Acetate Utilization in Sheep

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Acetate, an important metabolite in most species, has a unique place in ruminant metabolism. As the major product of ruminal and caecal fermentation, acetate provides much of the energy requirements of ruminant tissues. Little information is available, however, on the overall utilization of acetate in sheep. Intravenous-acetate tolerance tests in sheep (Jarrett, Potter & Filsell, 1952; Pugh & Scarisbrick, 1952; Reid, 1958) have indicated that raised concentrations of circulating acetate fall more slowly than in cats (Smyth, 1947) or dogs (Ciaranfi & Fonnesu, 1954). A serious limitation of tolerance tests is that utilization rates can be examined only at high, unphysiological and continuously varying concentrations. In the present studies an isotope-dilution technique has been used to determine overall utilization rates at normal concentration of circulating acetate. The constant-infusion technique employed was similar to that used by Steele, Wall, de Bodo & Altszuler (1956) for the measurement of glucose-utilization rates in dogs. In this technique the rate of entry of acetate into the circulation is measured, and under steady-state conditions this will equal the rate of acetate leaving the plasma, i.e. the utilization rate.

In addition, the utilization of acetate has been studied at normal and enhanced levels of blood acetate, high concentrations of circulating acetate being achieved by infusing unlabelled acetate with the ^{14}C -labelled material. Similarly, by infusing either glucose or insulin with labelled acetate, acetate-utilization rates at high and low levels of blood glucose have been measured. Comparison of the specific activity of expired carbon dioxide and plasma acetate during the infusion of [*carboxy*- ^{14}C]-acetate and [*methyl*- ^{14}C]-acetate has allowed rough estimates to be made of the contribution of acetate to the oxidative metabolism of sheep. A preliminary account of this work has been given to The Biochemical Society (Annison & Lindsay, 1958).

MATERIALS AND METHODS

Experimental animals. Mature Clun Forest ewes were fed once daily (1000 g. of chopped hay) and trained to consume their food within 2 hr. To accustom them to the conditions of the experiments, the animals were fitted with the face masks used for respiratory CO_2 collection and

placed in stocks for several hours daily. Sheep which failed to stand quietly under these conditions were rejected. In some experiments sheep with permanent rumen fistulae were used, and two sheep prepared with tracheal cannulae (Webster & Cresswell, 1957) were also available when it was necessary to exclude rumen gases from expired air. Sheep were fasted for 24 hr. before each experiment.

Experimental procedure. The general arrangement is shown in Fig. 1. Polythene catheters were inserted into both jugular veins under local anaesthesia (procaine), 2–3 hr. before the start of an experiment. The left-side catheter, which was used for the injection or infusion of labelled acetate, was inserted downwards, i.e. towards the heart, about 6–8 in. The second catheter, for the withdrawal of blood samples, was inserted upwards (3–4 in.). This procedure was adopted to avoid possible contamination of blood samples with infused material. Constant infusion was maintained by using an apparatus built in this Institute. It consisted of two stainless-steel cylinders facing one another which were alternately filled and emptied by movement of pistons on a common shaft. Valves ensured that one cylinder filled from a reservoir while the second was infusing solution into the animal. Constancy of rate of movement of the pistons was ensured by means of a Velodyne (Williams & Uttley, 1946).

Respired CO_2 was collected with a rubber face-mask at the end of which was a bifurcated Perspex tube fitted with thin rubber-flap valves. Expired air passed through an absorption train of NaOH , as shown in Fig. 2. Collections were made for 3–5 min. periods at intervals during the infusion period. The absorption train consisted of three towers (two are shown in Fig. 2) containing 20% (w/v) of NaOH (effective height of column of alkali in each tower was 5 cm., and total volume 100 ml.). Absorption of CO_2 was facilitated by dispersal of the gas mixture with sintered-glass (200–250 μ porosity) disks placed near the base of the towers (Fig. 2). Suction of the pump (Fig. 2) was arranged to match the expired air output of the animal by keeping the spirometer height constant. Further washing of the gas leaving the absorption train with $\text{Ba}(\text{OH})_2$ (0.2N) contained in additional absorption tubes indicated that at the flow rates of gas used in the experiments approx. 5% of the expired CO_2 escaped absorption.

