Metabolism of ketone bodies in pregnant sheep

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1. A combination of isotope-dilution and arteriovenous-difference techniques was used to determine the significance of ketones to energy homoeostasis in fasted pregnant ewes.

2. There was incomplete interconversion of D(−) 3-hydroxybutyrate (3HB) and acetoacetate (AcAc) and therefore neither entry rate nor oxidation of total ketone bodies could be estimated by assuming circulating ketone bodies represent a single metabolic compartment. Total ketone body metabolism was satisfactorily summarized using a three-compartment model. In fasted pregnant ewes the mean entry rate of total ketones was 1 mmol/h per kg body-weight and of the ketones entering the circulation 87% were promptly oxidized to carbon dioxide accounting for 30% of the total CO_2 production.

3. Ketone bodies are readily utilized by hind-limb skeletal muscle such that if completely oxidized, 18 ± 4 and 48 ± 3% of the oxygen utilized could be accounted for in fed and fasted pregnant ewes respectively. For both 3HB and AcAc there was a hyperbolic relationship between utilization and arterial concentration. The apparent Michaelis constant (K_m) values were 0·55 and 1·42 mM respectively and the maximum velocity (V_{max}) 2·9 and 5·6 mmol/h per kg muscle. The arterial concentration of AcAc is always below the K_m value and this limits the utilization rate. The D(−) 3HB concentration, however, may surpass that required for maximum utilization and ketoacidosis may be a consequence of this.

4. A two-compartment model was used to analyse ketone body metabolism by hind-limb skeletal muscle. The results suggested substantial interconversion and production of AcAc and 3HB.

5. The pregnant uterus utilized 3HB which if completely oxidized accounted for 12 ± 2 (fed) and 25 ± 4 (fasted) % of its O_2 consumption. At least 64% of the net 3HB utilized was oxidized. AcAc was not utilized in significant quantities.

Krebs (1966) coined the phrase 'physiological ketosis' to emphasize that ketone bodies can serve as efficient fuels of respiration. Pathological ketosis was reserved for those ketoacidotic conditions associated with a severe shortage of carbohydrate either as a result of insulin lack (e.g. diabetes) or because of excessive glucose demands as in bovine ketosis and ovine pregnancy toxaemia. Krebs (1966) suggested that such states are associated with excessive rates of ketogenesis.

The measurement of ketone body entry rate (and so ketogenesis) in vivo by isotope-dilution experiments poses problems, since acetoacetate (AcAc) and D(−) 3-hydroxybutyrate (3HB) are interconverted too rapidly to be considered as independent substrates, but generally too slowly for their specific radioactivities to become equal. McGarry *et al.* (1970) suggested that under the previously-mentioned circumstances, total ketone body entry rate can be determined by measuring the specific radioactivity of total ketones, that is, by assuming that circulating ketone bodies represent a single metabolic compartment. This assumption has been conclusively validated in the dog (Keller *et al.* 1978) but is subject to dispute in humans (Balasse & Delcroix, 1980; Barton, 1980). No comparable studies have been made in sheep. The experiments of Bergman and colleagues (Bergman *et al.* 1963; Bergman & Kon, 1964) that showed AcAc and 3HB attained similar specific radioactivities during the infusion of [3-^{14}C]AcAc are equivocal, since the method used has been shown subsequently by McGarry *et al.* (1970) not to discriminate between AcAc and 3HB specific radioactivities.

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It has been known for many years that most tissues can utilize ketone bodies (See Krebs *et al.* 1971). Thus the sheep hind-limb (Jarrett *et al.* 1976; Lindsay & Setchell, 1976), kidney (Kaufman & Bergman, 1971) and heart (Lindsay & Setchell, 1976) can gain large amounts of energy from ketone bodies when available in sufficient quantities. The sheep foetus does not ulitize ketone bodies as a significant fuel during maternal starvation (Morriss *et al.* 1974), although the utilization by the gravid uterus has not been reported.

Ketone utilization is considered to be controlled largely by the circulating concentration (Krebs *et al.* 1971). Nevertheless, many studies (e.g. Jarrett *et al.* 1974; Berger *et al.* 1978) indicate peripheral utilization (particularly by muscle) of ketones can be depressed, independently of blood concentration. Control of ketone body utilization by the tissues of pregnant ewes has not been studied.

In the present study the measurement of ketone body entry rate and tissue utilization is reported. The experiments were designed to determine:

1. If total ketone body entry rate in ketotic sheep can be determined by assuming circulating ketones to be a single metabolic compartment.
2. The contribution of ketones to energy metabolism in fasted pregnant ewes.
3. The contribution of ketones to the energy metabolism of the muscle and pregnant uterus.
4. The control of ketone body utilization by the tissues of the sheep.

MATERIALS AND METHODS

Experimental animals

Clun Forest ewes of between 3 and 4 years were used throughout. Pregnant sheep were housed singly in pens for 6–7 weeks before lambing and given 1000 g chopped hay (g/kg: 500 lucerne; 500 cocksfoot) and 100 g concentrates at 09.00 hours and 100 g concentrates at 16.00 hours each day. The composition of the concentrates is shown in Pethick & Lindsay (1982). Alloxan-diabetic, non-pregnant sheep were prepared as detailed in Pethick *et al.* (1981).

Pregnant ewes were surgically prepared with indwelling carotid arterial (Pethick *et al.* 1981) and uterine venous (Setchell *et al.* 1972) catheters 1 week before the experiments. The recurrent tarsal vein was cannulated 12–20 h before experiments as described previously (Domanski *et al.* 1974).

Experimental design

The procedure for measurement of ketone body metabolism in pregnant ewes was similar to that described for acetate metabolism by Pethick *et al.* (1981). Sheep, fed or fasted for 3–4 d were placed in an open-circuit calorimeter and approximately 100 μCi [U-^{14}C]3HB or 60 μCi [3-^{14}C]AcAc were infused for a period of 5 h. Hourly blood samples (for 10–12 h) were taken from carotid arterial, recurrent tarsal and uterine venous circulations for the measurement of the concentration of 3HB, AcAc, carbon dioxide, oxygen and specific radioactivities of 3HB, AcAc and CO_2. Muscle blood-flow was measured after the last blood sample as described by Pethick *et al.* (1981). In some experiments, ^{14}C-labelled ketone bodies were not infused during blood sampling from the previously-mentioned circulations.

