Rates of Entry and Oxidation of Acetate, Glucose, D(−)-β-Hydroxybutyrate, Palmitate, Oleate and Stearate, and Rates of Production and Oxidation of Propionate and Butyrate in Fed and Starved Sheep

By E. F. ANNISON,* R. E. BROWN,† R. A. LENG, D. B. LINDSAY AND C. E. WEST*
School of Rural Science, University of New England, Armidale, New South Wales, Australia

1. Rates of entry and oxidation of a range of metabolites have been measured in tracheostomized sheep (diet, 800g. of lucerne chaff and 100g. of maize/day) by combining isotope-dilution techniques with the continuous measurement of total respiratory gas exchange, and $^{14}CO_2$ production during the intravenous or intraruminal infusion of ^{14}C-labelled substrates. 2. Mean entry rates in fed and starved (24hr.) sheep respectively, expressed as mg./min./kg. body wt.$^{0.75}$, were: glucose, 5·0 (range 4·8–5·1, 2 observations) and 3·8 (3·2–4·2, 4); acetate, 10·8 (9·1–13·5, 4) and 5·8 (1); D(−)-β-hydroxybutyrate, 1·4 (1) and 1·5 (0·8–2·4, 4); palmitate, oleate and stearate (starved sheep only) 1·0 (0·6–1·9, 7), 0·9 (0·2–1·6, 10) and 0·9 (0·5–1·1, 11) respectively. 3. Production rates of propionate and butyrate in continuously feeding sheep were 6·4 (4·7–8·3, 4) and 4·3 (3·4–6·1, 4) mg./min./kg.$^{0.75}$ respectively, and in starved (24hr.) sheep were 2·5 (2·2–2·9, 2) and 1·0 (0·8–1·2, 2) mg./min./kg.$^{0.75}$ respectively. 4. Calculated terminal values for the specific radioactivity of respiratory $^{14}CO_2$ during measurements of entry rates and production rates were used to calculate the contributions of individual substrates to overall oxidative metabolism. Mean values for fed and starved sheep respectively were: glucose, 9·1 (8·6–9·6, 2) and 11·2 (5·9–15·1, 4)%; acetate, 31·6 (26·8–38·1, 4) and 22·1 (1)%; D(−)-β-hydroxybutyrate, 10·4 (1) and 4·8 (1·9–7·7, 4)%; propionate, 23·0 (13·8–29·9, 4) and 7·1 (6·8–7·4, 2)%; butyrate, 16·5 (13·7–20·5, 4) and 5·3 (5·2–5·3, 2)%; palmitate, oleate and stearate (starved sheep only), 4·7 (2·0–7·7, 7), 4·0 (1·2–6·6, 10) and 4·4 (3·8–5·8, 9)% respectively. The sum of these values for individual substrates in fed and starved sheep, excluding that of β-hydroxybutyrate and after correction of the glucose value for the known interrelations of this substrate with propionate, accounted for 76% and 58% respectively of total production of carbon dioxide. 5. Calculations based on the proportion of substrate entry directly oxidized indicated that the substrates studied accounted for 63% (fed sheep) and 43% (starved sheep) of total energy expenditure measured by oxygen uptake. The contribution of β-hydroxybutyrate was excluded, and corrections were made for glucose–propionate interrelations, and for the different rates of oxidation of the methyl and carboxyl fragments of acetate. 6. The present results have been combined with those obtained earlier in this Laboratory to examine the relationships between rates of substrate entry and oxidation, and concentrations of substrate in blood. Rates of entry of acetate, glucose, D(−)-β-hydroxybutyrate, palmitate and oleate (but not stearate) were well correlated with concentration in blood, and substrate contribution to production of carbon dioxide showed a similar correlation to blood concentration, except with glucose. 7. It was concluded that the general technique is of potential value in providing valid quantitative parameters of animal metabolism.

The role of the major energy-yielding substrates in ruminant metabolism can be evaluated by the use of isotope-dilution techniques in the undisturbed, conscious animal (see Annison, 1964).

* Present address: Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford.

† Present address: Department of Dairy Science, University of Illinois, Urbana, Ill., U.S.A.

In this Laboratory the rates of entry of glucose, acetate, propionate, butyrate, D(−)-β-hydroxy-butyrate and plasma FFA* have been measured by the continuous intravenous infusion of ^{14}C-labelled materials (Annison & White, 1961, 1962; Annison & Lindsay, 1961, 1962; West & Annison, 1964; Leng & Annison, 1964): rates of production of acetate, propionate and butyrate have been measured by the intraruminal infusion of labelled substrates in sheep fed at intervals of 1 hr. (Leng & Leonard, 1965). In some instances the specific radioactivity of expired $^{14}CO_2$, or of arterial blood $^{14}CO_2$, provided a measure of substrate oxidation. These techniques have been extended in the present studies to include the continuous measurement of both total respiratory gas exchange and $^{14}CO_2$ output. These additional data have allowed calculation of the contribution of each substrate to total production of carbon dioxide, and of the proportion of each substrate entering the body pool that was promptly oxidized. In some experiments total energy expenditure was calculated from the oxygen uptake.

The patterns of excretion of $^{14}CO_2$ during the infusions of ^{14}C-labelled substrates are complex, particularly in the fed animal, where there is considerable production of carbon dioxide in the rumen which exchanges with carbon dioxide produced in the tissues. We have used correction factors based on experimental data, and on information obtained in other investigations, but we must emphasize that the values obtained for substrate oxidation are no more than estimates. Attempts to obtain quantitative data in this way were prompted by the lack of alternative procedures for measuring substrate oxidation in relation to total oxidative metabolism in intact animals.

The present results, and those obtained earlier, have been considered in relation to overall metabolism. The importance of gluconeogenesis in ruminant tissues has been confirmed, and acetate has been shown to make the largest single contribution to oxidative metabolism.

The potential usefulness of isotope dilution in animal nutrition, and the validity of the general technique in providing quantitative parameters of metabolism, are discussed in relation to the data obtained by us and by other investigators.

MATERIALS AND METHODS

Experimental animals. Merino ewes and wethers (age 3–5 years) were housed indoors and fed with lucerne chaff (800 g./day) and whole maize (100 g./day) at 08.30 hr. The ration was generally consumed within 2 hr. The animals were trained to stand quietly in stocks for periods of 6–8 hr. Animals were tracheostomized under thiopentone anaes-

thesia at least 6–8 weeks before experimentation. The trachea was exposed ventrally by blunt dissection, and roughly half of one segment of cartilage was excised to allow the introduction of an endotracheal tube. A rigid plastic self-retaining cannula similar to that described by Blaxter & Joyce (1963) was positioned in the trachea, and the skin wound sutured tightly around the cannula. The fistula was cleaned at frequent intervals between experiments. Immediately before an experiment the cannula was removed and a McGill cuffed endotracheal tube (size 9; J. G. Franklin and Sons Ltd., London, E. 8) was inserted caudally into the trachea and the cuff inflated. Expired air, free from eructated rumen gas, was collected for analysis by using a three-way respiration valve (Max Planck Institute, Göttingen, West Germany).

Carotid loops were established at least 6–8 weeks in advance in the sheep used for experiments where arterial blood was required. Catheters were inserted into the carotid artery on the day of the experiment, a local anaesthetic (procaine) being applied at both ends of the loop. Jugular vein catheters were inserted on the day before the experiment where jugular vein blood was required. Rumen fistulae were established at least 6–8 weeks in advance in sheep used for experiments where infusion into and sampling from the rumen was required.

Experiments were carried out on sheep 24 hr. after feeding, 2–6 hr. after feeding and during feeding. For the experiments on feeding sheep, the sheep were transferred to a continuous feeding regime. The sheep were given one-twelfth of their daily ration (66·7 g. of lucerne and 8·3 g. of maize) at hourly intervals from 08.00 hr. to 19.00 hr. for 4 or 5 days. The experiments were carried out on the fourth or fifth day of each period. Between these periods, the sheep were returned to their once daily-feeding regime. Animals were allowed water *ad libitum* but it was withheld during infusions.