Urine was collected by bladder catheterization during the period of the experiment.

Heparin was used as an anti-coagulant throughout the investigation.

Chemical methods

Determination of plasma acetate. Acetate accounts for 80–90% of the volatile fatty acids of sheep blood, the remainder consisting largely of formate (Annison, 1954). The acids are not distributed equally between plasma and red cells, however, the latter containing a greater proportion of formate. Plasma formate levels rarely exceed 5% of

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Fig. 1. General arrangement of apparatus for constant intravenous infusion of isotope into sheep. *T*, Absorption towers; *S*, spirometer; *R*, reservoir of isotope; *P*, constant infusion pump; *V*, valves; *C*, sampling catheter.

Fig. 2. Apparatus used for collecting expired CO₂. *M*, Face-mask; *F*, flap valves; *S*, spirometer; *T*, absorption tower.

the total volatile fatty acid concentrations, and steam-distillation of deproteinized plasma filtrates at pH 4.0 decreases the amount of formate in the distillates to negligible proportions. The procedure of Annison (1954) was used in the deproteinization of plasma and the concentration and distillation of filtrates, except that the solutions were adjusted to pH 4.0, and not pH 3.0, before steam-distillation. Quantitative recoveries of acetate were obtained, and the acetate content of filtrates was occasionally checked by gas-liquid chromatography (James & Martin, 1952).

Assay of radioactive acetate. To steam-distillates, after titration with 0.01 N-NaOH, were added 0.2 ml. of N-NaOH. They were concentrated by boiling to a volume of 0.5–1.0 ml. The solutions were then dried in air on aluminium planchets and assayed for radioactivity with an end-window Geiger-Müller tube. If less alkali was added to the distillates before drying, acetate was lost from the planchets on standing in air. The quantity of sodium acetate (0.5–1.5 mg.) obtained in the steam-distillates was small relative to the amount of added alkali (8 mg.). A standard curve was made by plotting counts/min. against added radio-

activity whilst maintaining the total sodium acetate concentration constant at 1.5 mg. Alkali (0.2 ml. of N-NaOH) was added to the solutions, which were concentrated and dried as in actual assays. A linear plot was obtained.

Assay of carbon dioxide in expired air. A gas-transfer apparatus similar to that described by Kornberg, Davies & Wood (1952) was used to transfer CO_2 from the alkali in the absorption train to $\text{Ba}(\text{OH})_2$. Unchanged $\text{Ba}(\text{OH})_2$ was titrated with 0.2N-HCl with phenolphthalein as indicator, and the precipitated BaCO_3 was separated by centrifuging. The precipitate was washed twice with water, then with acetone, and transferred to planchets as a slurry in acetone. After drying in air, the BaCO_3 was counted with an end-window Geiger-Müller tube. Counts were corrected to infinite thickness.

Blood carbon dioxide. Blood (10 ml.) was withdrawn slowly into a syringe lubricated with liquid paraffin and containing heparin (150 units) as anti-coagulant. The samples were stored at 4° in the syringes until used, when about 10 ml. of blood, the precise volume being determined by change of weight of the syringe, was added to 0.5 ml. of 0.5N-NaOH (CO_2 -free) contained in a modified gas-transfer apparatus. The apparatus was evacuated and 3 ml. of 2N-lactic acid was slowly added to the blood, the liberated CO_2 being absorbed in $\text{Ba}(\text{OH})_2$ and treated as described above.

Wet oxidation of acetate. The solutions of [carboxy- ^{14}C]-acetate and [methyl- ^{14}C]-acetate (The Radiochemical Centre, Amersham, Bucks.) used in the infusion experiments were standardized by wet oxidation to $^{14}\text{CO}_2$ (van Slyke & Folch, 1940) which was absorbed in $\text{Ba}(\text{OH})_2$ and assayed as BaCO_3 . Absolute levels of radioactivity were obtained by reference to a standard source [^{14}C polymer reference source (CFP1) obtained from The Radiochemical Centre]. A portion of the standard was also converted into BaCO_3 by wet combustion and counted at infinite thickness.

Determination of blood glucose. Blood glucose was determined by the method of Somogyi (1952) with the colorimetric modification of Nelson (1944).