Laboratory procedures

D(−) [U-^{14}C]3HB (0·4 mCi/mmol) was prepared from $^{14}CO_2$ (Amersham International, Bucks) essentially as described by Sachan & Davis (1967) using *Hydrogenomonas* H.16. Less than 0·5% of the sample was acid-volatile in the freeze-transfer system described by Pethick *et al.* (1981) and 98% of the radioactivity eluted was in the 3HB position when chromatographed on Dowex AG1-X10 (see p. 551). Lithium [3-^{14}C]AcAc was prepared

from ethyl [3-^{14}C]AcAc (2–10 mCi/mmol, Amersham International) by the method of Hall (1962). The purified lithium AcAc was stored at −80° and used within 6 weeks of synthesis. On the morning of infusion a sample of lithium AcAc was converted to the sodium salt by passing through Dowex 50W (Sigma Chemical Co., St Louis, USA) as described by Hall (1962). AcAc and 3HB were separated for specific radioactivity determinations using an ion-exchange column chromatographic technique adapted from the method used by LaNoue *et al.* (1970) for the separation of tricarboxylic acid cycle intermediates from mitochondrial extracts. Plasma (2 ml) was deproteinized with 1·8 M-perchloric acid (0·5 ml) and centrifuged for 30 min at 2000 *g* and 4°. The supernatant fraction was adjusted to pH 6·5–7·0 with 3·5 M-potassium hydroxide and 2 M-potassium bicarbonate and similarly centrifuged for 10 min. The neutralized supernatant fraction was loaded on a 10 mm × 150 mm column of Dowex AG1-X10, 100–200 mesh anion-exchange resin (Bio-Rad Laboratories, Calif., USA) prepared in the formate form. Metabolites were eluted with a formic acid gradient made by the addition of 3 M-formic acid to 250 ml distilled water at a flow rate of 1–2 ml/min. Complete separation of 3HB and AcAc was always found. Acetic acid eluted at the same position as 3HB. In three experiments where [U-^{14}C]3HB was infused the 3HB fraction from column chromatography was separated from acetate by the freeze-transfer technique described in Pethick *et al.* (1981). Because there was no consistent change in specific radioactivity of 3HB ($98 \pm 3\%$) after this treatment it was not done routinely. Plasma samples used for ketone body specific radioactivity determination were stored at −80° and analysed within 10 d. AcAc decomposed at 2% per month in these circumstances. Ketone body concentration and radioactivity in the column chromatography fractions were measured as described previously (Pethick *et al.* 1981).

The concentrations of CO_2 and O_2 in the blood and the specific radioactivity of CO_2 in blood were determined as previously described by Pethick *et al.* (1981).

Calculations

The calculations of indices of ketone body metabolism were similar to the corresponding indices for acetate metabolism as described previously (Pethick *et al.* 1981) with the following additions: The apparent entry rate of total ketone bodies (mmol/h) was I/total ketone S_A, where I is the infusion rate of the ^{14}C-labelled tracer ketone body (disintegrations/min per h) and S_A is the arterial blood specific radioactivity (disintegrations/min per mmol). Total ketone specific radioactivity was calculated as

$$\frac{\text{disintegrations/min in AcAc} + \text{disintegrations/min in 3HB}}{\text{mmol AcAc} + \text{mmol 3HB}}.$$

The apparent proportion of respiratory CO_2 derived from total ketones was expired CO_2 S_A/total ketone S_{At}, where S_{At} is the arterial blood specific radioactivity (disintegrations/min per m atom C). The excretion of $^{14}CO_2$ in expired air was corrected to a steady-state equilibrium value as described previously (Pethick *et al.* 1981).

Direct oxidation of total ketone bodies by the muscle and pregnant uterus was estimated by determining the total tissue excretion of $^{14}CO_2$ (area method) or by assuming a steady-state excretion of $^{14}CO_2$ was attained during an infusion of ^{14}C-labelled trace ketone body (equilibrium method) as described in detail by Pethick *et al.* (1981). Thus for any tissue, the proportion of CO_2 produced that was derived from ketone bodies was calculated as:

$$\frac{[^{14}CO_2\,V] - [^{14}CO_2\,A]}{\text{weighted total ketone } S_{Vt}} \times \frac{1}{[CO_2\,V] - [CO_2\,A]}$$

where [$^{14}CO_2$ V] and [$^{14}CO_2$ A] are the radioactive concentrations of CO_2 in venous and arterial blood (disintegrations/min per ml) and [CO_2 V] and [CO_2 A] are the CO_2 concentrations in venous and arterial blood (μmol/ml). The weighted total ketone S_{Vt} (disintegrations/min per m atom C) was computed as:

$$\text{AcAc } S_{Vt} \times \frac{([A]-[V]) \text{ for AcAc}}{([A]-[V]) \text{ for total ketones}} + \text{3HB } S_{Vt} \times \frac{([A]-[V]) \text{ for 3HB}}{([A]-[V]) \text{ for total ketones}}$$

where [A] and [V] represent blood concentrations (μmol/ml) in arterial and venous circulations respectively. This calculation assumes that utilized ketone bodies are equally susceptible to oxidation. For calculations of pregnant uterus and muscle metabolism, V represents uterine venous and recurrent tarsal circulations respectively.

RESULTS

Ketone body specific radioactivity

The pattern of ketone body specific radioactivity during the infusion of [U-^{14}C]3HB or [3-^{14}C]AcAc is shown in Fig. 1 (*a* and *b* respectively). In both types of experiment blood ketone concentrations were relatively stable over the experiment and the specific radioactivities reached a stable equilibrium value within the 5 h infusion period. The turnover time (see Atkins, 1969) of 3HB during the infusion of [U-^{14}C]3HB (43 ± 6 min) was three times that of AcAc during [3-^{14}C]AcAc infusions (14 ± 2 min) in fasted ketotic sheep.

The concentrations and relative specific radioactivities of AcAc and 3HB are shown in Table 1. In no experiment did the two ketones reach an identical specific radioactivity. The specific radioactivity of the infused ^{14}C-labelled ketone body was usually approximately twice that of the non-infused ketone body.