Radioactive materials. [U-^{14}C]Palmitic acid, [U-^{14}C]-stearic acid, [U-^{14}C]oleic acid, [1-^{14}C]palmitic acid, sodium [1-^{14}C]acetate, sodium [2-^{14}C]acetate, sodium [1-^{14}C]-propionate, sodium [3-^{14}C]propionate, sodium [1-^{14}C]-butyrate, sodium [2-^{14}C]butyrate, NaH$^{14}CO_3$ and [U-^{14}C]-glucose were obtained from The Radiochemical Centre, Amersham, Bucks. Sodium [2-^{14}C]propionate was obtained from the California Corp. for Biochemical Research, Los Angeles 63, Calif., U.S.A. Aqueous solutions of long-chain fatty acids in albumin were prepared by the method of Laurell (1957) and diluted to the required activity/ml. Albumin (bovine serum albumin fraction V powder) obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A., was used. The long-chain fatty acids (A grade) were obtained from the California Corp. for Biochemical Research. Sodium [U-^{14}C]acetate, sodium [U-^{14}C]propionate and sodium [1,2-$^{14}C_2$]butyrate were prepared by mixing the appropriate quantities of the 1-^{14}C-, 2-^{14}C- and 3-^{14}C-labelled compounds. Sodium D(−)-β-hydroxy[1,2-$^{14}C_2$]butyrate was prepared from sodium [1,2-$^{14}C_2$]butyrate by the method of Leng & Annison (1964).

Experimental procedure. ^{14}C-labelled substrates (except propionate, butyrate and, in some experiments, bicarbonate) were infused intravenously as described by Annison & Lindsay (1961). In these experiments the left jugular vein was used for infusion, and blood samples were withdrawn from a catheter inserted into the right jugular vein, except when long-chain fatty acids or NaH$^{14}CO_3$ were

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* Abbreviation: FFA, free fatty acid.

infused, when arterial blood was obtained from a catheter inserted into the carotid loop. It has been shown (C. E. West, unpublished work) that during the infusion of labelled FFA there is a substantial arteriovenous difference across the head in specific radioactivity but not of concentration of FFA as measured by samples taken from the carotid artery and jugular vein. Labelled glucose was infused after a priming injection of 90 to 120 times the amount infused in 1 min. as described by Annison & White (1961). The labelled substrates for intravenous infusion were prepared in 0·9% NaCl to contain 0·1–0·4mg. and 0·1–0·5μc of labelled material/ml. and were infused at a rate of 0·37ml./min.

The general procedure for the infusion of ¹⁴C-labelled substrates into the rumen for the measurement of production rates of propionate and butyrate was described by Leng & Leonard (1965). Sodium [U-¹⁴C]propionate and sodium [1,2-¹⁴C₂]butyrate were prepared to contain 1μmole (0·5μc/ml.). NaH¹⁴CO₃ was prepared to contain 0·1–0·2mg. and 0·1–0·2μc/ml. Solutions were infused into the rumen at the rate of 1·0–1·3ml./min. Infusions into feeding sheep were started at approximately 12.30hr., when the sheep had consumed five hourly feeds, and respiratory pattern analysis commenced at 14.30hr., when the feeding sheep had eaten seven hourly feeds. The feeding sheep consumed their ration in 15–20min. and were trained to eat during an infusion until connected to the respiration pattern analyser. During the 5hr. period of infusion rumen fatty acid concentrations remained constant in both feeding and starved sheep.

The equipment used to measure and record total gas exchange, and ¹⁴CO₂ production, is shown in Fig. 1. Expired air was collected by a respiration valve attached to an endotracheal tube inserted into the tracheal fistula as described above. The inhalation side of the valve connected by flexible tubing (1 in. diam.) to a dry-gas meter to measure total inflow. Expired air was passed first into a water trap

cooled with acetone–solid CO₂ and then into a spirometer filled with a low-viscosity lubricating oil. The gas was finally dried by passage through a wide-bore (2in. diam.) Perspex tube containing granulated anhydrous CaCl₂.

The ¹⁴CO₂ content of the dried gas was assayed with a 4·3l. ion chamber and a vibrating-reed electrometer (Cary model 31, Applied Physics Corp., Monrovia, Calif., U.S.A.). A low-capacity pump was used to withdraw 200ml. of air/min. from the gas stream for CO₂ and O₂ analysis (Fig. 1). The CO₂ content was continuously measured with a katharometer calibrated over the range 0–6% (Cambridge Instrument Co. Ltd., London), and the O₂ concentration with a paramagnetic oxygen analyser (Beckman Inc., Fullerton, Calif., U.S.A.). A thermocouple placed in the outlet tube from the ion chamber and connected to a three-channel multi-point recorder provided a continuous record of temperature. Signals from the vibrating-reed electrometer and the CO₂ gas analyser occupied the remaining two channels on the recorder, and O₂ concentrations were continuously recorded on an independent 0–10mv recorder (Fig. 1). The katharometer was calibrated before and after each experiment.

Ion chambers are usually calibrated with ¹⁴CO₂ liberated from known amounts of standardized Ba¹⁴CO₃ (Tolbert, Kirk & Baker, 1956). In addition to this procedure the ion chamber used here was calibrated indirectly by using a flow system identical with normal experimental conditions. The ion-chamber response to ¹⁴CO₂ in respiratory gases obtained during the intravenous infusion into sheep of NaH¹⁴CO₃ (0·05–0·15μc/min. for 400min.) was checked by passing the gases leaving the ion chamber through CO₂-free 0·5N-NaOH. The trapped ¹⁴CO₂ was assayed for specific radioactivity as Ba¹⁴CO₃.

Chemical methods. Compounds were estimated, isolated and specific radioactivities determined as described earlier: plasma glucose (Lindsay & Brown, 1966); blood acetate (Davis, Brown, Staubus & Nelson, 1960); individual
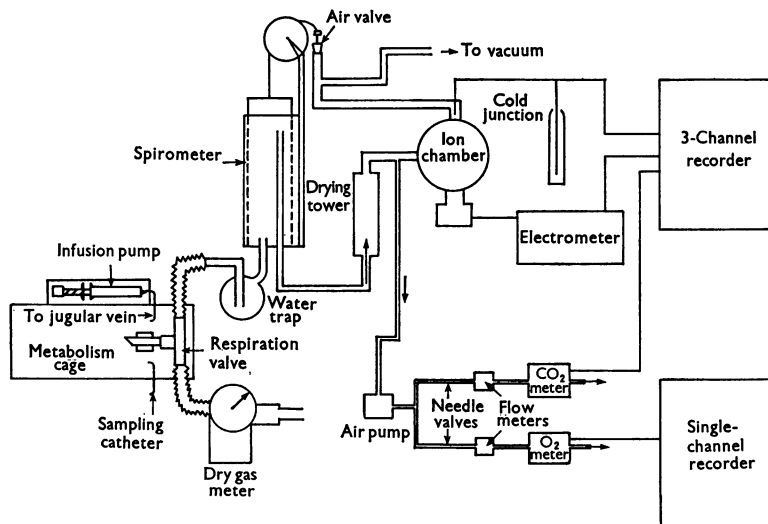

Fig. 1. Diagram of equipment used to measure rates of entry and oxidation of substrates in sheep.

plasma FFA (West & Annison, 1964); blood β-hydroxy-butyrate (Leng, 1965); individual rumen volatile fatty acids (Leng & Leonard, 1965); specific radioactivity of blood and of rumen CO_2 (Annison & White, 1961; Leng & Leonard, 1965).

Statistical analysis. The plot of specific radioactivity against time for respiratory $^{14}CO_2$ was fitted to the expression $y = A - Be^{-Cx}$ (a Mischerlich curve) where y is the specific radioactivity at any time x and A, B and C are constants, A being the calculated specific radioactivity at infinite time. This calculation was carried out by means of computers. Two programmes were used. The first was written for the IBM 1620 computer by Mr B. Butterfield of the University of New England Computer Centre and the second was from the University of California in Los Angeles BIMED series. Linear regression analysis was carried out as described by Snedecor (1956).

RESULTS

Intravenous and intraruminal infusion of $NaH^{14}CO_3$. The output of respiratory $^{14}CO_2$ was continuously monitored during the intravenous infusion of $NaH^{14}CO_3$ into continuously feeding and starved (24–30hr.) sheep. The rate of respiratory output of $^{14}CO_2$ reached a constant value after about 300 min. and at this time was $83 \pm 2\%$ (mean and range of 2 experiments) of the rate of infusion of radioactivity as $NaH^{14}CO_3$ in the feeding sheep, and $93 \pm 2\%$ in the starved sheep. The assumption was made that at this time the blood HCO_3^-–carbon dioxide had completely equilibrated with other tissue HCO_3^-–carbon dioxide pools (except bone), and that the difference between the radioactivity infused and that excreted as respiratory $^{14}CO_2$ was largely accounted for by loss in eructated rumen gases and retention in bone. The excretion of $H^{14}CO_3^-$ in urine accounted for only 0·3% of that infused. During these infusions the specific radioactivity of $^{14}CO_2$ in rumen fluid was also followed,

and when plotted against time (Fig. 2) showed a roughly constant value after 300 min. During the terminal stages of the infusion the specific radioactivity of rumen $^{14}CO_2$ was 40% of that of respiratory $^{14}CO_2$.

