Rate of Utilization of Acetate in the Ruminant.* (26889)

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McClymont(1) identified acetic acid as the major volatile fatty acid in the peripheral blood of the ruminant, and McCarthy *et al.* (2) showed that concentration of blood acetate is directly proportional to concentration of acetate in the rumen. In the present experiments, the rate at which acetate comes from the rumen (and from endogenous sources), the rate at which acetate leaves the body pool, and the rate at which acetate is oxidized to CO_2 in sheep have been investigated using isotope dilution procedures.

In these experiments constant intravenous infusions of radioacetate with fed sheep were carried out since preliminary single-injection experiments did not appear to be interpretable.

Only in the case of the fed animal is there a sizeable acetate pool due to acetate's being absorbed continuously from the rumen. This phenomenon assures an appreciable quantity of acetate in the peripheral blood. Thus, it is under these conditions rather than starvation that we felt it most desirable to carry out the isotope dilution experiments(3). Only very small amounts (1 mg/min.) of acetate were infused in these experiments and served only as a tracer to label the acetate pool.

Experimental. Two yearling grade wethers (weighing 105 and 144 lb), which had been trained to wear a face mask for prolonged periods of time, were used in these experiments. About 3.5 hr before starting infusion, each was fed 1.25 kg of a ration composed of 46.7% chopped alfalfa hay, 47.0% cracked corn, 3.3% soybean meal, 1.0% salt and 2.0% steamed bone meal. The animals had been maintained on this ration for 2 months prior to these experiments. They were given a tranquilizer (tetrahydrozoline, 0.3 mg/lb body wt) 1 hr before the operation, at which time polyethylene catheters (I.D., 0.062 inch, O.D., 0.082 inch) were placed in both jugulars, by a modification of the procedure of Ralston(4), to permit simultaneous infusion and removal of blood samples.

Potassium acetate-C^{14} was infused into the left jugular vein for about 1 hr at a constant rate.‡ The infusion solution was prepared by dissolving 1 mM of potassium acetate, with a total radioactivity of 2.04 mc, in 50 ml of sterile saline. The activity of the resulting solution was 40.8 μc/ml, or 0.02 mM, and 2040 μc/mM, or 4.488×10^9 dpm/mM. The infusion was 2 mc in 60 min. in Experiment 1, and 2 mc in 67 min. in Experiment 2.

Approximately 50 ml of blood were drawn at each withdrawal (20 ml being used for each duplicate assay) through the polyethylene catheter inserted in the right jugular, using a 50-ml syringe fitted with a 16-gauge needle. In Exp. 1, samples of blood were taken just before infusion was begun, at 10-min. intervals during the hour infusion, and at 15-min. intervals thereafter for 45 min. In Exp. 2, samples of blood were taken at 15-min. intervals during the hour infusion, and at 2-min. intervals thereafter for 7 min.

In Exp. 1, the samples were freed of protein by the tungstate procedure of Hawk, Oser and Summerson(5), and the volatile fatty acids in the protein-free filtrate were measured by the procedures of Oppermann *et al.*(6) and Sheppard(7), using a 300×12 mm column packed with Celite 535. In Exp. 2, total volatile fatty acids in whole blood were separated by steam distillation in a Markham still (Markham, 8), a method which gave 98% recovery when a standard acetate solution was added to whole blood. Titration of this fraction gave widely varying values, possibly due to the fact that the dis-

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‡ Infusions were performed by use of a continuous automatic infusion-withdrawal pump, Model 600-950, obtained from Harvard Apparatus Co. Inc., Dover, Mass.

tillation was carried out at too low a pH, causing pyruvate to be included in the distillate. Therefore, the distillates were made alkaline and were then concentrated and partitioned on Celite columns as in Exp. 1.

Five-ml eluates from the Celite column were collected quantitatively and placed in scintillation bottles. Five ml of the scintillation solution, prepared by dissolving 6 g of 2,5-diphenyloxazole in 1 liter of redistilled reagent grade toluene, was then added. Radioactivity was measured in a liquid scintillation counter (Packard), and the eluates were then titrated to a bromthymol blue end point with 0.0105 N alcoholic KOH to estimate the acetic acid content.

A face mask, constructed from a metal cup 3 inches in diameter by 2½ inches in depth with a 1 inch diameter T-tube protruding from the end, was used for collection of expired CO_2. The outside of the cup was covered with a piece of rubber innertube, cut to the shape of the face and vulcanized into a tubular shape. The inside of the mask was lined with foam rubber to insure a close fit.

