Acetone Metabolism in Sheep

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1. Entry rates of acetone were estimated in normal and ketonaemic sheep by using a constant-infusion technique with [^{14}C]acetone. Entry rates were less than 1 mg./min. in normal and 2–6 mg./min. in ketonaemic sheep. 2. Only 1–2% of plasma glucose is derived from acetone. 3. Labelling in lactate is consistent with the conversion of acetone into glucose through lactate. 4. There is significant labelling of blood but not rumen volatile fatty acids.

Much of the dietary carbohydrate ingested by ruminants is fermented to volatile fatty acids (Annison & Lewis, 1960). Circulating glucose has thus to be synthesized by gluconeogenesis. The possible contribution of butyrate has proved controversial (Lindsay, 1959). Any plausible pathway for conversion of butyrate into glucose would seem to involve a C_3 intermediate. The existence of such a pathway should be demonstrable through a difference in the labelling pattern in glucose after injection of [^{14}C] or [^{14}C]butyrate. Annison, Leng, Lindsay & White (1963) were unable to find such a difference, but Kronfeld & Brown (1962) produced evidence for such a pathway on the basis of labelling in muscle glycogen after injection of DL- β -hydroxy[3- ^{14}C]butyrate into sheep. They suggested that the pathway involved acetone and propanediol. There is evidence for the existence of such a pathway in rats (Sakami & Rudney, 1952; Mourkides, Hobbs & Koeppe, 1959). We have examined the quantitative significance of such a pathway in sheep, by using an isotope-dilution technique to estimate the entry rate of acetone in normal and ketonaemic sheep, and the extent of conversion of acetone into glucose and lactate. A major difficulty in making such determinations stems from the very small quantities of free acetone present in sheep blood. Even in ketonaemic sheep the concentration of acetone is often as low as 1 mg./100 ml. We have overcome this difficulty by infusing appreciable amounts of carrier acetone (1–5 mg./min.). This procedure makes it impossible to obtain accurate estimates of the entry rate in normal sheep, but it is possible to set an upper limit to this rate.

MATERIALS AND METHODS

Animals. These were Merino wethers and ewes (weight 30–40 kg.). Normal animals received 700 g. of lucerne

chaff/day. Ketonaemia was induced in pregnant ewes by withholding food for 48 hr. during the last 3 weeks of pregnancy.

Chemicals. [1,3- ^{14}C]Acetone (specific activity 116 μC /mg.) was obtained from The Radiochemical Centre, Amersham, Bucks.

Determination of entry rates. The general arrangement was similar to that described by Annison & Lindsay (1961) for the determination of acetate entry rates. [^{14}C]Acetone was infused intravenously with carrier acetone at a constant rate for 130–150 min. The specific activity of acetone in expired air or plasma was constant in the last 60 min. and measurements were made in that time. Total acetone entry was calculated as the ratio (specific activity of infused acetone/specific activity of circulating acetone) $\times$ mg. of acetone infused/min. Endogenous acetone entry was obtained by subtracting the rate of infused acetone. Further details are given with the results of individual experiments.

Plasma glucose. The specific activity was determined after isolation as potassium gluconate, by the method of Blair & Segal (1960). The gluconate was dissolved in 3 ml. of water; 10 ml. of Bray's solution (Bray, 1960) was added, and radioactivity determined in a Nuclear-Chicago 725 liquid-scintillation counter. Corrections for quenching were applied by using the channels-ratio method (Baillie, 1960).

Plasma lactate. The specific activity was determined after isolation as zinc lactate, as described by Annison, Lindsay & White (1963). Radioactivity of zinc lactate was determined as described for potassium gluconate.

Total ketones in blood. These were determined by the method of Bakker & White (1957).

Blood and rumen volatile fatty acids. Specific activities were determined by the method of Davis, Brown, Staubus & Nelson (1960a) except that silicic acid was used in place of Celite.

Acetone in blood. This was determined in the early experiments by difference. Compounds reacting with salicylaldehyde (Behre & Benedict, 1926) were determined in deproteinized blood filtrates before and after aspiration for 10 min. at 37°. For specific-activity determinations, 2 ml. of blood was frozen in a thin film in a 100 ml. flask by rotating the flask in liquid air. The flask containing frozen blood was connected directly to a receiving flask immersed in liquid air and the whole unit evacuated. Water and acetone were completely transferred to the flask in liquid

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air in about 2 hr. Since the contents of the distilling flask remain frozen until transfer is complete, acetoacetic acid does not break down. A 0.2 ml. sample of the transferred aqueous acetone was taken for measurement of acetone concentration, and 0.2 ml. was added to 10 ml. of Bray's solution for measurement of radioactivity.