The interchange of CO_2 produced by tissue metabolism with that arising by fermentation in the rumen was also investigated by following the rate of increase of the specific radioactivity of respiratory $^{14}CO_2$ during the intraruminal infusion of $NaH^{14}CO_3$ into continuously feeding and starved sheep. Roughly constant specific radioactivities were achieved within 250–300 min. (Fig. 3), when that of respiratory carbon dioxide was 15% and 10% respectively of the specific radioactivity of rumen fluid $^{14}CO_2$ in feeding and starved sheep. This result implied that, under the conditions of the experiments, 15% and 10% of the respiratory carbon dioxide was produced by fermentation in the rumen.

These data clearly indicate the complexities of carbon dioxide exchange in sheep. Rumen carbon dioxide produced by fermentation is lost from the rumen by eructation or by transfer across the rumen wall and excretion by the lungs. The fraction of rumen carbon dioxide reduced to methane is lost almost entirely by eructation since there is negligible transfer of methane across the rumen wall, and entry of rumen gases into the lungs via the trachea, which occurs to a considerable extent in normal sheep, is prevented by the tracheal cannula. Metabolic carbon dioxide produced by the oxidation
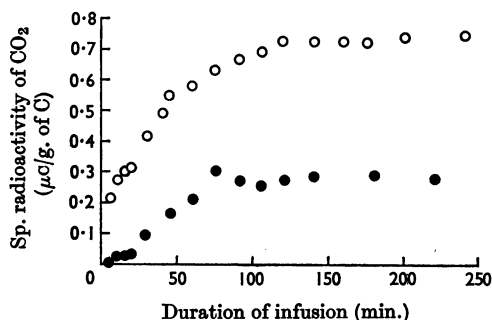

Fig. 2. Time-course of the increase in specific radioactivity of $^{14}CO_2$ in expired air and rumen contents during the intravenous infusion of $NaH^{14}CO_3$ at 0·33 μc/min. Wt. of sheep, 33·0kg. O, Sp. radioactivity of respiratory $^{14}CO_2$; ●, sp. radioactivity of rumen $^{14}CO_2$.
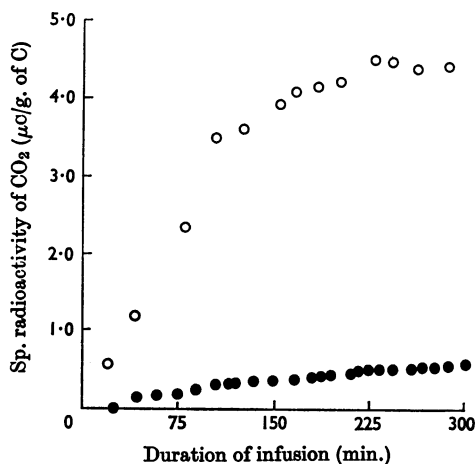

Fig. 3. Time-course of the increase in specific radioactivity of $^{14}CO_2$ in rumen contents and expired air during the intraruminal infusion of $NaH^{14}CO_3$ at 0·21 μc/min. Wt. of sheep, 34·8kg. O, Sp. radioactivity of rumen $^{14}CO_2$; ●, sp. activity of respiratory $^{14}CO_2$.

of substrates in tissues is lost as respiratory carbon dioxide, and by entry into the rumen by direct transfer across the rumen wall, or in saliva. In addition, metabolic carbon dioxide may enter bone, where the low turnover rate of the bone carbon dioxide pool relative to the time of the infusion experiments results in the virtual fixation of carbon dioxide, or may enter carbon dioxide-fixation reactions.

We have assumed that $NaH^{14}CO_3$, when infused intravenously, follows the same patterns of fixation and excretion as metabolic carbon dioxide. The recovery of radioactivity infused as $NaH^{14}CO_3$ therefore provided correction factors which were applied to the calculated terminal values of the specific radioactivity of respiratory $^{14}CO_2$ during the infusion of ^{14}C-labelled substrates. The data on the interchange of metabolic and fermentation carbon dioxide gave no information on the influence of the rumen on total respiratory output of carbon dioxide, and we have assumed that there is no net increase. Additional correction factors for the dilution of respiratory carbon dioxide with rumen carbon dioxide are unnecessary if this assumption is correct, since the replacement of a fraction of metabolic carbon dioxide by rumen carbon dioxide is accounted for in the correction factor based on the recovery of infused radioactivity. In the starved (24 hr.) sheep the lower rate of production of fermentation carbon dioxide reduces the possibility of errors due to the inadequate correction for the interchange of metabolic and fermentation carbon dioxide.

The correction factors 1·2 (1/83) and 1·08 (1/93)

were applied to the calculated specific radioactivities of respiratory $^{14}CO_2$.

Excretion of respiratory $^{14}CO_2$. The patterns of excretion of respiratory $^{14}CO_2$ during the continuous infusion of ^{14}C-labelled bicarbonate, glucose (with priming dose), acetate, oleate (as a representative FFA) and $D(-)-\beta$-hydroxybutyrate and the intraruminal infusion of butyrate and propionate are shown in Fig. 4. The increases in specific radioactivity with time cannot be completely represented by a single exponential function, since many body carbon dioxide pools are involved. Single-injection experiments with $NaH^{14}CO_3$ in cats and rats produced data which best fitted a three-compartment system (Kornberg, Davies & Wood, 1952; Steele, 1955), and in the sheep the additional major complication of the rumen carbon dioxide pool suggested that an expression based on four exponential functions (representing four compartments) should be tested. Unfortunately the reproducibility of the time-course of respiratory $^{14}CO_2$ excretion between experiments on the same substrates was too great to allow detailed interpretation of the curves, and mathematical analysis was confined to the use of a single exponential function to provide a terminal value for the specific radioactivity of respiratory $^{14}CO_2$. Values were fitted into the curve $Y = A - Be^{-Cx}$, where Y is the specific radioactivity at time x, and A, B and C are constants. The terminal value (A) differed only slightly (3–10%) from the value at 300 min. in experiments based on the intravenous infusion of ^{14}C-labelled substrates, but was much higher in the intraruminal infusions (Table 3), reflecting the additional time necessary to reach constant specific radioactivity of substrate in the rumen.
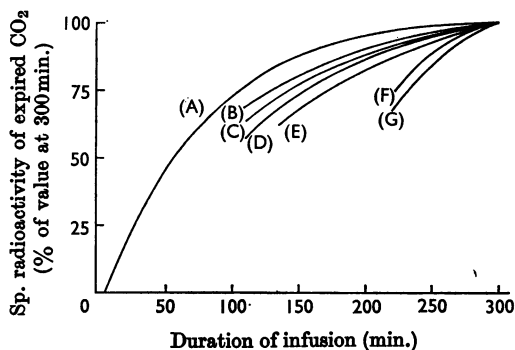

Fig. 4. Specific radioactivity of expired CO_2 during the continuous intravenous (A–E) or intraruminal (F, G) infusion of: (A) $NaH^{14}CO_3$; (B) [U-^{14}C]oleic acid; (C) [U-^{14}C]acetic acid; (D) [U-^{14}C]glucose, after a priming 90 times that of the rate of infusion/min.; (E) $D(-)-\beta$-hydroxy[1,2-$^{14}C_2$]butyric acid; (F) [1,2-$^{14}C_2$]-butyric acid; (G) [U-^{14}C]propionic acid. Results have been expressed as percentages of the specific radioactivity at 300 min.

Table 1. *Comparison of specific radioactivity of carbon dioxide in arterial blood and expired air during the intravenous infusion of* $NaH^{14}CO_3$

Wt. of sheep, 33·0 kg.; infusion rate, 0·092 μc/min. Significance of difference 0·05 > P > 0·02.