The air to be inhaled by the animal was passed through shell caustic to remove CO_2 and was drawn through the mask at a rate sufficient to maintain a negative pressure to prevent loss of expired air around the closely fitting mask. A one-way flutter valve was situated between caustic and mask.

CO_2 was collected from the expired air by means of 2 glass bubbling towers (150 × 900 mm), each having a sintered-glass disc near the bottom to cause dispersion of the incoming gas through 6 liters of 6.25 N sodium hydroxide. The expired air passed through a flutter valve between the mask and the CO_2 collection towers. Twenty-ml samples of the solution were removed from each tower simultaneously with each collection of blood sample. These samples of the sodium hydroxide solution were analyzed for radioactivity and for CO_2. A 50-μl aliquot was diluted with 6 ml of absolute alcohol and 8 ml of toluene containing 3 g 2,5-diphenyloxazole/liter and counted for 10 min. The amount of CO_2 was determined gravimetrically as barium carbonate.

Results and discussion. Blood acetate. The radioactivity of the various acid fractions measured in Exp. 1 showed that virtually all the radioactivity was in the acetate fraction. The activity of propionate, butyrate and the higher fatty acids was extremely small, usually less than twice background, indicating very little formation from acetate. Although Annison(9) reported finding formate in the blood, it was not found on any of our chromatograms. It was found that a reasonably constant level of radioactivity in the blood acetate had already been reached 10 min. after beginning infusion and that this level was maintained until infusion stopped (Table I). The 50% drop in the 60-min. sample is probably explained by the fact that infusion was stopped some 30 to 60 sec. before the 60-min. blood sample was withdrawn. During this brief period a considerable portion of the radioacetate could have disappeared, as was indicated by the turnover times found.

TABLE I. Acetate Concentration and Radioactivity in Acetate Fraction of Sheep Jugular Blood (Experiment 1).

Time (min.) after starting infusion	Total acetate conc., μe/100 ml blood	Total radioactivity of blood (acetate fraction), dpm/100 ml blood (thous.)	Specific activity of blood acetate, dpm /μM acetate
0	141	0	—
10	148	900	6100
20	103	819	7940
30	136	1183	8680
40	138	1129	8160
50	149	942	6330
60*	149	533	3570
75	125	11	90
90	143	9	60
105	145	7	50

* Infusion stopped.

The data for Exp. 1 indicated that essentially all the radioacetate had disappeared from the jugular blood 15 min. after infusion was stopped. Such radioactivity as was present was presumably coming into the blood as tissue metabolic acetate.

Table II gives similar data for Exp. 2 and shows that the specific activity of radioacetate fell to 1/10th its equilibrium value 4 min. after infusion was stopped. Fig. 1 gives the logarithm of disintegration in min./ml of

TABLE II. Acetate Concentration and Radioactivity in Acetate Fraction of Sheep Jugular Blood (Experiment 2).

Time (min.) after starting infusion	μM/100 ml	dpm/100 ml (thousands)	Specific activity, dpm/μM (thousands)
0	166	—	—
15	123	580	4710
30	155	801	5170
45	178	890	5000
60	108	754	6980
65	225	983	4370
67*	—	—	—
69	222	405	1830
71	466	218	470
74	336	154	460

* Infusion stopped.

blood plotted against time in min. There is a rectilinear decrease during the first 4 min. after stopping infusion, which corresponds to a half-life of about 2.1 min. This corresponds to a turnover time of 3 min. for the body pool $\left(\frac{2.1}{1n2}\right)$. This is in good agreement with the value of 3.5 to 4 min. found by Annison and Lindsay(3).

The rate of acetate turnover D, in mM/min., under steady state conditions was calculated as $D = i.c.A/a$

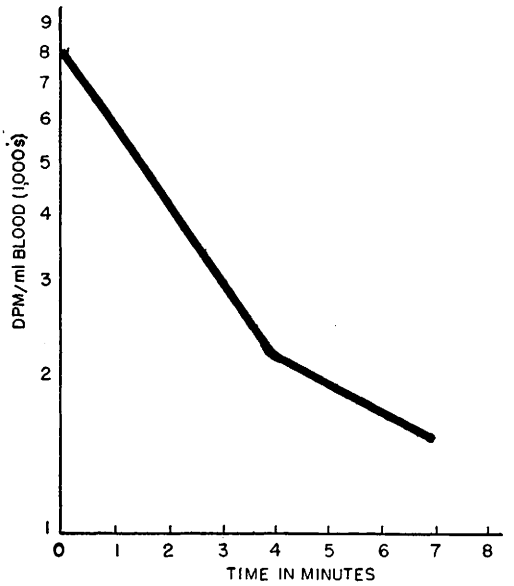

FIG. 1. Disappearance curve for radioacetate in blood.

where

D = acetate turnover rate in mM/min.
i = mM radioacetate infused/min.
c = dpm/mM of infused acetate
A = mM acetate/ml of blood
a = dpm in blood acetate/ml of blood.