Entry rate and oxidation of ketones in the whole animal

The entry rates of the infused ketone body and the adjusted values for apparent total ketone body entry rate allowing for the disparity in ketone body specific radioactivity are shown in Table 2. Fig. 2*a* shows the entry rate of both 3HB and AcAc related to the arterial concentration of 3HB for all animals. For either ketone body at a given arterial concentration the entry rate is similar and follows a hyperbolic relationship. However, estimated rates of apparent total ketone body entry rate differ (Table 2) depending on which ^{14}C-labelled ketone body was infused. Thus the entry rate of AcAc and 3HB is less than the apparent total ketone body entry rate (56 ± 3 and 90 ± 1% respectively). The results for ketone body oxidation to respiratory CO_2 can be interpreted similarly to those of ketone body entry rate (see Table 2, Fig. 2*b*).

A three-compartmental model has been constructed for 3HB, AcAc and CO_2 metabolism in sheep using the results for fasted pregnant ewes. The method is described by Mann & Gurpide (1966) and how it relates to a three-compartment system is shown in detail by Nolan *et al.* (1976). Theoretically, the entry rate (called irreversible loss by Nolan *et al.* 1976) and intercompartmental conversion of all three metabolites should be determined. However, in the present study, the entry rate of CO_2 was assumed to be 5% greater than the respiratory production (Whitelaw *et al.* 1972) to allow for CO_2-fixation reactions. In addition the incorporation of CO_2 into ketone bodies is assumed to be zero.

There are, therefore, nine unknown constants (Fig. 3), with an assumed value for R_{03}. From experiments in which [U-^{14}C]3HB is infused, by considering ^{14}C balance in each compartment, three equations are obtained. Three further equations are obtained from ^{14}C balance figures in each of these compartments when [3-^{14}C]AcAc is infused. Finally, there are three equations from C balance in each compartment. The nine simultaneous equations were solved using a micro-computer by means of an iterative technique.

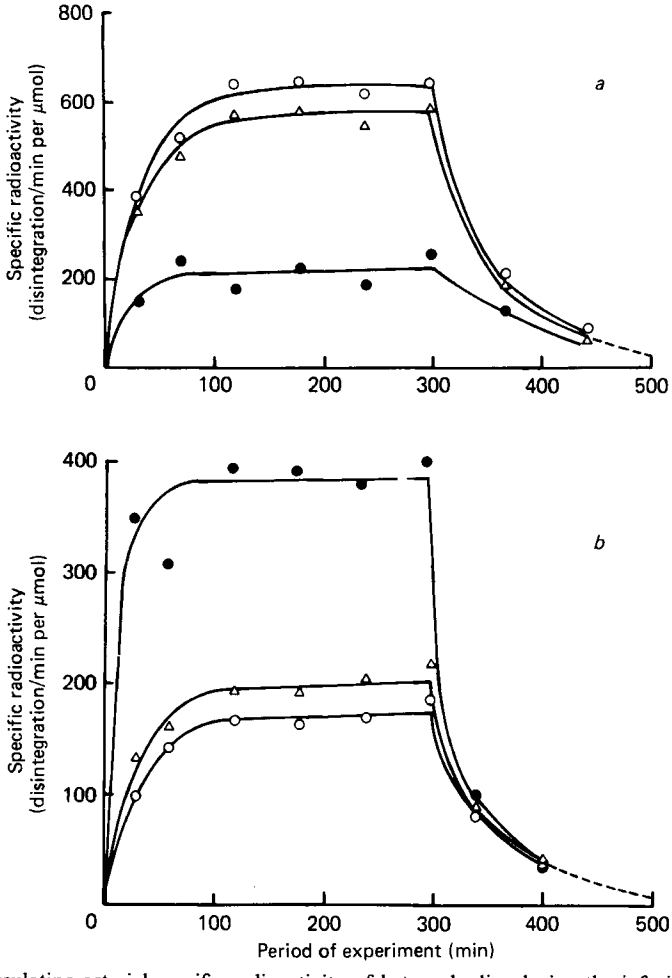

Fig. 1. Circulating arterial specific radioactivity of ketone bodies during the infusion of ^{14}C-labelled ketone bodies into fasted pregnant ewes. (○), 3-Hydroxybutyrate (3HB) specific radioactivity; (●), acetoactetate (AcAc) specific radioactivity; (△), total ketone bodies specific radioactivity. (*a*) Infusion of D(−) [U-^{14}C]3HB into the jugular vein (0·23 μCi/min). The mean arterial concentrations were: 3HB 1·95 mM, AcAc 0·3 mM. If x is the period after infusion and y is the ketone body specific radioactivity (disintegrations/min per μmol) then the best-fit functions are: AcAc, $y = 217(1\text{-}e^{-0\cdot046x})$; 3HB, $y = 635(1\text{-}e^{-0\cdot029x})$; total ketone bodies, $y = 569(1\text{-}e^{-0\cdot031x})$. (*b*) Infusion of [3-^{14}C]AcAc into the jugular vein (0·12 μCi/min). The mean arterial concentrations were: 3HB 1·80 mM, AcAc 0·29 mM. The best-fit functions were: AcAc, $y = 380(1\text{-}e^{-0\cdot070z})$; 3HB, $y = 170(1\text{-}e^{-0\cdot028z})$; total ketone bodies, $y = 199(1\text{-}e^{-0\cdot033x})$. Fitted functions were calculated (and are valid) only for values obtained during the period of infusion (0–300 min).

The results of the compartmental analysis are shown in the legend to Fig. 3. Total ketone body entry rate is represented as the sum of R_{10} and R_{20}, i.e. 1 mmol/h per kg body-weight. Of the ketones entering the circulation, 87% are promptly oxidized to CO_2 accounting for 30% of the total CO_2 production.