Acetone in expired air. The apparatus used was modified from that used in this Laboratory for the determination of the specific activity of expired CO_2 (see Annison, 1965). Expired air from a tracheal tube (Davis, Brown, Staubus & Nelson (1960b)) passes into one of a pair of flasks kept in a water bath at 45° . At intervals a flask is removed for determination of the acetone content in the expired air. The expired air leaving the flask passes immediately into an ion chamber on which a stream of warm air is directed, to prevent condensation within the chamber. A fraction of the expired air then passes to a CO_2 -meter for determination of CO_2 content; the remainder passes through a CO_2 absorber (NaOH) and then is expelled through a vacuum pump.

The specific activity of the CO_2 absorbed was determined by the procedure of Nathan, Davidson, Waggoner & Berlin (1958). From this and the percentage CO_2 in expired air the $^{14}\text{CO}_2$ in the ion chamber was calculated. The difference between this and the total radioactivity in the ion chamber was assumed to be due to acetone.

The acetone content of expired air was determined by adding 5 ml. of 5N-NaOH to the flask containing expired air and leaving it overnight to absorb acetone. Then 1 ml. of 10% (v/v) salicylaldehyde in ethanol was added and the solution heated for 45 min. at 50° . The colour was read at $490\text{m}\mu$ and compared with standard acetone solutions in alkali, which contained an appropriate amount of NaHCO_3 .

RESULTS

Initial attempts to isolate acetone from blood without breakdown of acetoacetic acid were unsuccessful. In normal sheep the specific activity of acetone was measured in expired air. Table 1 shows the specific activity of acetone in expired air, obtained after 75 min. infusion, in experiments

carried out on six sheep. Although it was necessary to infuse carrier acetone (1.7–4.6 mg./min.) to obtain a measurable concentration of acetone (about $20\text{ }\mu\text{g./l.}$) in expired air, the acetone concentration in blood remained low (0.4–1.6 mg./min.). The infusions were made at times from 3 to 48 hr. after feeding, but in none of the experiments is the measured specific activity of acetone significantly different from that infused. From the calculated s.e.m. it would seem that a fall in specific activity to about 75% of that infused would have been detectable (outside the 95% confidence limits). Thus acetone entry rate must be below 25% of the rate of infusion of unlabelled acetone, i.e. less than 1 mg./min.

Experiments of this type could not be made on ketonaemic sheep, since attempts to obtain such animals fitted with a tracheal tube were unsuccessful. The method described in the Materials and Methods section was finally devised for the determination of the specific activity of acetone in blood, and determinations of entry rate in ketonaemic sheep were made by using such specific activities. Carrier acetone was infused with ^{14}C acetone, since acetone concentrations may be low (about 1 mg./100 ml.) in blood, even when total ketones are 20 mg./100 ml. Results of infusions of this type, carried out on three sheep, are shown in Table 2. In each experiment the specific activity of acetone in blood was substantially below that infused. Calculated values for endogenous entry rates of acetone are 2.4, 5.0 and 6.5 mg./min.

Specific activities of plasma glucose, plasma lactate and expired carbon dioxide were obtained at the end of infusion of ^{14}C acetone in the experiments described above. Table 3 shows these specific activities, expressed as percentages of the specific activity of circulating acetone. The percentage of the metabolites derived from acetone is

Table 1. *Comparison of the specific activity of acetone in expired air with that infused, after constant intravenous infusion of ^{14}C acetone*

Results are from six normal sheep. In each infusion ^{14}C acetone was infused for 130–150 min. and the specific activity was determined in the last 60 min. Each value is the mean $\pm$ s.e.m. of the no. of estimations shown in parentheses.

Time of experiment in relation to feeding	Specific activity of acetone ($\text{m}\mu\text{C/mg.}$)		Acetone infused (mg./min.)	Concn. of acetone in blood (mg./100 ml.)
	Infused	Found in expired air		
3–5 hr. after feed	190	166 ± 20 (3)	1.7	0.4
	153	176 ± 14 (4)	4.5	1.6
24–28 hr. after feed	194	211 ± 21 (3)	3.3	0.9
	142	141 ± 11 (4)	4.6	1.6
48 hr. after feed	198	215 ± 15 (4)	3.6	1.0
	187	188 ± 8 (3)	3.6	1.0

Table 2. *Comparison of the specific activity of acetone in blood with that infused, after constant intravenous infusion of [¹⁴C]acetone*

Results are from three ketonaemic sheep. In each experiment [¹⁴C]acetone was infused for 130–150 min. and specific activity was determined in the last 60 min. Each value is the mean $\pm$ s.e.m. of the no. of estimations shown in parentheses.