The acetate concentration in the blood was taken as representative of its concentration in the total "acetate space." Using the average blood values obtained during the equilibrium period, values for rate of acetate turnover in the whole animal were obtained. These are given in Table III.

TABLE III. Rate of Acetate Turnover (Rate of Disappearance of Labeled Acetate from Blood).

Exp.	In mM per min.	In g per hr	In cals per hr	In cals per day*
1	9 7	35	122	2930
2	10.9	39	137	3290

* Calculated on basis of same rate for 24 hr as was found during 3rd-4th hour after feeding.

The "acetate space," V, *i.e.*, size of the body compartment containing acetate in transit under steady state condition can be calculated as follows:

$$V = \frac{D \cdot t\frac{1}{2}}{A \cdot 1n2}$$

where

V = acetate space in ml
A = mM acetate/ml of blood
D = turnover rate in mM/min.
$t\frac{1}{2}$ = half-time of labeled acetate in blood (assumed the same as that in total space).

When calculated, this proves to be 21-22 liters, or about 30% of body weight of the sheep used, in reasonable agreement with figures of approximately 40-45% reported for glucose space in the non-ruminant(10). The acetate space calculated for the weight of the animal minus the rumen would be of the same order.

From the data in Exp. 2 on size of the acetate space and concentration of acetate in the space, as represented by blood concentration, a total pool size of acetate of 33 mM was obtained.

Respiratory CO_2. In both experiments (Table IV) the peak in specific activity of

TABLE IV. Radioactivity of Expired CO_2 from Sheep Given Acetate-1-C^{14} in Jugular Vein.

Time after starting infusion	Exp. 1		Exp. 2	
	% of C^{14} dose in CO_2	Specific activity, dpm/μM	% of C^{14} dose in CO_2	Specific activity, dpm/μM
0	0	0	0	0
10	2.7	2384		
15			6.8	4768
20	5.7	5573		
30	9.3	9234	11.0	8663
40	13.3	13486		
45			13.0	10994
50	16.8	16923		
60	18.4	18412	14.0	13310
75	29.6	22840	22.6	18109
90	38.3	24653	23.4	15631
105		26856	31.6	17628
120	46.8	22967	35.1	18045
135	51.3	22131	37.6	17700
150		19913		
165	57.6	20434		
180	59 2	19545		

respiratory CO_2 occurred almost an hour after infusion was stopped, indicating that equilibrium had never been achieved. Hence, no value for the half life of acetate from time of injection to time of loss of CO_2 in the expired air can be given. However, it was greater than 70 min. (*i.e.*, the approximate time from cessation of infusion to 50% recovery of CO_2) and less than 130 min. in Exp. 1. In Exp. 2 it was also greater than 70 min., but no upper limit can be set in this second experiment due to termination before 50% recovery had been obtained. Thus it appears that acetate, in moving from the blood to its metabolic end products (water and CO_2), passes through intermediary compounds which have much slower turnover rates than does body acetate. The half life of at least 70 min. is longer than that given by Popják *et al.*(11), who reported less than 50 min. in the lactating goat, and that given by Annison and Lindsay(3), who found that most of the CO_2 coming from a single injection of radioacetate was expired by fasting sheep within 1 hr after injection.

The oxidation of acetate occurs *via* the citric acid cycle and requires oxaloacetic acid, which can be synthesized from glucose *via* pyruvic acid and, in the ruminant particularly, from propionic acid *via* succinate. Acetate not metabolized directly to CO_2 and water could serve as precursor of a number of compounds, such as, acetoacetic acid, β-hydroxy butyric acid, higher fatty acids, glycogen, wool fat, cholesterol, and proteins, in the ruminant as it does in other species(12).

Summary and conclusions. Yearling lambs which had been trained to wear a face mask were used in experiments to study the metabolism of acetate. The rate of turnover of acetate in the body, that is, rate of acetate influx from the rumen into the blood, its transfer from the blood into other tissues, its conversion into other compounds, and its subsequent appearance as CO_2 were measured in 2 sheep. Acetate-1-C^{14} disappeared from the body pool with an extremely short turnover time, approximately 3 min., while 50% recovery as expired CO_2 required well over an hour. These results indicate the formation of intermediary compounds which turn over much more slowly than body acetate. The calculated acetate flux during the fourth hour after feeding was 9.7 and 10.9 mM/min/sheep, equivalent to 35 and 39 g of acetic acid/hr.

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