Utilization of ketone bodies by the muscle and pregnant uterus

The net utilization of 3HB and AcAc by hind-limb skeletal muscle in ketotic and non-ketotic sheep is related to arterial concentration, as shown in Fig. 4*a*,*b*. The arterial blood concentration of 3HB at half-maximal utilization was estimated as 0·55 mM, a value at the low end of the physiological range which can reach approximately 12 mM; however,

Table 1. *Summary of ketone body infusion experiments*

Ewes	No. of animals	Infusate	Body-wt (kg)	Arterial concentration (mM) 3HB	Arterial concentration (mM) AcAc	Ketone specific activity ratio†
Pregnant, fed, twin foetuses	2	[U-^{14}C]3HB	82 72	0·83 0·70	0·11 0·16	ND ND
Pregnant, fasted 3–4 d, single foetus	2	[U-^{14}C]3HB	64 76	1·51 2·16	0·40 0·31	0·26 0·41
Pregnant, fasted 3–4 d, single foetus	1	[3-^{14}C]AcAc	72	1·8	0·29	0·45
Pregnant, fasted 3–4 d, twin foetuses	5	[U-^{14}C]3HB	80·2‡ 5·9‖	3·02‡ 0·41‖	0·57‡ 0·09‖	0·49‡ 0·06‖
Pregnant, fasted 3–4 d, twin foetuses	1	[3-^{14}C]AcAc	76	3·39	0·53	0·55
Alloxan-diabetic. Food and insulin withheld 2 d	1	[3-^{14}C]AcAc	49	2·17	0·36	0·49

3HB, D(−) 3-hydroxybutyrate; AcAc, acetoacetate.
ND, specific activity of acetoacetate not determined.
† $\frac{\text{Arterial specific activity of ketone not infused}}{\text{Arterial specific activity of infused ketone}}$.
‡ Mean, ‖ SE.

Table 2. *Entry rate and oxidation of ketone bodies*

Ewes	No. of animals	Infusate	Apparent entry rate of ketone bodies (mmol/h per kg body-wt) Infused ketone	Apparent entry rate of ketone bodies (mmol/h per kg body-wt) Total ketones	Proportion of respiratory carbon dioxide derived from: Infused ketone	Proportion of respiratory carbon dioxide derived from: Total ketones	Proportion of infused ketone directly oxidized
Pregnant, fed, twin foetuses	2	[U-^{14}C]3HB	0·31 0·44	ND ND	0·06 0·13	ND ND	0·79 0·88
Pregnant, fasted 3–4 d, single foetus	2	[U-^{14}C]3HB	0·56 0·67	0·66 0·72	0·19 0·27	0·22 0·29	0·92 0·82
Pregnant, fasted 3–4 d, single foetus	1	[3-^{14}C]AcAc	0·60	1·18	0·17	0·34	0·68
Pregnant, fasted 3–4 d, twin foetuses	5	[U-^{14}C]3HB	0·75† 0·08‡	0·83† 0·08‡	0·23† 0·04‡	0·26† 0·04‡	0·84*† 0·07‡
Pregnant, fasted, twin foetuses	1	[3-^{14}C]AcAc	0·9	1·47	0·27	0·43	0·87
Alloxan-diabetic. Food and insulin withheld 2 d	1	[3-^{14}C]AcAc	0·79	1·42	ND	ND	ND

ND, not determined. * Only four observations. † Mean. ‡ SE.

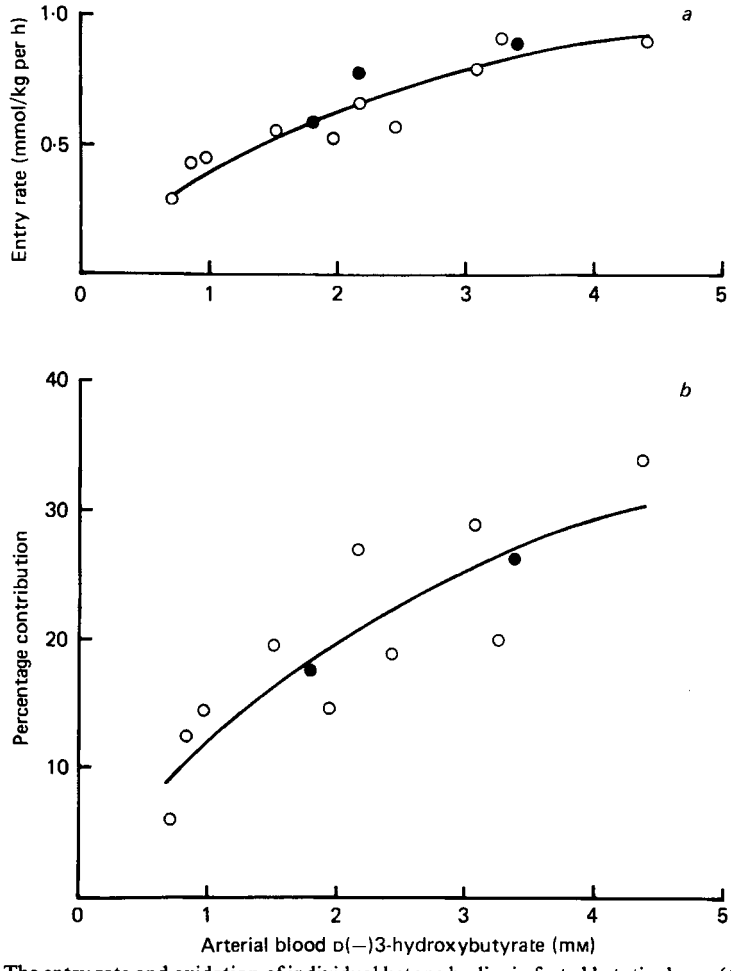

Fig. 2. The entry rate and oxidation of individual ketone bodies in fasted ketotic sheep. (○), Experiments in which D(−) [U-^{14}C]3-hydroxybutyrate (3HB) was infused; (●), experiments in which [3-^{14}C]acetoacetate (AcAc) was infused. (*a*) Entry rate. If x is the arterial concentration of 3HB (mM), y is the entry rate of AcAc or 3HB (mmol/h per kg body-weight), V is the maximum entry rate and K is the concentration at which $y = 0{\cdot}5V$, then the best-fit curve for $y = Vx/K+x$ (see Bliss, 1970) with 3HB concentration as the abscissa gives V 1·49±0·023 mmol/h per kg and K 2·65±0·08 mM. If the abscissa is AcAc concentration, V 1·06±0·19 mmol/h per kg and K 0·22±0·11 mM. (*b*) Contribution to respiratory carbon dioxide production. If x is the arterial concentration and y the percentage contribution of AcAc or 3HB to CO_2 production then, when 3HB concentration is the independent variable, the corresponding values are: V 55·6±18·9% and K 3·57±2·1 mM. If arterial blood AcAc is the independent variable, values are V 44·2±13·2% and K 0·42±0·26 mM.