Measurement of utilization rate. The term 'utilization' has been used in this paper to mean the rate of removal of metabolite from the plasma. In practice, isotope dilution measures the rate at which a metabolite is added to the plasma. Under steady-state conditions when the concentration of, say, acetate is constant, rates of addition and removal are equal. Utilization rates (U , m-moles/min.) have been calculated from the expression $U = X/Y$, where X is rate of infusion of isotope ($\mu\text{C}/\text{min.}$), and Y is specific activity of plasma acetate ($\mu\text{C}/\text{m-mole}$).

RESULTS

Equilibration of [^{14}C]-acetate between red cells and plasma. Plasma was used instead of whole blood in the assay of acetate to avoid interference by formate. Before adopting this procedure, however, it was necessary to establish that injected labelled acetate quickly equilibrated with the acetate in red cells. A small volume (1 ml.) of labelled acetate (20 μC) in 0.9% sodium chloride solution was added to 50 ml. of sheep blood maintained at 37° . After mixing thoroughly, a portion of blood was re-

moved and centrifuged to separate red cells and plasma. The haematocrit was noted. The remainder of the blood was shaken gently at 37° for 30 min., when a further portion was separated into red cells and plasma. Analysis of each of the fractions for acetate showed that the specific activity was the same in all fractions, indicating rapid equilibration of added acetate between red cells and plasma.

Half-time of injected [carboxy- ^{14}C]-acetate. The specific activity of acetate in sheep plasma was determined at intervals of 2–3 min. (accurately measured), after the injection of 100 μC of sodium [carboxy- ^{14}C]-acetate (40 μmoles) in 5 ml. of solution. The rapid turnover of acetate was indicated by the results shown in Fig. 3, where specific activity was plotted against time on a semi-logarithmic scale. The slope was clearly not fixed with any great precision but a half-life of 3–4 min. was indicated. From the intercept, the apparent volume of distribution was approx. 70 l. This is about 1.5 l./kg. body wt.

Utilization rates of acetate. Utilization rates of acetate at normal and enhanced levels of plasma acetate were conveniently determined by the constant-infusion method during the same experiment. A solution of labelled acetate containing negligible amounts of carrier acetate was infused for 3 hr., as Tables 1 and 2 show; the specific activity of plasma acetate had reached a constant level within 2 hr. A solution containing the same amount of radioactivity per unit volume, but in addition a high concentration of unlabelled sodium acetate, was now infused at the same rate, care being taken to limit the interruption of isotope inflow to 5–10 sec. Steady-state conditions of plasma acetate were usually attained within 30–50 min. The short half-time of acetate found by single injection experiments made it unnecessary to use an initial priming dose of acetate, a technique found useful by Steele *et al.* (1956) in the establishment of constant specific activity of plasma glucose in the dog.

In a typical experiment with a 44.0 kg. ewe, [carboxy- ^{14}C]-acetate was infused for 167 min. at an inflow rate of 0.494 $\mu\text{C}/\text{min.}$ (2 $\mu\text{moles}/\text{min.}$). For the next 150 min. the same infusion of [carboxy- ^{14}C]-acetate was maintained, together with carrier acetate at 1.31 m-moles/min. Plasma acetate concentrations remained constant during the infusion of essentially carrier-free [carboxy- ^{14}C]-acetate at 0.25 m-mole/l. but gradually increased during the second period to 2.20 m-moles/l. (Table 1). The mean utilization rate during the first period (0.51 m-mole/min.) increased to 1.83 m-moles/min. when steady-state conditions had been reached at the raised level of plasma acetate. The increase in mean utilization rate ($1.83 - 0.51 = 1.32$ m-moles/min.) was in excellent agreement with the rate of infusion of carrier acetate (1.31 m-moles/min.).

When methyl-labelled acetate was used in a similar experiment with a 27.1 kg. sheep, inflow rate 0.506 $\mu\text{C}/\text{min.}$ during first period, and carrier acetate infused at 1.30 m-moles/min. during the second period, the results shown in Table 2 were

obtained. The agreement between infusion rate and increase in utilization rate was again good.

Effect of glucose on acetate utilization rates. The effects of raised or diminished levels of blood glucose on acetate utilization rates were examined

Fig. 3. Radioactivity in plasma acetate after a single intravenous injection of 100 μC of [carboxy- ^{14}C]acetate. Concn. of acetate, 0.47 m-mole/l. throughout the period.