Time (min.)	Sp. radioactivity (μc/g. of C)	
	Blood CO_2	Expired CO_2
9	0·23	0·19
22	0·32	0·26
32	0·39	0·31
70	0·44	0·41
81	0·50	0·48
92	0·54	0·52
103	0·54	0·49
111	0·54	0·50
122	0·55	0·56
128	0·53	0·47
Mean	0·46	0·42

Table 2. *Collated results from experiments in which ^{14}C-labelled substrates were infused intravenously into fed and starved sheep*

Substrate	Time after feeding (hr.)	Sheep no. and sex (wether or ewe)	Wt. of sheep (kg.)	Plasma concn. of substrate§ (mg./100ml.)	Entry rate (mg./min./kg.$^{0.75}$)	Entry rate (mg./hr.)	Substrate oxidized (mg./hr.)	Substrate oxidized (% of entry rate)	Substrate oxidized (% of CO$_2$ output)	Total CO$_2$ production (mg. of C/hr.)	Total O$_2$ uptake* (mole/hr.)	Respiratory quotient*	Total energy production† (kcal./hr.)	Energy from substrate (kcal./hr.)‡	Energy from substrate (%)
Glucose	3-7	W 27	34.8	72	4.78	4120	1182	28.7	8.6	5514	(0.64)	(1.03)	67	4.42	6.6
	3-7	W 29	29.2	89	5.12	3860	1436	37.2	9.6	5960	(0.69)	(1.03)	73	5.36	7.4
	24-30	W 32	34.6	70	3.95	3380	1272	37.6	15.1	3382	(0.42)	(0.97)	44	4.76	10.8
	24-30	E 5	34.8	62	4.20	3610	1313	36.4	14.5	3621	(0.45)	(0.97)	47	4.91	10.4
	24-30	W 27	34.8	78	3.21	2760	593	21.5	5.9	4050	(0.50)	(0.97)	53	2.22	4.2
	24-30	W 27	34.2	67	3.73	3170	1178	37.2	9.3	5090	(0.63)	(0.97)	66	4.40	6.7
Acetate	3-7	W 93	28.5	5.7	9.10	6740	4375	64.9	26.8	6540	(0.76)	(1.03)	80	12.80	16.0
	3-7	W 27	35.2	4.9	9.91	8000	4809	55.9	32.5	5912	(0.69)	(1.03)	72	14.07	19.5
	3-7	W 32	34.0	6.1	10.85	9180	4308	46.9	32.2	5357	(0.62)	(1.03)	66	12.61	19.1
	3-7||	W 24	31.0	7.3	13.48	10590	4259	40.2	38.1	6071	(0.71)	(1.03)	74	12.45	16.8
	24-30	W 93	29.8	2.7	5.79	4210	2708	64.3	22.1	4896	(0.61)	(0.97)	64	7.92	12.4
β-Hydroxy-butyrate	3-7	W 56	38.0	4.2	1.38	1265	1097	86.7	10.4	4872	(0.42)	0.96	48	5.14	10.7
	24-30¶	W 20	45.0	3.2	1.09	1135	331	29.2	4.5	3384	(0.42)	(0.97)	44	1.55	3.5
	24-30¶	W 20	45.2	5.5	2.42	2530	719	28.4	7.7	4288	(0.53)	(0.97)	56	3.37	6.0
	24-30	W 72	33.6	1.6	0.79	661	265	40.1	1.9	6540	(0.60)	0.91	63	1.25	2.0
	24-30	W 56	37.5	2.9	1.87	1700	574	33.8	4.9	5172	(0.50)	0.87	52	2.69	5.2
Palmitate	24-30	E 35	33.0	0.9	0.58	477	147	30.8	3.1	3564	(0.44)	(0.97)	46	1.40	3.0
	24-30	E 35	34.8	1.3	0.65	559	150	26.8	3.9	2907	(0.36)	(0.97)	38	1.43	3.8
	24-30	W 16	34.2	0.7	0.41	345	—	—	2.0	—	—	(0.97)	—	—	—
	24-30**	E 5	35.2	2.6	1.21	1048	388	37.0	7.7	3750	(0.46)	(0.97)	49	3.70	7.5
Oleate	24-30	W 52	33.0	2.2	1.20	990	301	30.4	5.0	4480	(0.39)	0.96	51	2.87	5.6
	24-30	W 85	25.6	1.9	1.33	906	270	29.8	4.4	4630	(0.57)	(0.97)	60	2.68	4.5
	24-30	E 85	35.4	3.0	1.93	1680	518	30.8	6.7	5807	(0.72)	(0.97)	75	4.95	6.6
	24-30	E 43	26.0	2.1	0.58	398	186	46.7	2.8	5068	(0.63)	(0.97)	66	1.75	2.7
	24-30	W 52	30.2	1.3	0.24	183	72	39.3	1.2	4701	(0.58)	(0.97)	61	0.68	1.1
	24-30	W 60	31.8	3.4	0.78	680	246	39.0	3.6	5285	(0.65)	(0.97)	68	2.32	3.4
	24-30	W 60	33.4	4.6	1.23	990	411	41.5	6.2	5095	(0.63)	(0.97)	66	3.88	5.9
	24-30	W 46	33.4	8.5	1.58	1323	490	37.0	6.6	5652	(0.70)	(0.97)	73	4.63	6.3
	24-30	W 46	33.4	4.1	1.06	878	297	33.8	4.7	4854	(0.60)	(0.97)	63	2.80	4.4
	24-30	W 91	34.8	4.9	0.74	617	239	38.7	3.5	5244	(0.65)	(0.97)	68	2.25	3.3
	24-30	E 85	35.0	3.0	0.93	805	378	47.0	5.0	5724	(0.56)	0.85	59	3.57	6.0
	24-30	E 115	32.2	4.6	0.90	774	309	39.9	4.2	5610	(0.52)	0.90	55	2.92	5.3
Stearate	24-30	E 115	32.2	1.7	0.71	576	214	37.2	2.7	6000	(0.43)	1.17	45	2.10	4.7
	24-30	W 45	35.2	4.0	1.00	877	307	35.0	3.9	5901	(0.73)	(0.97)	77	2.92	3.8
	24-30	W 45	35.2	3.9	0.81	708	285	40.3	3.8	5724	(0.71)	(0.97)	74	2.72	3.8
	24-30	W 46	32.8	5.7	1.12	923	322	34.9	4.1	6012	(0.74)	(0.97)	78	3.07	3.9
	24-30	W 46	32.8	4.5	1.02	844	338	40.0	4.6	5940	(0.73)	(0.97)	77	3.22	4.2
	24-30	E 96	31.6	3.3	0.68	546	261	47.8	5.8	3417	(0.42)	(0.97)	44	2.48	5.6
	24-30	E 96	31.6	8.3	0.68	546	255	46.7	5.2	3708	(0.46)	(0.97)	48	2.43	5.1
	24-30	E 115	33.2	3.1	0.97	804	260	32.3	4.1	4782	(0.46)	0.87	48	2.48	5.1
	24-30	E 115	34.4	1.4	0.96	823	300	36.5	4.4	5136	(0.48)	0.89	50	2.86	5.7
	24-30	E 52	30.2	1.5	0.87	675	250	37.0	4.0	4806	(0.45)	0.90	47	2.38	5.1

* Mean respiratory quotients for fed and starved (24hr.) sheep are shown in parentheses where oxygen consumption was not measured. These values were used to calculate oxygen uptakes and thus energy expenditure.

† Total energy production has been calculated from oxygen consumption: 105 kcal./mole of oxygen (Blaxter, 1963, p. 43).

‡ Factors for deriving energy produced from substrate oxidized were obtained and derived from Blaxter, (1963, p. 41). Values (kcal./g. of substrate oxidized): glucose, 3.738; acetic acid, 2.925 (for calculation, see the text); β-hydroxybutyric acid, 4.692; palmitic acid, 9.350; oleic acid, 9.421; stearic acid, 9.527.

§ The concentrations of acetate and D(−)-β-hydroxybutyrate are expressed in mg. of acetic acid and mg. of β-hydroxybutyric acid/100ml. of blood respectively.

|| Sodium [1-14C]acetate was infused.

¶ Sodium D(−)-β-hydroxy[2-14C]butyrate was infused.

** Potassium [1-14C]palmitate was infused.

Comparison of the calculated terminal value of the specific radioactivity of metabolic $^{14}CO_2$ (obtained by correcting the values for respiratory $^{14}CO_2$) with that of substrate provided values for the contribution of substrate to oxidative metabolism.