half-maximal utilization of AcAc was at an arterial concentration of 1·4 mM, a value greater than any observed. Thus the utilization of 3HB relative to arterial concentration tends to decline more rapidly than AcAc as the respective blood ketone body concentration increases. Ketones could significantly contribute to the energy metabolism of muscle since, if completely oxidized, they can account for 18 and 48% of the oxygen utilized by muscle in fed and fasted pregnant ewes respectively (Table 3). The utilization of AcAc is greater than 3HB with respect to arterial concentration; thus the extraction of AcAc by muscle is nearly two times greater than 3HB in fed sheep and nearly 2·5 times greater in fasted sheep (Table 3). However, because the estimated arterial concentration of 3HB is greater

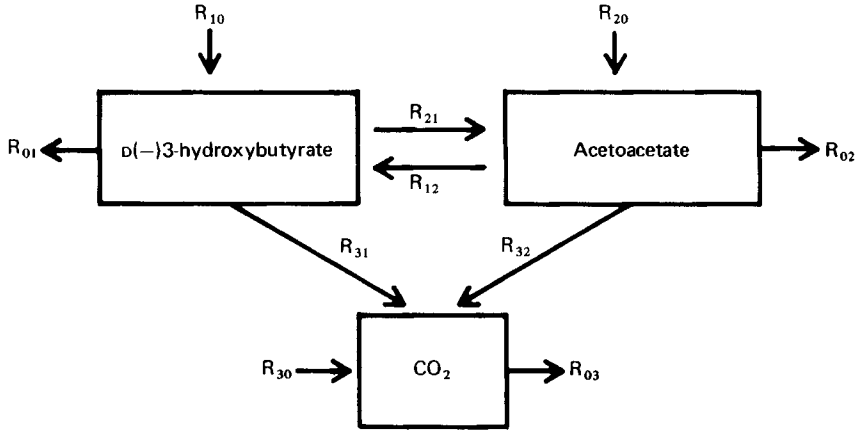

Fig. 3. Three compartment model for calculation of the synthesis, interaction and oxidation of circulating ketone bodies in fasted pregnant ewes. The model has been computed from the results shown in Tables 1 and 2 for 3–4 d fasted pregnant ewes. Respiratory production of carbon dioxide was 10·73 ± 0·6 mmol/h per kg body-weight (mean ± SE, nine animals) and thus R_{03} has been taken as 11·27 (see p. 552). Complete results were available for seven experiments with 3-hydroxybutyrate (3HB) and two experiments with acetoacetate (AcAc). Transfer rates are therefore presented as mean ± SE of the fourteen possible solutions, being expressed as m atom/h per kg body-weight. Values are: R_{01} − 0·04 ± 0·19, R_{02} 0·54 ± 0·10, R_{10} 1·85 ± 0·13, R_{20} 2·13 ± 0·16, R_{30} 7·81 ± 0·17, R_{12} 1·86 ± 0·14, R_{21} 1·79 ± 0·23, R_{31} 1·95 ± 0·29, R_{32} 1·52 ± 0·22. Total ketone body synthesis, $R_{10} + R_{20}$ = 3·98 m atom/h per kg body-weight; total ketone body oxidation, $R_{31} + R_{32}$ = 3·47, i.e. 31% of R_{03}. Utilization of ketones by other than oxidation is given by R_{01} (not significantly different from zero) and R_{02}, and interaction of ketones is given by R_{21} and R_{12}.

than AcAc the absolute net utilization by muscle of 3HB was 5·3 and 2·2 times greater in fed and fasted ewes respectively.

The pregnant uterus can utilize ketones but in a manner different to that found in skeletal muscle (Table 3). Thus 3HB is utilized in proportion to arterial concentration while AcAc is not utilized and tends to be excreted in small quantities (Fig. 5*a*, *b*). D(−) 3HB, if completely oxidized, could account for 12 and 25% of the O_2 utilized by the pregnant uterus in fed and fasted ewes respectively.

The utilization of ketone bodies was confirmed by direct measurements of $^{14}CO_2$ production from utilized ^{14}C-labelled ketones. Estimates of ketone body oxidation in muscle using the equilibrium calculation were consistently only 71 ± 3% (mean ± SEM; seven animals) of the area calculation (see p. 551), confirming the findings for measurements of acetate oxidation in previous work (Pethick *et al.* 1981). Only values using the area calculation are therefore reported. Determination of oxidation was similar whether [U-^{14}C]3HB or [3-^{14}C]AcAc was used as the tracer ketone body. Oxidation of ketone bodies in fasted pregnant ewes accounted for 42 ± 3% (mean ± SEM; seven animals) of the CO_2 produced by muscle, representing 66 ± 5% of the ketone body taken up by the muscle. In two experiments with ^{14}C-precursors, ketone body uptake (as 3HB) by the pregnant uterus accounted for 13 and 23% of the CO_2 production representing 63 and 65% of the net ketone body uptake.

Extrahepatic ketogenesis

During the infusion of [U-^{14}C]3HB or [3-^{14}C]AcAc the extraction of ^{14}C-labelled and unlabelled ketone bodies by muscle differed as shown in Table 4. These results suggest isotopic exchange between AcAc and 3HB and possible synthesis of ketones from another source. A two-compartment system similar to that used by Wolff & Bergman (1972) for amino acids has been fitted to the values in Table 4 to determine the fate of 3HB and AcAc in muscle. Consider the two-compartment system shown in Fig. 6 with $R_1, \ldots R_{10}$ transfer