Table 1. *Measurement of utilization rate of acetate at normal levels of plasma acetate (0–167 min.), and at high acetate concentrations (208–322 min.) by infusion of sodium [carboxy- ^{14}C]acetate*

Labelled acetate (0.494 $\mu\text{C}/\text{min.}$) was infused for 330 min. Carrier acetate was infused from 0 to 167 min. and from 167 to 317 min. at the rates 2 μmoles and 1.31 m-moles/min. respectively. Mean utilization rates: 102–167 min. = 0.51 m-mole/min.; 249–322 min. = 1.83 m-moles/min. The ewe weighed 44 kg.

Time after start of infusion (min.)	Plasma acetate (m-moles/l.)	Specific activity of acetate ($\mu\text{mc}/\text{m-mole}$)	Specific activity of blood CO_2 ($\mu\text{mc}/\text{m-mole}$)	Blood glucose (mg./100 ml.)
23	—	—	—	37.0
102	0.25	775	28.2	42.0
130	0.25	970	33.0	—
145	0.25	1000	35.0	—
155	0.25	987	36.8	43.0
167	0.25	945	34.6	—
208	—	—	43.6	32
240	—	—	39.6	—
249	2.19	277	—	—
264	—	—	40.7	—
280	2.22	286	—	—
285	—	—	37.2	—
297	2.20	278	—	38
304	2.22	265	46.3	—
322	2.20	250	53.1	—

by infusing carboxyl-labelled acetate at a constant rate (a) alone, (b) with added glucose and (c) with added insulin. In each of the three periods of the experiment sufficient time was allowed for steady-state conditions to be obtained. The results of a typical experiment are shown in Table 3. A moderate elevation of blood glucose levels increased acetate utilization. Infusion of insulin lowered blood-sugar levels, and depressed acetate utilization. At very high levels of blood glucose, acetate utilization was depressed, as the results of an experiment shown in Table 4 indicate. In this experiment infusion of insulin again depressed acetate utilization.

Specific activity of expired carbon dioxide. Expired air in ruminants may contain carbon dioxide eructated from the rumen in addition to expired carbon dioxide. To eliminate the contribution of ruminal carbon dioxide, sheep prepared

with tracheal cannulae were used in early experiments. Later it was found (Fig. 4.4) that there was close agreement between the specific activities of carbon dioxide from expired air and in venous (jugular) blood during infusion of $\text{NaH}^{14}\text{CO}_3$. This allowed the collection of expired carbon dioxide to be dispensed with in experiments in which only the specific activity of carbon dioxide, but not total output, was required. However, more recent experiments have shown that the agreement of blood and expired air specific activities is not found when labelled acetate is used. Fig. 5 shows that on average (jugular) blood carbon dioxide has a specific activity about 15% lower than that of expired air. Since expired air is of higher specific activity than blood carbon dioxide, this can hardly be due to dilution of expired air with eructated (inactive) rumen gas.

In order to interpret the relationships between

Table 2. *Measurement of utilization rates of acetate at normal (0–167 min.), and raised (167–330 min.) levels of plasma acetate by the infusion of sodium [methyl- ^{14}C]acetate*

Labelled acetate ($0.506\ \mu\text{C}/\text{min.}$) was infused for 330 min. Carrier acetate was infused from 0 to 167 min. and from 167 to 330 min. at the rates $2\ \mu\text{moles}$ and $1.30\ \text{m-moles}/\text{min.}$ respectively. Mean utilization rates (a) (124–157 min.) $0.43\ \text{m-mole}/\text{min.}$, (b) (207–330 min.) $1.80\ \text{m-moles}/\text{min.}$ The ewe weighed $27.1\ \text{kg.}$

Time after start of infusion (min.)	Plasma acetate (m-moles/l.)	Specific activity of acetate ($\mu\text{mc}/\text{m-mole}$)	Specific activity of blood CO_2 ($\mu\text{mc}/\text{m-mole}$)	Blood glucose (mg./100 ml.)
124	1.09	1090	—	57
137	1.01	1290	20.6	—
146	1.03	1250	22.0	—
157	1.01	1110	22.8	55
207	—	—	25.6	51
243	—	—	28.8	—
275	—	—	26.4	—
285	2.34	235	26.0	—
303	2.45	229	25.4	—
314	2.32	289	28.3	—
330	2.35	268	27.4	55