During the infusion of ^{14}C-labelled bicarbonate the specific radioactivity of $^{14}CO_2$ in arterial blood was consistently slightly higher than in expired air (Table 1). Possible errors due to the use of different techniques for the assay of $^{14}CO_2$ in blood and air were eliminated by using essentially the same method of analysis in each case, i.e. collection of respiratory $^{14}CO_2$ and arterial blood $^{14}CO_2$ in carbon dioxide-free sodium hydroxide, and assay as $Ba^{14}CO_3$. Similar results were obtained when [U-^{14}C]stearic acid was infused. The probable explanation was slight leakage of rumen gases past the endotracheal fistula and into the lungs (Colvin, Wheat, Rhode & Boda, 1957).

Experiments were discarded when the rate of output of respiratory carbon dioxide showed changes of more than 10% of the mean value during the infusion. Slight changes in rate of output of carbon dioxide, reflecting changes in carbon dioxide pool size or carbon dioxide production rate, undoubtedly contributed to the failure to obtain consistent and reproducible patterns of respiratory $^{14}CO_2$ production in ostensibly similar experiments.

Entry and oxidation of glucose. Values were obtained for four starved (24 hr.) sheep, and for two sheep 3–5 hr. after feeding (Table 2). Mean glucose entry rates in fed and starved sheep were 5·0 (range 4·8–5·1, 2 observations) and 3·8 (3·2–4·2, 4) mg./min./kg.$^{0·75}$ respectively, and corresponding values for the contribution of glucose to oxidative metabolism were 9·1 (8·6–9·6, 2) and 11·2 (5·9–15·1, 4)% respectively. Terminal specific radioactivities of $^{14}CO_2$ were obtained by calculation, with a single exponential function, as with the other substrates.

The results of this investigation have been combined with those reported earlier (Annison & White, 1961, 1962) to obtain mean values for glucose entry over 24 hr., in animals fed once a day, by assuming that this value is represented by half the sum of the values obtained for fed and starved (24 hr.) sheep. These values were 2·5 (14 experiments) and 1·5 (23) mg./min./kg. for the fed and starved (24 hr.) respectively, suggesting a daily entry rate of 120 g./day for a 40 kg. animal. Good correlation between glucose entry rate and plasma glucose concentration was shown when all the data from this Laboratory were examined (Fig. 5). The small number of results available relating glucose oxidation to plasma glucose concentration showed the absence of significant correlation between these parameters.
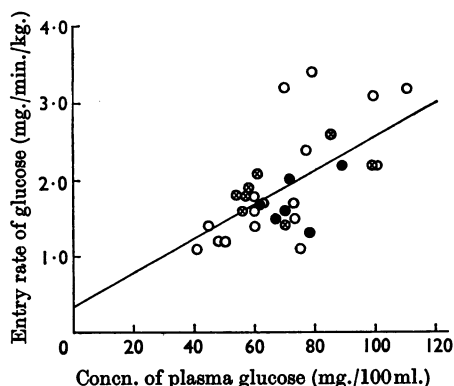

Fig. 5. Regression of glucose entry on plasma glucose concentration. ○, Results from Annison & White (1961, 1962); ⊗, two identical results from similar experiments; ●, results from present study; $y = 0·225x + 0·35$ ($s_b = 0·0045$; $P < 0·001$).

When the experiments on glucose were carried out the oxygen-analysis equipment was not available.

Entry and oxidation of acetate. Experiments were carried out on one starved (24 hr.) sheep, and on four sheep 3–5 hr. after feeding, and the rates of entry and oxidation of acetate measured (Table 2). Mean acetate entry rates in fed and starved sheep were 10·8 (9·1–13·5, 4) and 5·8 (1) mg./kg.$^{0·75}$ respectively, and corresponding values for the contribution of acetate to oxidative metabolism were 31·6 (26·8–38·1, 4) and 22·1 (1)% respectively. In one experiment (W24) [1-^{14}C]acetate was used instead of [1,2-$^{14}C_2$]acetate, and the output of $^{14}CO_2$ was corrected to the calculated output from uniformly labelled acetate. Data obtained earlier showed that the ratio

$$\frac{^{14}CO_2 \text{ production from } [1\text{-}^{14}C]\text{acetate}}{^{14}CO_2 \text{ production from } [2\text{-}^{14}C]\text{acetate}}$$

was 1·58 (Lindsay & Ford, 1964), giving a correction factor $1/1·29 = 0·78$.

The correlation between acetate concentration in blood and acetate entry rates when the present results and earlier data (Annison & White, 1962) were plotted is shown in Fig. 6. Good correlation between acetate concentration and acetate oxidation was seen when the small number of results obtained in this investigation were plotted (Fig. 7). Oxygen uptakes were not measured in these experiments.

Entry and oxidation of plasma long-chain free fatty acids. Continuous infusion experiments with [U-^{14}C]palmitic acid, [U-^{14}C]stearic acid and [U-^{14}C]oleic acid were made on starved (24 hr.)
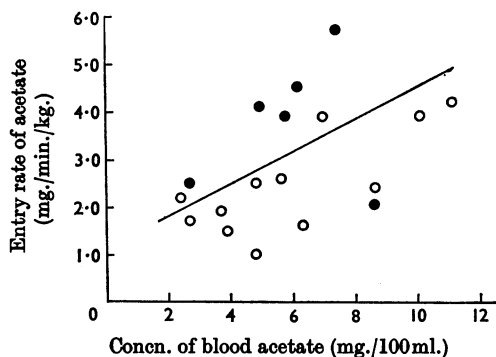
Fig. 6. Regression of acetate entry on blood acetate concentration. O, Results from Annison & White (1962); ●, results from present study; $y=0.345x+1.017$ ($s_b=0.131$; $0.02>P>0.01$).
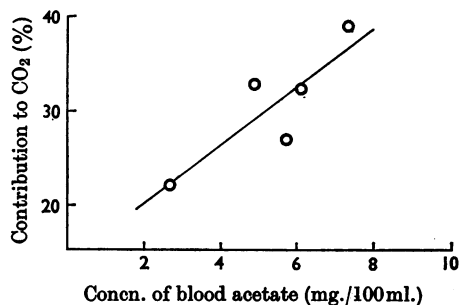

Fig. 7. Regression of the percentage contribution to CO_2 production of acetate on blood acetate concentration. $y=3.09x+13.9$ ($s_b=1.03$, $0.1>P>0.05$).
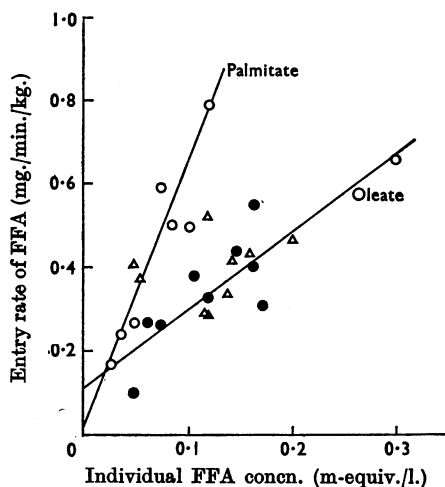

Fig. 8. Interrelations of the entry rates of palmitate, oleate and stearate and plasma palmitate, oleate and stearate concentrations respectively. O, Palmitate, $y=5.59x+0.0203$ ($s_b=1.76$; $0.02>P>0.01$). ●, Oleate, $y=1.86x+0.115$ ($s_b=0.398$; $0.02>P>0.01$). △, Stearate, no significant regression.
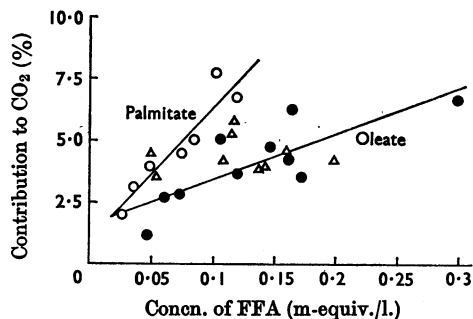

Fig. 9. Interrelations of the percentage contribution to CO_2 production of palmitate, oleate and stearate and plasma palmitate, oleate and stearate concentrations respectively. O, Palmitate, $y=53.4x+0.94$ ($s_b=9.0$; $0.01>P>0.001$). ●, Oleate, $y=18.2x+1.59$ ($s_b=4.65$; $0.01>P>0.001$). △, Stearate, no significant regression.

sheep to provide data on the rates of entry and oxidation of these individual fatty acids. Seven experiments with palmitate showed a mean entry rate of 1.0 (0.6–1.9) mg./min./kg.$^{0.75}$, and a contribution to carbon dioxide output of 4.7 (2.0–7.7, 7)% at an average plasma concentration of 1.8 mg./100 ml. Corresponding results for oleate and stearate were: entry rates 0.9 (0.2–1.6, 10) and 0.9 (0.7–1.1, 9) mg./min./kg.$^{0.75}$; contribution to carbon dioxide, 4.0 (1.2–6.6, 10) and 4.4 (3.8–5.8, 9)% at plasma concentrations of 3.8 and 3.4 mg./100 ml. respectively. With palmitate and oleate there were good correlations between plasma fatty acid concentration and rates of entry and oxidation (Figs. 8 and 9), but not with stearate. At similar plasma concentrations, the turnover and oxidation of palmitate was considerably greater than that of stearate or oleate.