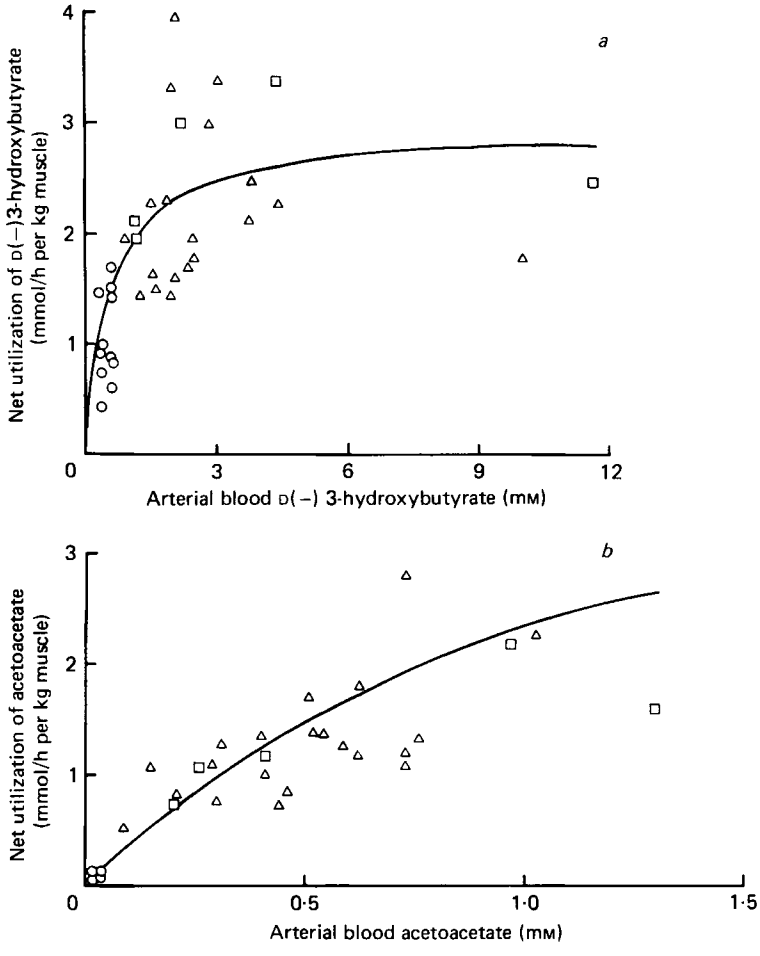

Fig. 4. Net utilization of ketone bodies by hind-limb skeletal muscle of sheep. (○), Lactating and non-lactating (non-pregnant) sheep. Values from Pethick & Lindsay (1982). (□), Alloxan-diabetic sheep, food and insulin withheld 2–3 d (Pethick *et al.* 1981). (△), 3–4 d fasted pregnant ewes used in the present study. (*a*) Net utilization of 3-hydroxybutyrate (3HB). If x is the arterial concentration of 3HB, y is net utilization of 3HB by skeletal muscle (mmol/kg per h), V is maximum utilization rate and K is the concentration at which y is $0{\cdot}5V$, then the best-fit gives V $2{\cdot}9 \pm 0{\cdot}26$ mmol/kg per h and K $0{\cdot}55 \pm 0{\cdot}19$. (*b*) Net utilization of acetoacetate (AcAc). If x is the arterial concentration of AcAc and y is the net utilization of AcAc by muscle, then the best-fit gives V $5{\cdot}6 \pm 0{\cdot}85$ mmol/h per kg muscle and K $1{\cdot}42 \pm 0{\cdot}15$ mM.

rates (mmol/h per kg muscle). During the infusion of [U-^{14}C]3HB two equations can be derived, relating balance in 3HB and AcAc respectively:

$$[A]^*_{3HB} \times BF + AcAc_{SA}\, R_{10} = 3HB_{SA}\, R_2 + 3HB_{SA}\, R_5 + [V]^*_{3HB} + BF$$
$$[A]^*_{AcAc} \times BF + 3HB_{SA}\, R_5 = AcAc_{SA}\, R_7 + AcAc_{SA}\, R_{10} + [V]^*_{AcAc} \times BF$$

where $[A]^*$ and $[V]^*$ represent radioactive concentrations (disintegrations/min per ml blood), SA the arterial blood specific radioactivity (disintegrations/min per mmol) and BF the blood flow (ml/h per kg muscle). Similarly, two equations can be formed for experiments employing an infusion of [3-^{14}C]AcAc. The resulting four simultaneous equations can then be solved for R_2, R_5, R_7 and R_{10}. The transfer rates R_1, R_4, R_6 and R_9 are already known

Table 3. *Utilization of ketone bodies by hind-limb skeletal muscle and the pregnant uterus in fed and fasted pregnant ewes*

(Mean values with their standard errors; no. of animals given in parentheses)

Index	Regime	D(−) 3-Hydroxybutyrate Mean	SE	Acetoacetate Mean	SE
Arterial blood concentration (mM)	Fed	0·86	0·09 (7)	0·09	0·04 (5)
	Fasted	2·72	0·46 (19)	0·51	0·05 (19)
Extraction of ketone bodies (%)*					
Muscle	Fed	8·8	1·8 (3)	15·6	6·9 (3)
	Fasted	10·2	2·0 (19)	25·0	3·0 (19)
Uterus and contents	Fed	6·5	0·9 (7)	−4·0	2·2 (5)
	Fasted	4·6	0·8 (7)	−5·8	3·8 (7)
Maximum contribution to oxygen consumption if completely oxidized†					
Muscle	Fed	16	3 (3)	2	0·9 (3)
	Fasted	31	2 (19)	17	1·0 (19)
Uterus and contents	Fed	12	2 (7)	−0·7	0·4 (5)
	Fasted	25	4 (7)	−5	3 (7)

* Extraction calculated as $\frac{(A)-(V)}{(A)} \times 100$, where A, V represent arterial and venous concentrations respectively.

† Calculated assuming 1 mol of D(−) 3-hydroxybutyrate or acetoacetate require 4·5 or 4 mol of oxygen respectively for complete oxidation.

(blood concentration of ketone body × muscle blood flow). Finally two equations relating carbon balance derive R_3 and R_8, i.e. 3HB C balance

$$R_1 + R_3 + R_{10} = R_2 + R_5 + R_4$$

and AcAc C balance

$$R_6 + R_5 + R_8 = R_{10} + R_7 + R_9.$$

The legend to Fig. 6 shows the computed transfer rates. Ketone body formation was 1·3 mmol/h per kg muscle which represented 22% of the gross utilization of ketones by the muscle.

DISCUSSION

Ketone body production

The results of the present study suggest that in experiments where the entry rate is determined by infusion of radioactive ketones over 5 h, total ketone body entry rate in ketotic sheep cannot be calculated simply by determining the specific radioactivity of total ketone bodies as in the dog (Keller *et al.* 1978). The findings of the present study suggest that a two- or three-compartment model rather than a single-compartment model should be used to calculate ketone body synthesis in fasted pregnant ewes. The mean entry rate of total ketones (1 mmol/kg per h) is quite similar to the total ketone output by the liver of alloxan-diabetic ewes (1·1 mmol/kg per h) (Pethick *et al.* 1981) with comparable 3HB and AcAc blood concentrations.