Table 3. *Measurement of utilization rates of acetate in a 42.0 kg. sheep*

Rates were measured at (a) normal levels of blood glucose (0–137 min.), (b) raised levels produced by the infusion of glucose ($0.067\ \text{g.}/\text{min.}$) over period of 137–284 min., and (c) depressed glucose levels resulting from the infusion of insulin ($0.23\ \text{unit}/\text{min.}$) over a period of 284–412 min. Sodium [carboxy- ^{14}C]acetate was infused continuously throughout all three periods at $0.580\ \mu\text{C}/\text{min.}$

Time after start of infusion (min.)	Plasma acetate (m-mole/l.)	Specific activity of acetate ($\mu\text{mc}/\text{m-mole}$)	Blood glucose (mg./100 ml.)	Mean acetate utilization rate (m-mole/min.)
83	0.77	160	57	3.58
98	0.78	181	52	
120	0.78	163	48	
137	0.79	144	48	
248	0.78	100	73	4.93
265	0.79	143	71	
281	0.79	128	64	
284	0.80	98	64	
375	0.91	159	24	2.96
385	0.92	203	23	
395	0.90	233	25	
412	0.91	203	25	

Table 4. *Measurement of utilization rates of acetate in a 31.5 kg. sheep*

Rates are measured at (a) normal levels of blood glucose (0–120 min.), (b) raised levels produced by the infusion of glucose (0.17 g./min.) (120–247 min.), and (c) depressed glucose levels (insulin infused at 0.54 unit/ml.) (247–363 min.). Sodium [*methyl*- ^{14}C]acetate was infused at 0.536 $\mu\text{C}/\text{min.}$ throughout.

Time after start of infusion (min.)	Plasma acetate (m-mole/l.)	Specific activity of acetate ($\mu\text{mC}/\text{m-mole}$)	Blood glucose (mg./100 ml.)	Mean acetate utilization rate (m-mole/min.)
90	0.59	168	60	2.93
98	0.50	214	62	
111	0.64	186	62	
120	0.56	169	63	
218	0.70	188	170	2.29
230	0.63	250	178	
241	0.57	269	178	
247	0.54	247	173	
340	0.52	235	17	1.94
350	0.61	295	16	
360	0.56	299	14	
363	0.58	283	18

Fig. 4. Changes in specific activity of $^{14}\text{CO}_2$ during continuous intravenous infusion of $\text{NaH}^{14}\text{CO}_3$. A, Ewe (35 kg.) infused at the rate of 0.465 $\mu\text{C}/\text{min.}$; B, wether (29 kg.) infused at the rate of 0.320 $\mu\text{C}/\text{min.}$ Carrier bicarbonate was negligible in both. ●, Expired-air CO_2 ; ○, jugular-blood CO_2 .

the specific activities of expired carbon dioxide and infused [^{14}C]acetate, it was necessary to investigate the rate of mixing of labelled carbon dioxide with the carbon dioxide pool of the animal. The results shown in Fig. 4 (A and B) were obtained when labelled bicarbonate was infused intravenously at a constant rate (0.465 $\mu\text{C}/\text{min.}$). Infused labelled carbon dioxide was largely equilibrated with the exchangeable carbon dioxide of the body within 2–3 hr. from the start of the infusion.

The specific activities of blood carbon dioxide

Fig. 5. Changes in specific activity of CO_2 during continuous intravenous infusion of [*carboxy*- ^{14}C]acetate. Infusion was at the rate of 0.555 $\mu\text{C}/\text{min.}$ with negligible carrier acetate. The ewe weighed 70 kg. ○, Expired-air CO_2 ; ●, jugular-blood CO_2 .

shown in Tables 1 and 2 were used to obtain approximate estimates of the fractional contribution of acetate to the carbon dioxide output. This was given by the ratio specific activity of $\text{CO}_2/0.5$ of specific activity of plasma acetate, where the factor 0.5 was used because it was assumed that both acetate C atoms contributed to carbon dioxide, although only one was labelled. Thus in Table 1, with mean values of the specific activities of acetate and carbon dioxide over the period 130–167 min., the ratio was 0.07, and for the period 304–322 min., 0.4. That is, with the plasma acetate concentration 0.25 m-mole/l., about 7% of expired carbon dioxide may be considered as derived directly from acetate; with the plasma acetate

concentration 2.2 m-moles/l., the value was about 40 %.