The oxygen analyser was available for some of the experiments with long-chain acids, and from the measured oxygen uptakes and corrected carbon dioxide outputs respiratory quotients were calculated (Table 2). Values for total energy expenditure were calculated from oxygen uptakes by using the factor 105 kcal./mole of oxygen (Blaxter, 1963, p. 43).

Entry and oxidation of D(−)-β-*hydroxybutyrate.* Sodium D(−)-β-hydroxy[1,2-$^{14}C_2$]butyrate was infused into one fed and four starved (24 hr.) sheep. Mean entry rates were 1.4 (1) and 1.5 (0.8–2.4, 4)

mg./min./kg.$^{0.75}$, and corresponding values for the contribution of this substrate to production of carbon dioxide were 10·4 (1) and 4·8 (1·9–7·7, 4)% respectively.

The possible interrelation of rates of entry and oxidation, and concentration of β-hydroxybutyrate in blood, were examined by combining the five results of the present study with those of the eight experiments reported earlier (Leng & Annison, 1964), when the dependence of both entry rate and contribution to carbon dioxide on concentration were clearly shown (Figs. 10 and 11).

Production rates of propionate and butyrate, and the contribution of these acids to oxidative metabolism. Uniformly ^{14}C-labelled propionate and butyrate were infused into the rumen of fed and starved sheep to measure rates of fatty acid production, as described by Leng & Leonard (1965). Reasonably constant specific radioactivities of fatty acid in the rumen were achieved after 200 min., and the infusions were continued for at least 300 min. The specific radioactivity of respiratory ^{14}CO$_2$ was followed during these infusions, and when plotted against time gave curves which had not reached a plateau by 300 min. Terminal values for the specific radioactivity of respiratory ^{14}CO$_2$ were calculated as before, and corrected for loss of radioactivity, and dilution with rumen carbon dioxide. In calculating the contribution of propionate and butyrate to production of carbon dioxide it was assumed that the specific radioactivities of the acids reaching the tissues were the same as that in the rumen. Although there is no evidence of endogenous

propionate or butyrate production, the absorption of caecal fatty acids undoubtedly diluted the acids reaching the liver, but this effect was difficult to assess since (*a*) rates of absorption of fatty acids from the caecum are not known, and (*b*) a substantial fraction of the fatty acids absorbed from the rumen is metabolized by the rumen wall.

When rumen contents (50 ml.) were incubated at 39° in an atmosphere of nitrogen with ^{14}C-labelled propionate and butyrate, only negligible amounts of ^{14}CO$_2$ were produced.

Mean values for propionate and butyrate production rates were 2·5 (2·2–2·9, 2) and 1·0 (0·8–1·2, 2) mg./min./kg.$^{0.75}$ in the starved (24 hr.) sheep, and 6·4 (4·7–8·3, 4) and 4·3 (3·4–6·1, 4) mg./min./kg.$^{0.75}$ in the continuously feeding animal (Table 3). Corresponding values for the contribution to oxidative metabolism were 7·1 (6·8–7·4, 2) and 5·3 (5·2–5·3, 2)% and 23·0 (13·8–29·9, 4) and 16·5 (13·7–20·5, 4)% respectively.

The interrelations of rates of production and oxidation of propionate and butyrate and their concentration in the rumen are shown in Figs. 12 and 13.

Substrate contribution to total carbon dioxide production. When attempting to account for the sources of metabolic carbon dioxide, the summation of the contribution of individual substrates is only justified if the pathways of dissimilation are independent, which is not so in all cases. β-Hydroxybutyrate arises almost entirely from butyrate and plasma FFA in sheep (R. A. Leng & C. E. West, unpublished work), the proportional contribution of butyrate being 80% in fed sheep and 20% in the starved (24 hr.) animal. Since a substantial fraction of both butyrate and plasma FFA (mainly palmitate, oleate and stearate) is oxidized after conversion
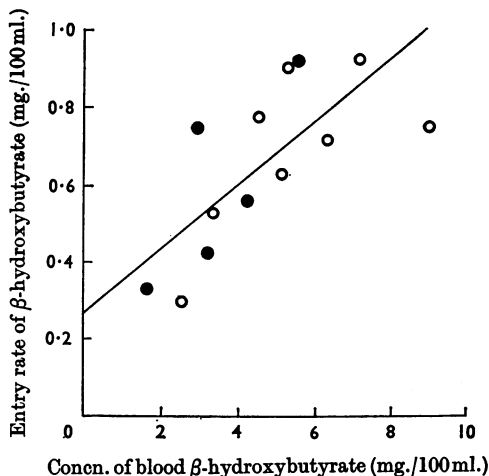

Fig. 10. Regression of D(−)-β-hydroxybutyrate entry on blood D(−)-β-hydroxybutyrate concentration. ○, Results from Leng & Annison (1964); ●, results from present study; $y = 0·0841x + 0·268$ ($s_b = 0·0230$; $0·01 > P > 0·001$).
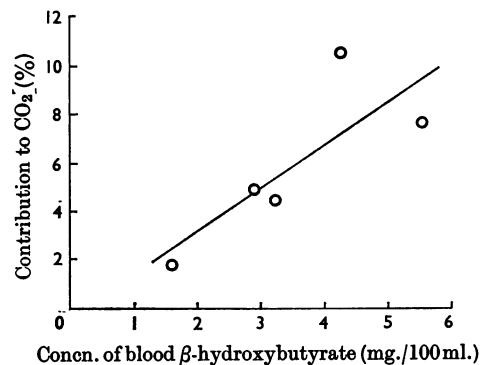
Fig. 11. Regression of the percentage contribution to CO$_2$ production of D(−)-β-hydroxybutyrate on blood D(−)-β-hydroxybutyrate concentration. $y = 1·80x − 0·38$ ($s_b = 0·78$; $0·2 > P > 0·10$).

Table 3. *Collated results from experiments in which sodium [U-14C]propionate and sodium [U-14C]butyrate were infused intraruminally into feeding and starved sheep*

Substrate	Time after feeding (hr.)	Sheep no. and sex (wethers)	Wt. of sheep (kg.)	Concn. of rumen substrate (mg./100 ml.)	Production rate (mg./min./kg.$^{0.75}$)	Production rate (mg./hr.)	Substrate oxidized (mg./hr.)	Substrate oxidized (% of entry rate)	Substrate oxidized (% of CO2 output)	Total CO$_2$ production (mg. of C/hr.)	Total O$_2$ uptake* (mole/hr.)	Respiratory quotient*	Total energy production† (kcal./hr.)	Energy from substrate (kcal./hr.)	Energy from substrate (%)	Asymptotic correction factor)§‡
Propionate	0–2	W 32	32·0	200·3	6·24	5050	1462	29·0	13·8	5150	(0·60)	(1·03)	63	7·36	11·7	2·250±0·134
	0–2	W 92	36·8	136·4	6·18	5540	2762	49·9	29·9	4500	(0·52)	(1·03)	55	13·90	25·3	1·280±0·356‖
	0–2	W164	34·8	161·8	4·71	4050	3445	85·0	28·2	5940	(0·49)	1·07	51	17·85	34·0	1·710±0·474
	0–2	W167	33·4	170·0	8·31	6940	2267	32·7	20·0	5520	(0·46)	0·98	48	11·41	23·8	1·000¶
	24–30	W193	30·2	61·6	2·85	2200	596	27·1	6·8	4270	(0·53)	(0·97)	55	3·00	5·4	1·451±0·141
	24–30	W 32	31·2	90·7	2·23	1765	668	32·9	7·4	4860	(0·54)	(0·97)	57	3·36	5·9	1·635±0·202
Butyrate	0–2	W 32	39·0	64·5	3·47	3245	1480	45·7	14·8	4950	(0·58)	(1·02)	61	8·90	14·6	1·090±0·060
	0–2	W 32	32·4	61·8	3·84	3135	1292	41·2	13·7	5140	(0·60)	(1·02)	63	7·79	12·4	1·250±0·082
	0–2**	W141	31·8	71·5	6·06	4875	1639	33·6	20·5	4370	0·47	1·17	49	9·86	20·1	1·095±0·045
	0–2**	W167	32·3	56·0	4·02	3280	2087	63·6	17·0	6700	0·57	0·98	60	12·55	20·9	1·050±0·042
	24–30	W193	30·2	36·1	1·19	915	527	57·8	5·3	4927	(0·61)	(0·97)	64	3·18	5·0	1·090±0·082
	24–30	W 92	38·0	32·0	0·82	755	369	48·8	5·2	3840	(0·47)	(0·97)	50	2·22	4·4	1·022±0·110