Total blood ketones (mg./100 ml., as acetone)	Concn. of acetone in blood (mg./100 ml.)	Specific activity of acetone (m μ C/mg.)		Acetone infused (mg./min.)	Total entry rate (mg./min.)
		Infused	Found in blood		
20.0	5.7	124.0	55.1 $\pm$ 2.1 (6)	5.2	11.7
17.7	3.2	31.6	16.1 $\pm$ 1.0 (4)	5.2	10.2
15.6	4.6	31.4	21.4 $\pm$ 1.5 (4)	5.2	7.6

Table 3. *Relative specific activity of expired carbon dioxide and plasma lactate and glucose*

The values were obtained at the end of the infusions described in Tables 1 and 2. Relative specific activities are expressed as percentages of the specific activity of infused acetone for normal animals and as percentages of the specific activity of blood acetone in ketonaemic sheep. Each value is the mean $\pm$ s.e.m. from the no. of animals shown in parentheses.

Sheep	Specific activity (as % of that of acetone)		
	CO ₂	Lactate	Glucose
Normal	0.05 $\pm$ 0.003 (6)	1.15 $\pm$ 0.35 (4)	0.45 $\pm$ 0.18 (4)
Ketonaemic	0.15 $\pm$ 0.04 (2)	2.7 $\pm$ 0.7 (3)	2.2 $\pm$ 1.0 (3)

small (less than 3% in all cases). In six out of seven experiments the specific activity of lactate was greater than that of glucose, which is consistent with the formation of glucose through lactate. The relative specific activity of carbon dioxide was much less than that of glucose and lactate, so that labelling in these compounds cannot be explained through carbon dioxide fixation.

Significant radioactivity was also found in the blood volatile fatty acids. In blood samples taken at the end of infusion of [¹⁴C]acetone into two normal sheep, the specific activity of propionate averaged 6%, acetate 1% and formate only a trace of that of infused acetone. In similar studies with two ketonaemic sheep, neither formate nor propionate was detectable in blood, and the specific activity of acetate averaged 3% of that of circulating acetone. In the two experiments with normal sheep, rumen volatile acids were also examined, but only a trace of radioactivity was found in them (about 0.1% relative specific activity).

DISCUSSION

Although the infusion of carrier acetone made accurate determination of the entry rate of acetone in normal sheep impossible, this must be less than 1 mg./min. In ketonaemic animals values of 2.4–6.5 mg./min. were obtained. Bergman, Kon & Katz (1963) and Bergman & Kon (1964) have determined

entry rates of acetoacetic acid in normal (0.6–0.9 g./hr.) and ketonaemic sheep (3.4–10.4 g./hr.). It was also shown that acetoacetic acid decarboxylates at about 5%/hr. at 40°. Thus the entry rate of acetone may be estimated as 0.5–0.75 mg./min. in normal sheep and 2.8–8.6 mg./min. in ketonaemic sheep. These values are in excellent agreement with the present ones.

The values correspond to acetone entry rates of less than 1 g./day in normal sheep and up to 10 g./day in ketonaemic sheep. It appears, however, that only a small part of the acetone available is converted into glucose: our results show that about 0.4% of plasma glucose of normal sheep and 2% of that in ketonaemic sheep is derived from acetone. Even in ketonaemic sheep the contribution of acetone to glucose needs must be small (about 1.5 g./day). Such glucose as is derived from acetone could well be derived through the propanediol pathway, since the labelling in lactate is consistent with this. Although appreciable labelling is observed in the blood volatile fatty acids, absence of label in the rumen acids shows that reactions in the rumen play no part in the transfer of label to these acids.

Black & Luick (1965) have reported on the incorporation of labelled acetone into milk lactose in cows: 14–20% of the injected dose appeared in milk lactose of normal cows, and 2–3% in that of ketonaemic ones. The substantial transfer of label in normal cows presumably reflects a very small

entry rate of acetone. However, the small transfer in ketonaemic cows shows that the contribution of acetone to glucose requirements is as insignificant in cows as it is in sheep.

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