Corresponding values when methyl-labelled acetate was used (Table 2) were 3.5 % when the plasma acetate level was 1.1 m-moles/l. and 20 % at 2.3–2.5 m-moles/l. These values are about 15 % low for reasons discussed above. Corrected values are therefore 8.1 and 46 % for carboxyl-labelled acetate and 4.0 and 23 % for methyl-labelled acetate.

Excretion of acetate in urine. Urine was collected by bladder catheterization during the course of an infusion experiment. Acetate was isolated from urine by steam-distillation (Annison, 1954) and assayed for radioactivity. In a typical experiment in which 109 μC of [carboxy- ^{14}C]acetate was infused in 120 min., 95 ml. of urine was excreted containing labelled acetate at the concentration of 1.07 μmoles , 0.41 $\mu\text{mC/ml}$. It was concluded that losses of acetate by urinary excretion were of negligible proportions.

DISCUSSION

Utilization of glucose in the dog has been extensively studied by the method of isotope dilution, both by single injection (e.g. Baker, Shipley, Clark & Incefy, 1959; Wrenshall, 1955) and by constant-infusion techniques (Searle, Strisower & Chaikoff, 1956; Steele *et al.* 1956) and the limitations of these techniques are now fairly well understood (e.g. Wrenshall & Hetenyi, 1959). For acetate, the use of a single-injection system is even more restricted than for glucose. Such a method requires that the rate of mixing be rapid compared with the rate of utilization. The short half-life of acetate (2–3 min.), suggests that this requirement is not met, and this is also supported by the improbably large value for the volume of distribution of acetate (70 l.). Such an enhanced value would be found if there were significant loss of injected radioactivity before mixing was completed. Constant infusion of labelled acetate eliminates this difficulty, sufficient time being allowed before samples were taken for a steady state to be reached between infusion of label and utilization. Preliminary experiments suggested that this steady state was reached within 20–30 min. from the beginning of infusion. However, for the experiments reported, at least 1 hr. elapsed before samples were taken, and the constancy of specific activity supports the view that a steady state had indeed been reached.

Acetate entering the plasma may be considered as coming from two sources, (a) from the gastrointestinal tract (mainly from the rumen), and (b) from endogenous metabolism (from the liver, fat depots, etc.). Some preliminary experiments by one of the authors (D.B.L.) suggest that endogenous acetate production is about 1–1.5 m-moles/min. in a fed animal. It is probable therefore that

the major part of the acetate entering the blood in the initial experiments shown in Tables 1 and 3 was endogenous. In the experiments reported in Tables 3 and 4, rumen acetate presumably made a significant contribution to the total acetate inflow, up to 2 m-moles/min. This may be compared with a value of 1–1.5 m-moles/min., which may be inferred from data of Annison, Hill & Lewis (1957) obtained by an entirely different method. There is no obvious reason why there should be a difference between the initial rates in Tables 1 and 2 and Tables 3 and 4. In all cases the animals had not been fed for 24 hr.

The distinction between acetate entry (production rate) and removal (utilization rate) would seem to be important in considering the effects of glucose and insulin on acetate utilization (Tables 3 and 4). The results suggest that this was reduced by infusion of insulin, or by considerably raised levels of blood sugar (which would, of course, stimulate endogenous insulin output), whereas utilization rates were increased by small rises in blood sugar. Since these treatments altered the specific activity of the plasma acetate, they must have influenced acetate entry into the blood. However, the outflow of acetate must also have been affected, otherwise the concentration would have changed. This is not unexpected since Reid (1950) has shown that utilization of acetate in the sheep is proportional to its concentration. Thus changes in concentration due to changes in rate of entry of acetate will tend to be self-limiting. An effect of insulin or glucose infusion on acetate production by the rumen is unlikely and it seems more probable that the effect is on the endogenous output of acetate.