* Mean respiratory quotients for fed and starved (24 hr.) sheep are shown in parentheses where oxygen consumption was not measured. The values were used to calculate oxygen uptakes and thus energy expenditure.
† Total energy production was calculated from oxygen consumption: 105 kcal./mole of oxygen (Blaxter, 1963, p. 43).
‡ Energy produced from substrate: 5·032 kcal./g. of propionate; 6·018 kcal./g. of butyrate (from Blaxter, 1963, p. 41).
§ S.D. of factor has P<0·001, unless otherwise stated.
¶ Linear regression of the plot of specific radioactivity against time curve between 260 and 480 min. gave slope 0 (P<0·001).
‖ 0·2>P>0·1.
** 480 min. infusion.
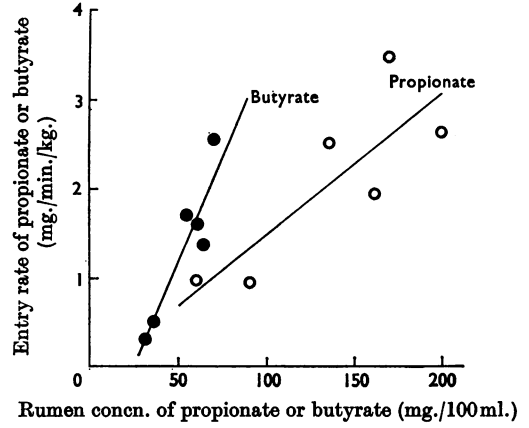

Fig. 12. Regressions of propionate and butyrate entry on rumen propionate and butyrate concentrations respectively. O, Propionate, $y=0.0157x-0.76$ ($s_b=0.0054$; $0.05>P>0.02$). ●, Butyrate, $y=0.0479x-1.23$ ($s_b=0.0089$; $0.01>P>0.001$). The combined regression of the results is not significant.
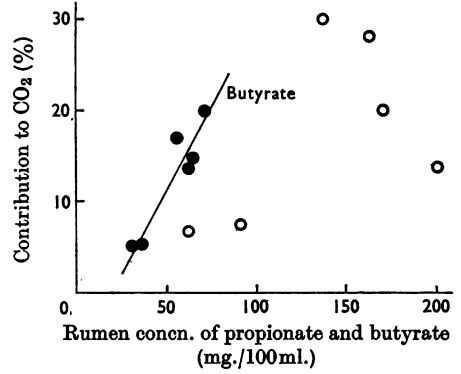

Fig. 13. Interrelations of the percentage contribution to CO$_2$ production of propionate and butyrate and rumen propionate and butyrate concentrations respectively. O, Propionate, no significant regression. ●, Butyrate, $y=0.37x-7.0$ ($s_b=0.065$; $0.01>P>0.001$).

into β-hydroxybutyrate, it would be incorrect to include the contribution of β-hydroxybutyrate to carbon dioxide production when accounting for total carbon dioxide in terms of individual substrates, if values for butyrate and FFA are included. Propionate is a major precursor of glucose, recent data of Bergman, Roe & Kon (1966) and Leng, Steel & Luick (1967) showing that in the fed sheep 30–50% of glucose arises from propionate. We must therefore reduce values for the contribution of glucose to total carbon dioxide production in the

fed animal by 40%, giving a corrected figure of 5·4%. Corresponding data on the starved (24hr.) sheep are not available, but since ruminal propionate concentrations fall to roughly 50% of the post-feed levels we have assumed that under these conditions 20% of body glucose arises from propionate. The value of 12·7 for the contribution of glucose to total carbon dioxide production was reduced by 20%, i.e. 10·2, to allow for the contribution of propionate in the starved (24hr.) sheep.

Interconversions between glucose, acetate and plasma FFA are usually small. The specific radioactivity of blood acetate at the end of a labelled glucose infusion (160min.) was about 1·5% of that of glucose, and in similar infusions with labelled acetate the specific radioactivity of blood glucose was about 3% of that of acetate (Annison & White, 1962). Only slight labelling of blood acetate was observed during the infusion of labelled palmitate (West & Annison, 1964), and there was negligible transfer of radioactivity from labelled blood acetate to plasma FFA (C. E. West, unpublished observations). Leng & Annison (1964) showed that the labelling of blood glucose and acetate during the infusion (120min.) of labelled $D(-)-\beta$-hydroxybutyrate was negligible. These observations suggest that the values obtained for the contribution of acetate, and palmitate, oleate and stearate, to total carbon dioxide production can be summed without correction. The sums of the corrected values for glucose, acetate, propionate, butyrate, palmitate, oleate and stearate were 76% in fed sheep and 58% in starved (24hr.) sheep. The uncertainties and assumptions inherent in these rough estimates, particularly for fed sheep, must again be emphasized.

Substrate contribution to overall energy expenditure. The proportion of substrate entry that was directly oxidized during the experiment was calculated from the total respiratory carbon dioxide output, and the specific radioactivities of respiratory $^{14}CO_2$ (Tables 2 and 3) and substrate. These data allowed calculation of the energy provided by each substrate when corrected (where possible) for incomplete substrate oxidation. The energy released during complete oxidation is independent of metabolic pathway, but corrections must be made when fragments of a molecule are oxidized at different rates, and there are differences in the energy values of the fragments. When the oxidation of [1-^{14}C]acetate and [2-^{14}C]acetate in the sheep were compared, the ratio

$$\frac{^{14}CO_2 \text{ production from } [1\text{-}^{14}C]\text{acetate}}{^{14}CO_2 \text{ production from } [2\text{-}^{14}C]\text{acetate}}$$

was 1·58 (Lindsay & Ford, 1963), and this value was used to correct calculated energy expenditures from acetate (Table 2). The energies of the methyl and carboxyl portions of the acetate

molecule were assumed to be 180·0 and 29·4kcal./mole respectively. These values were calculated from the heats of combustion of oxalic acid (60kcal./mole) and acetic acid (209·4kcal./mole) (Blaxter, 1963, p. 41). Thus the energy derived from the oxidation of acetate was calculated to be 175·5kcal./mole or 2·925kcal./g. The relatively large size and symmetry of the molecules of glucose and long-chain fatty acids suggests that only small errors arise when substrate contribution to energy expenditure is calculated from oxidation data, irrespective of the partial or complete oxidation of these substrates. Total energy expenditure was calculated from oxygen consumption, by using the factor 105kcal./mole of oxygen (Blaxter, 1963, p. 43), but unfortunately measurements of oxygen uptake were made in only 14 of the 54 experiments reported here. The mean respiratory quotients for fed and starved (24hr.) sheep were 1·03 (5 observations) and 0·94 (11 observations), and these values were used to calculate the oxygen uptakes, and thus total energy expenditure, in those experiments where only total carbon dioxide output was measured (Tables 2 and 3). The energy accounted for by the oxidation of individual substrates in fed and starved sheep was 63% and 43% respectively of the energy expenditure calculated from total oxygen uptakes.

