate, when it was very slightly higher.

5. The infusion of [$1\text{-}^{14}\text{C}$]acetate, [$1\text{-}^{14}\text{C}$]propionate, [$1\text{-}^{14}\text{C}$]butyrate or [$3\text{-}^{14}\text{C}$]butyrate gave rise to labelling mainly in C-3 and C-4 of glucose and C-1 of lactate. The infusion of [$2\text{-}^{14}\text{C}$]acetate, [$2\text{-}^{14}\text{C}$]propionate or [$2\text{-}^{14}\text{C}$]butyrate labelled mainly C-1, C-2, C-5 and C-6 of glucose, and C-2 and C-3 of lactate.

6. The specific radioactivity of blood β -hydroxybutyrate was about 50 % of that of infused [^{14}C]butyrate, about 7–15 % of infused [^{14}C]acetate and about 2 % of infused [^{14}C]propionate.

7. The specific radioactivity of blood formate was about 5 % of that of infused [^{14}C]acetate, [^{14}C]propionate or [^{14}C]butyrate.

8. The specific radioactivity of blood acetate was less than 1 % of that of infused [^{14}C]propionate but about 6–10 % of that of infused [^{14}C]butyrate.

9. The results are consistent with the operation of metabolic pathways similar to those known to occur in non-ruminants.

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Biosynthesis of Nucleic Acids in *Bacillus megaterium*

2. THE FORMATION OF RIBONUCLEIC ACID BY NUCLEAR MATERIAL *IN VITRO**

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Butler & Godson (1963) have described the preparation of nuclear material, from *Bacillus megaterium*, containing nearly all the DNA of the cell and a small proportion of the RNA, which had been shown (Godson & Butler, 1962) to be highly active metabolically and to a great extent the site of the initial synthesis of RNA when protoplasts are incubated with the appropriate radioactive precursors. In the experiments reported below the various cell fractions were incubated *in vitro* with a radioactive RNA precursor, and the conditions under which the synthesis of RNA takes place and the nature of the product formed are examined in detail.

EXPERIMENTAL

Materials. ATP, GTP, CTP, UTP, UDP and barium 3-phosphoglycerate were obtained from the Sigma Chemical Co., [³H]uridine of specific radioactivity 3c/m-mole from The Radiochemical Centre, Amersham, Bucks., and deoxyribonuclease from the Worthington Biochemical Corp. Streptomycin sulphate was obtained from The Distillers Co. (Biochemicals) Ltd., and protamine sulphate (from salmon milt) from British Drug Houses Ltd. All other materials were of AnalaR grade or were the purest available.

Growth and fractionation of cells. *Bacillus megaterium* was grown, harvested in logarithmic-growth phase and converted into protoplasts as described by Butler & Godson (1963). The fractionations were also done as described by Butler & Godson (1963), with the following modifications: lysis by Lubrol was carried out in a medium consisting of 0.1M-tris-HCl buffer, pH 7.6, containing sucrose (0.3M) and MgCl₂ (5 mM), and in the presence of 0.03% of Lubrol W; nuclear material was sedimented by centrifuging at 100 000 g for 1 hr. As prepared in this way, nuclear material contained ribosomes (see Butler & Godson, 1963), which could be removed by centrifuging at 100 000 g for 2 hr.

Purification of ribonucleic acid polymerase. The method of Chamberlin & Berg (1962) was used: portions (10 ml.) of nuclear material containing 50 mg. of protein were made 0.1 mM with respect to EDTA and 10 mM with respect to 2-mercaptoethanol. Deoxyribonuclease (10 µg.) was added and the material digested at 37° for 30 min. A portion (0.5 ml.) of a 10% (w/v) solution of streptomycin sulphate was added with stirring and the solution set aside for 15 min. The solution was centrifuged at 30 000 g for 15 min. and 1 ml. of a 1% (w/v) solution of protamine sulphate was added to the supernatant. The precipitate was collected by centrifuging at 30 000 g for 15 min. and washed with buffer solution A [5 mM-tris-HCl, pH 7.6, containing MgCl₂ (10 mM) and 2-mercaptoethanol (10 mM)]. The precipitate was then extracted with 1 ml. of 0.1M-ammonium sulphate in buffer A and the residue removed by centrifuging at 30 000 g for 15 min. The supernatant was removed and stored at -25°. Storage for 3 days resulted in a 30% loss of RNA-polymerase activity.

Preparation of [³H]uridine triphosphate. Spleen (0.5 g.) obtained from Aki mice bearing a virus-induced leukaemia (Bianchi, Butler, Crathorn & Shooter, 1961) was homogenized in 1 ml. of a medium consisting of 10 mM-tris-HCl buffer, pH 8, containing sucrose (0.23M) and KCl (5 mM), and centrifuged at 100 000 g for 1 hr. The supernatant was used to phosphorylate [³H]uridine to [³H]UTP and showed no loss of activity on storing for 6 months at -25°, even after repeated freezing and thawing. The incubation mixture contained, in a volume of 1 ml.: 0.6 ml. of mouse-spleen supernatant fraction, 53 µmoles of ATP, 64 µmoles of sodium 3-phosphoglycerate, 53 µmoles of MgCl₂, 180 µmoles of KCl, 3.8 m-moles of tris-HCl, pH 8, 83 m-moles of sucrose and 200 µC of [³H]uridine. The time of incubation to produce the maximum yield of [³H]UTP was determined for each batch of enzyme and was between 15 and 30 min. at 37°. The incubation mixture was made 5% (w/v) with respect to trichloroacetic acid and the acid-soluble nucleotides were separated by chromatography. The overall yield of [³H]UTP from [³H]uridine was 40-50%, and batches of 100 µC in a volume of 1 ml. were easily isolated by washing the chromatograms by downward flow of ethanol and eluting the appropriate band into a tube by downward

* Part 1: Butler & Godson (1963).