The measurement of acetate utilization in the sheep in the period immediately after feeding would be of considerable interest. Ruminal fermentation and acetate production rise to a peak 3–6 hr. after feeding, however (cf. Annison & Lewis, 1959), and then decline for a further 12–18 hr. Reasonably constant levels of plasma acetate are only obtained after a day's fasting, and utilization rates can be measured by the isotope dilution method only when the rate of acetate entry to the system is fairly constant, i.e. under conditions of constant specific activity of plasma acetate. In any one animal, however, the measurement of acetate utilization at various concentrations of acetate achieved by the infusion of unlabelled acetate should allow acetate entry at any instant to be deduced subsequently by reference only to the plasma acetate concentration, provided that the experimental conditions are unchanged. In the experiment reported in Table 1, utilization rates were measured in the starved animal (plasma acetate, 0.25 m-mole/l.; utilization rate, U , 0.51 m-mole/min.) and at plasma acetate concentrations

similar to those observed 3–6 hr. after feeding (plasma acetate, 2.2 m-moles/l.; U , 1.83 m-moles/min.).

Estimation of the contribution of acetate to the oxidative metabolism of the sheep must be regarded as approximate, since the interpretation of carbon dioxide specific-activity curves is subject to many uncertainties. Difficulty arises because of the existence of a large pool of body carbon dioxide with which metabolically produced carbon dioxide has to mix to a variable extent before it is expired. The rate of mixing appears to be comparable with the rate of production of metabolic carbon dioxide (Fig. 4).

We have interpreted the ratio, carbon dioxide specific activity/plasma acetate specific activity, as indicating the fraction of total carbon dioxide produced which is contributed by the direct oxidation of acetate. The validity of this depends on at least two important factors: (1) that mixing of labelled carbon dioxide with body carbon dioxide is complete; (2) that the conversion of acetate into other metabolites and their subsequent oxidation are negligible compared with the direct oxidation of acetate. To some extent the two assumptions are self-cancelling. Secondary oxidation of acetate will be negligible initially but increasingly important as the time of infusion continues; whereas mixing errors become less important as infusion continues. The shape of curve *A* (Fig. 4) suggested that mixing errors were small after 2–3 hr., but in another experiment (curve *B*, Fig. 4) a stable plateau was not reached even after infusion of bicarbonate for 4 hr., although the value at 2–3 hr. was 80 % of the 4 hr. value. These variations may have been due to differences in the size and rate of mixing of the carbon dioxide pool, perhaps because there is a variable rate of mixing between the rumen carbon dioxide pool and the body carbon dioxide pool.

It has been shown in the course of another investigation that the specific activities of plasma glucose, and of the free fatty acid and triglyceride fractions of plasma, after the constant infusion of [*carboxy*- ^{14}C]acetate for 4 hr. were less than 1 % of the specific activity of plasma acetate. The data of Harper, Neal & Hlavacek (1953) suggested that, in the dog, labelling of tissue lipids was of the same order as that of plasma lipids. It seems likely, therefore, that even if lipids or glucose or both were oxidized as rapidly as acetate, about 95 % of the ^{14}C in expired carbon dioxide up to 3 hr. after infusion will be derived from the oxidation of acetate. Mixing errors are probably more serious than secondary-oxidation errors.

The smaller amount of carbon dioxide expired from methyl-labelled, compared with carboxyl-labelled acetate is in line with observations on other animals (see Weinman, Strisower & Chaikoff,

1957). As these authors showed, if carbon dioxide arises principally from the tricarboxylic acid cycle, then methyl- and carboxyl-labelled acetate would give rise to equal amounts of labelled carbon dioxide when there is no turnover of di- and tricarboxylic acids. When there is such a turnover, more methyl- than carboxyl-carbon is carried away into synthetic reactions, and less appears as carbon dioxide. Taking account of this, our results would suggest that in the starved sheep (i.e. 24 hr. after feeding) about 6 % of the expired carbon dioxide may be derived from the carbon of acetate, but that at plasma acetate levels found in sheep after feeding 35 % or more may be so derived.