The entry rate of total ketone bodies contributes significantly to the energy needs of fasted pregnant ewes, 30% of the energy metabolism being derived from ketones. This represents virtually complete oxidation of ketone bodies in contrast to an earlier study by Bergman & Kon (1964) where only 45–60% of ketone bodies were apparently oxidized.

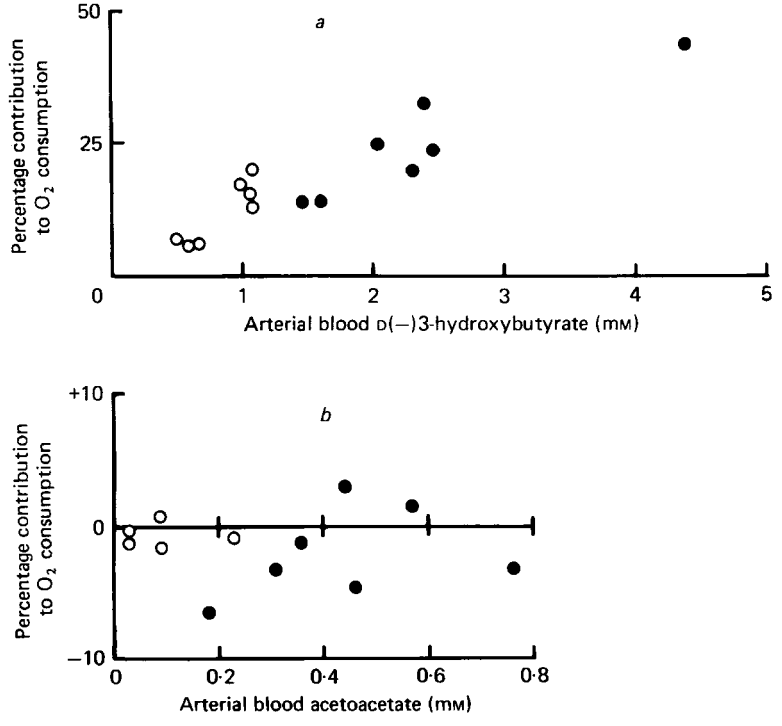

Fig. 5. Utilization of ketone bodies by the pregnant uterus, expressed as the maximum contribution of ketone bodies (if completely oxidized) to the oxygen consumption of the pregnant uterus. All ewes were approximately 4 weeks from parturition. (○), Fed animals; (●), fasted animals. (*a*) Contribution of D(−) 3-hydroxybutyrate (3HB). If x is the arterial 3HB concentration (mM) and y is the contribution of 3HB to oxygen consumption (%) then for fed animals $y = 20{\cdot}9\,x - 5{\cdot}9$ (r^2 0·90) and for fasted animals $y = 10{\cdot}2\,x + 0{\cdot}9$ (r^2 0·92). (*b*) Contribution of acetoactetate (AcAc). There was no significant regression.

Table 4. *Metabolism of ketone bodies by the hind-limb skeletal muscle of pregnant ewes fasted for 3–4 d after which either [3-^{14}C]acetoacetate (AcAc) or D(−) [U-^{14}C] 3-hydroxybutyrate (3HB) were infused intravenously*

(Mean values with their standard errors, no. of animals given in parentheses)

Infused isotope	[U-^{14}C]3HB (6) Mean	SE	[3-^{14}C]AcAc (2) Mean
Muscle venous: arterial specific activity ratio			
3HB	0·95	0·008	0·99
AcAc	1·12	0·02	0·92
Total ketone bodies	0·98	0·007	0·96
Arterial concentration			
3HB (mM)	2·77	0·42	2·59
Extraction (%)	7·9	2·0	11·6
AcAc (mM)	0·54	0·08	0·41
Extraction (%)	24·2	6·1	27·6
Percentage extraction of radioactivity			
3HB	12·7	2·4	12·6
AcAc	15·3	6·1	33·0

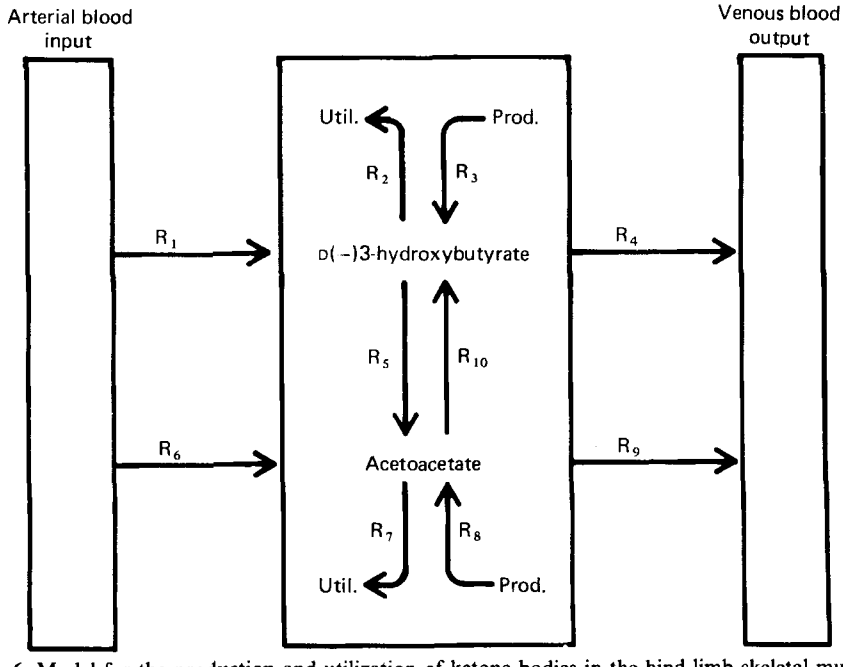

Fig. 6. Model for the production and utilization of ketone bodies in the hind-limb skeletal muscle of fasted pregnant ewes. The model has been computed from the results shown in Table 4. Mean muscle blood flow was 12·98 ± 1·47 l/h per kg muscle (mean ± SE). Util., gross utilization; Prod., production. For details of the calculations, see pp. 556–558. Values known are: R_1 35·95, R_4 33·11, R_6 7·01, R_9 5·31. Values obtained were: R_2 4·26, R_3 1·18, R_5 0·62, R_{10} 0·85, R_7 1·58, R_8 0·12. All values are expressed as mmol/h per kg muscle.