DISCUSSION

The metabolism of ruminants is greatly influenced by the extensive microbial fermentation of ingested foodstuffs which occurs in the rumen. Dietary carbohydrates are largely converted into short-chain fatty acids, and little glucose is absorbed from the alimentary tract. Isotope-dilution techniques have been used to obtain quantitative information on the rates of ruminal production of acetate, propionate and butyrate (Leng & Leonard, 1965; Bergman, Reid, Murray, Brockway & Whitelaw, 1965), and on the rates of entry into body pool of glucose (see Ford, 1965) and fatty acids (see Annison, 1964). Entry rates of glucose have been shown to be equivalent to 120g./day for a sheep of 40kg. body wt. [mean of average values for fed and starved (24hr.) sheep: present results and Annison & White (1961)], indicating that gluconeogenesis is a major metabolic activity in sheep since the amounts of glucose absorbed from the alimentary tract are unlikely to exceed 20g./day (see Lindsay, 1959). Leng & Leonard (1965) showed that ruminal production rates of propionate, a major precursor of glucose, were about 70g./day, and that part of this fraction was directly oxidized. Since acetate and butyrate cannot give rise to net synthesis of glucose (see Annison, 1964), the glucose deficit must be made good by gluconeogenesis from protein and triglyceride glycerol.

Circulating acetate in ruminants is of alimentary (exogenous) and metabolic (endogenous) origin, and entry rates determined in the whole animal represent total entry. Earlier studies showed that endogenous acetate production was markedly affected by the exogenous entry rate, the latter rate being roughly proportional to the concentration of acetate in the rumen (Annison & White, 1962). All metabolic processes proceeding via acetyl-CoA are potential contributors to endogenous acetate, and the relatively high rates of endogenous entry suggest that a proportion of acetyl-CoA is in equilibrium with blood acetate. Supporting evidence for this hypothesis is the rapidity with which injected ^{14}C-labelled acetate is oxidized to $^{14}CO_2$ (presumably via acetyl-CoA), and the widespread distribution of acetyl-CoA deacylase. Protein almost certainly contributes substantially to endogenous acetate, since those amino acids which are not precursors of pyruvate are metabolized largely through acetyl-CoA. Total acetate entry constitutes a useful parameter of overall metabolism, but since it cannot be equated with ruminal production it is of limited significance in nutritional studies.

When ^{14}C-labelled substrates are infused intravenously into sheep, the specific radioactivity of circulating substrate reaches a relatively constant value within 180 min. For substrates with a small body pool, such as FFA, steady values are achieved in 30–60 min. (West & Annison, 1964). During these infusions, substrate oxidation in body tissues is indicated by the appearance of radioactivity in arterial blood carbon dioxide and respiratory carbon dioxide. The plot of specific radioactivity of respiratory $^{14}CO_2$ against time shows that the rate of increase with time reaches a minimum value after 3–6 hr. (Fig. 4), but never falls to zero. The 'plateau' value of the specific radioactivity of $^{14}CO_2$ after 3–6 hr. has been used in earlier work (see Annison, 1964) to provide rough estimates of the contribution of substrate to total carbon dioxide production. The equipment available in the present studies has allowed $^{14}CO_2$ production to be measured continuously during the constant infusion of ^{14}C-labelled materials, and the curves relating the specific radioactivity of respiratory $^{14}CO_2$ to time have been analysed mathematically. If there was only one carbon dioxide pool of constant size in the body, and recycling of substrate was absent, then the increase in specific radioactivity with time could be represented by a simple exponential function. The body, however, is a multi-compartment system with many carbon dioxide pools, including bone carbon dioxide, which equilibrates very slowly with blood carbon dioxide, and the rumen, a large pool which equilibrates fairly slowly with blood carbon dioxide

and greatly complicates the interpretation of the respiratory $^{14}CO_2$–time curves. Recycling is undoubtedly important for some substrates, e.g. glucose, which gives rise to lactate, which in turn contributes to glucose production (Annison, Lindsay & White, 1963). These factors have undoubtedly contributed to our failure to obtain data consistent enough to justify analysis of the $^{14}CO_2$–time curves by a multi-exponential expression relating the shape of the curve to the multi-compartment system comprising the total body carbon dioxide pool.

Tracheostomized sheep were used to minimize interference from rumen gases. Methane was largely eliminated by this procedure, since only small amounts pass across the rumen wall into blood, and are cleared by the lungs (Hoernicke, Williams, Waldo & Flatt, 1965). Carbon dioxide produced by ruminal fermentation, however, cannot be differentiated from that produced by tissue metabolism, since there is a continuous two-way transfer of carbon dioxide across the rumen wall, and substantial amounts of metabolic carbon dioxide enter the rumen in saliva (Hoernicke et al. 1965). Eructated rumen gases were not collected or assayed for radioactivity, and some loss of $^{14}CO_2$ of metabolic origin inevitably occurred. The magnitude of the effects of these factors will depend on the relative sizes and rates of turnover of the rumen and body carbon dioxide pools. These parameters were not measured in the present investigations, but values for metabolic $^{14}CO_2$ output were calculated from respiratory $^{14}CO_2$ outputs by using correction factors based on the output and specific radioactivity of respiratory $^{14}CO_2$ during the intravenous infusion of $NaH^{14}CO_3$. We have assumed that the output of respiratory carbon dioxide was not influenced by transfer of rumen carbon dioxide, i.e. no net increase in respiratory carbon dioxide. Hoernicke et al. (1965) have shown that although calculations suggest that the rate of absorption of carbon dioxide from the rumen is roughly balanced by the entry of carbon dioxide into the rumen, there is a net gain in the respiratory carbon dioxide output of fed tracheostomized cattle. The magnitude of the resulting errors in the measurement of output of carbon dioxide were estimated to be ±5%, and this figure probably represents the accuracy of the values we have obtained for contributions to total output of carbon dioxide, and energy expenditures, in the present studies.

Total metabolic carbon dioxide production is the end result of the oxidative metabolism of many substrates, some of which are closely interrelated, but in summing the contributions of individual substrates to total metabolic carbon dioxide production allowance was made for the interrelations

of propionate and glucose, and for the dependence of β-hydroxybutyrate on butyrate and plasma FFA metabolism. Interrelations between the other major substrates appear to be too low to cause more than minor errors and the failure to account for more than 76 and 58% of total carbon dioxide in fed and starved sheep was supporting evidence for this conclusion.

Respiratory quotients for fed and starved sheep were 1·03 (5 observations) and 0·94 (11 observations), but these values are more than usually difficult to interpret in ruminants, where a significant proportion of the total carbon dioxide output arises by anaerobic fermentation in the rumen. A large part of total rumen carbon dioxide, which arises both by fermentation and extraruminal tissue metabolism, is reduced to methane, but precise information is lacking on the sizes and rates of turnover of rumen and tissue carbon dioxide pools, rates of fermentation carbon dioxide production and rates of methane production in animals on different diets, and at varying times after feeding. This information can only be obtained by isotope dilution in animals in which total carbon dioxide and methane outputs, and total respiratory carbon dioxide outputs, are measured continuously.

We have been unable to account for all of the energy production and carbon dioxide output. A small part of the deficit in starved sheep was undoubtedly due to the oxidation of long-chain fatty acids other than palmitate, oleate and stearate, which together account for 80–85% of the long-chain fatty acid fraction. The remainder of the carbon dioxide must arise by the oxidation of material which is not in equilibrium with acetate, glucose, plasma FFA or ketone bodies, or their immediate precursors. In starvation, fat is the major source of energy and there is unequivocal evidence that adipose tissue is mobilized largely as plasma FFA (Steinberg, 1963). Nevertheless, the present data are consistent with the hypothesis that a fraction of adipose tissue, protein or glycogen, is mobilized and oxidized directly without equilibrating with plasma FFA, or with the main pools of acetyl-CoA or C3 compounds.

The utility of measurements of entry rate based on isotope dilution is well established, but the extension of this approach to the quantitative assessment of the oxidation of individual metabolites is more controversial. We have concluded that substrate-oxidation rates constitute useful parameters of metabolism. The values obtained are inevitably somewhat imprecise, but provide valuable comparative data when animals of different nutritional and physiological status are compared. The techniques can be extended to certain tissues and organs, when substrate uptake, production and oxidation can be examined in relation to overall metabolism (Annison, 1964). It must be stressed that measurements of substrate entry and oxidation can be superimposed on classical nutritional techniques of energy evaluation, and in particular can provide valuable supplementary information when linked with indirect calorimetry.

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