SUMMARY

1. Rates of utilization and oxidation of acetate have been studied in sheep with [^{14}C]acetate.

2. The specific activity of acetate in red cells and plasma, after the addition of labelled acetate *in vitro*, was the same within 30 sec. of addition as after 30 min.

3. Estimation of the utilization rate by making a single injection of isotope and following the change of specific activity with time was found unsatisfactory.

4. Satisfactory results were obtained by constantly infusing isotope until the specific activity of plasma acetate was constant. Fasting utilization rates of about 0.5 m-mole/min. were obtained. At higher plasma acetate concentrations, of the order reached after feeding, this increased to 1.8 m-moles/min.

5. Utilization rates were altered by changes in carbohydrate metabolism, e.g. infusion of glucose or insulin.

6. Both [*carboxy*- ^{14}C]acetate and [*methyl*- ^{14}C]acetate were used. Taking account of results from both, it would seem that in starved sheep about 6 % of expired carbon dioxide is derived by direct oxidation of acetate. At the higher levels found after feeding, 35 % or more may be so derived.

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Condensed Tannins

8. THE ISOLATION AND DISTRIBUTION OF INTERRELATED HEARTWOOD COMPONENTS OF *SCHINOPSIS* SPP.*

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Roux & Maihs (1960a) showed that the distribution of C₁₅ 'flavonoid' compounds of the resorcinol series was confined mainly to certain genera of the Leguminosae and two genera of the Anacardiaceae. Roux (1958, 1959) also demonstrated the association between monomeric flavan-3:4-diols and polymeric tannins of the same structural pattern in both families, and later Clark-Lewis & Roux (1959) found that (+)-7:3':4'-trihydroxyflavan-3:4-diol of wattle heartwood (Keppler, 1957) *Acacia mearnsii* (syn. *A. mollissima*) and (-)-7:3':4'-trihydroxyflavan-3:4-diol of quebracho heartwood *Schinopsis quebracho-colorado* (syn. *S. lorentzii*) (Roux, 1958; Freudenberg & Weinges, 1958) were enantiomorphs. Similar distribution of enantiomorphs in the Leguminosae and Anacardiaceae are to be found in the (+)-fustin of *A. mearnsii* (Roux & Paulus, 1960) and (-)-fustin of *Cotinus coggygria* (Freudenberg & Weinges, 1959; Weinges, 1959).

Recent demonstration of the association of the stereochemically interrelated group of flavonoids (+)-7:3':4'-trihydroxyflavan-3:4-diol, (+)-fustin and (-)-fisetinidol in *A. mearnsii* (Roux & Paulus, 1960, 1961), *Acacia decurrens*, *Acacia dealbata* and *Acacia pycnantha* (Roux, Maihs & Paulus, 1961) (Leguminosae) heartwoods now lends interest to a detailed examination of *S. quebracho-colorado* and

Schinopsis balansae (Anacardiaceae) heartwoods for the presence of these and possibly other related substances of opposite rotation.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Mixed melting points were on molecular mixtures of substances (Roux & Maihs, 1960a). Analyses of C, H and methoxyl are by Weiler and Strauss, Oxford. Infrared-absorption curves are by Dr J. R. Nunn, National Chemical Research Laboratory, C.S.I.R., Pretoria. A 2 dm. tube was used for optical rotations.

Isolation of fustin, protocatechuic acid, 2-benzyl-4'-methoxy-2:6:3'-trihydroxycoumaran-3-one, gallic acid and catechin from commercial quebracho extract

Powdered 'ordinary' (i.e. un sulphited) quebracho extract (250 g.) prepared commercially from both *S. balansae* (mainly) and *S. quebracho-colorado* (Howes, 1953) was dissolved in 600 ml. of boiling methanol; the mixture was cooled and 800 ml. of chloroform was slowly added with shaking. The precipitate formed was sucked off and the filtrate evaporated to dryness under vacuum in a rotary evaporator (144.5 g.). The powdered solids were dispersed in 400 ml. of commercial boiling ethyl acetate, and ethanol (75 ml.) was added to effect complete solution. After cooling, 200 ml. of chloroform was added as before. The filtrate gave 40 g. of solids after removal of the precipitate.

Solids (80 g.) obtained by the above method were separated in a Craig machine as described by Roux & Paulus

* Part 7: Roux & Paulus (1961).