Utilization of ketone bodies through reactions other than oxidation is approximately zero for 3HB (R_{01}, Fig. 3) but significant for AcAc (R_{02}) where it represents 14% of the gross AcAc synthesis ($R_{20} + R_{21}$). This non-oxidative utilization might represent acetone formation through AcAc decarboxylation (Reichard *et al.* 1979). Lindsay & Brown (1966) estimated the entry rate of acetone to be 0·13 mmol/h per kg body-weight (taking sheep weight as approximately 40 kg); this is 72% of R_{02}. Any remaining ketone bodies were probably excreted in the urine since in ketoanaemic pregnant ewes Leng (1966) estimated a total urinary ketone loss of 0·04 mmol/h per kg body-weight.

Extrahepatic ketogenesis

From 10 kg skeletal muscle, ketone body synthesis would represent 16% of the total ketone body entry rate. Hepatic ketogenesis is accepted as the major source of circulating ketone bodies (see Krebs, 1966) and so the estimated magnitude of extrahepatic (muscle) ketogenesis is unexpected and potentially considerable. Ketone body formation in human forearm muscle is related to exercise (Hagenfeldt & Wahren, 1971) and so the previously-mentioned values may be an over-estimate for all muscle since hind-limb muscle would be exercising to maintain posture even in a resting, standing sheep. Nevertheless, there is other evidence to support the existence of ketone body formation by skeletal muscle (Pethick *et al.* 1983). Further experiments are required to determine the extent of total ketone body entry rate in conjunction with hepatic and extrahepatic ketogenesis.

Utilization of ketones by the tissues

In fasted pregnant ewes at least half the ketones synthesized can be used by muscle and this tissue, therefore, dominates the whole animal metabolism of ketones. At concentrations of ketone bodies normally occurring in fasted pregnant ewes, 3HB utilization is much closer to the maximal value than is AcAc, so that although the fractional extraction is less, the absolute rate is greater. The wide range in utilization rates of 3HB at a given concentration suggests there may be much variation in maximum velocity (V_{max}) in different animals. Ruderman & Goodman (1973) found that rat muscle used AcAc more readily than 3HB relative to circulating concentrations. These authors suggested that this was due to limited D(−) 3HB dehydrogenase activity, since their results indicate that both ketones readily permeate muscle. Human forearm muscle utilized both ketones similarly (Wicklmayr & Dietze, 1979). However, in the same study, conditions leading to increased fatty acid utilization by muscle (i.e. catecholamine infusion) resulted in an increased AcAc uptake and a decreased 3HB uptake. It is possible that fatty acid utilization may have changed the redox state of the mitochondria and so inhibited D(−) 3HB dehydrogenase (*EC* 1.1.1.30) activity. It would seem probable that fatty acid oxidation in muscle might set a maximum limit to 3HB utilization, especially since non-esterified fatty acids (NEFA) are utilized readily over a wide range of physiological concentrations by sheep muscle (Pethick *et al.* 1983).

Changes in the rate of D(−) 3HB utilization by muscle may be an important determinant of the extent of ketoanaemia found in a fasted pregnant ewe.

The results in Fig. 4*a* suggest that only a small change in 3HB production is needed above an arterial concentration of 4 mM to produce a large change in arterial levels. Further, the ratio, 3HB : AcAc in arterial blood of mildly-ketotic ewes (approximately 4 mM) was 5 : 1 while in two markedly ketoanaemic ewes (3HB > 10 mM, see Fig. 5*a*) the value was 9 : 1, emphasizing the disproportionate increase in 3HB. Further experiments similar to those described by Wicklmayr & Dietze (1979) are needed to substantiate the possible interaction of 3HB and NEFA in fasted pregnant ewes.

These results raise serious doubt as to the commonly held, but unsubstantiated, view that AcAc is a deleterious ketone. This was suggested by Behnke (1964) because it is a stronger acid than 3HB (pK_a: AcAc 3·6, 3HB 4·4) and led Koundakjian & Snoswell (1970) to sugg st that ruminants may be susceptible to ketoacidosis because they have a limited ability to convert ('detoxify') AcAc to 3HB. In addition, Bergman *et al.* (1963) noted that during sodium AcAc infusions (sufficient to increase circulating ketones above 4 mM) clinical signs such as drowsiness, stupor and occasional hyperaesthesia sometimes developed. This observation is probably complicated by a significant alkalosis produced by sodium AcAc infusions in sheep (see Lindsay & Setchell, 1976). Assuming that the major deleterious property of ketones is their acid nature then, on a quantitative basis, 3HB should be more important than AcAc. During the present study a significant negative regression between blood 3HB and bicarbonate concentrations was observed, indicating an alteration of the acid–base balance in relation to 3HB concentration. Thus if x is arterial 3HB concentration (mM) and y is the arterial blood bicarbonate concentration (mM) then $y = -1{\cdot}56x + 25{\cdot}10$ (r^2 0·64, $P < 0{\cdot}001$). No significant regression was found between blood AcAc and bicarbonate concentrations.

The utilization of ketones by the pregnant uterus was strikingly different from the muscle in that AcAc was not consistently utilized. D(−) 3HB appeared to be utilized in a proportional manner (at least up to arterial levels of 4 mM) while AcAc was not always removed. Assuming a uterine blood flow in twin pregnant sheep of approximately 2 l/min

(Silver, 1976), then 3HB utilization in a fasted pregnant ewe (75 kg) would be approximately 19 mmol/h or nearly 25% of the total ketones synthesized.

The actual site of ketone utilization remains controversial since there is evidence that the sheep foetus does not utilize ketones because only small amounts cross the placenta (Alexander *et al.* 1969). However, the utilization could occur in the uterus and the placenta particularly since of the total O_2 consumption by the pregnant uterus 55% may be due to the foetus and 45% to the uterus and placenta (Meschia *et al.* 1979). Maximum utilization might, therefore, be expected at an arterial 3HB concentration of approximately 4 mM (i.e. at this concentration 3HB uptake can maximally account for 40% of the O_2 consumption. Further experiments are needed to verify these suggestions.

The findings are further complicated by a relatively low activity of 3-ketoacid CoA transferase (*EC* 2.8.3.5) in the sheep placenta (Edwards *et al.* 1977). Total placental O_2 consumption is probably 10 times higher than that of uterine muscle (Comline & Silver, 1974). Either the placental enzymic activity is underestimated or there is an as yet unidentified pathway for ketone body utilization by the pregnant uterus.

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