Enzymes Involved in Acetoacetate Formation in Various Bovine Tissues

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1. The activities of acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA synthase and lyase and acetoacetyl-CoA deacylase were measured in homogenates of samples of liver, rumen epithelium (long papillae), kidney and lactating mammary gland derived from slaughtered cows. 2. The activities of the four enzymes in bovine liver were similar to the activities previously reported for the corresponding enzymes in rat liver. 3. Acetoacetyl-CoA thiolase and hydroxymethylglutaryl-CoA synthase and lyase were present in rumen epithelium. The activities of the enzymes were all lower on a wet weight basis than in liver. Only very slight deacylase activity was detected. 4. Kidney contained acetoacetyl-CoA thiolase, hydroxymethylglutaryl-CoA lyase and acetoacetyl-CoA deacylase, but only trace amounts of hydroxymethylglutaryl-CoA synthase. 5. Mammary gland contained acetoacetyl-CoA thiolase and some hydroxymethylglutaryl-CoA lyase, but virtually no hydroxymethylglutaryl-CoA synthase or acetoacetyl-CoA deacylase. 6. Since physiologically significant ketogenesis probably occurs solely via the hydroxymethylglutaryl-CoA pathway, it is evident that, of the four tissues examined, such ketogenesis must be restricted to the liver and the rumen epithelium. 7. All the enzymes except hydroxymethylglutaryl-CoA lyase were also assayed in the four tissues derived from cows suffering from bovine lactational ketosis. Ketosis did not cause a statistically significant change in the activity of any of the enzymes measured. 8. Hepatic hydroxymethylglutaryl-CoA synthase and lyase were found to be associated mainly with the particulate fraction, as in the rat. A considerably greater proportion of these enzymes was found to be present in the cytoplasmic fraction from rumen epithelium, although it was not excluded that this was due to mitochondrial damage during homogenization. 9. Appreciable hydroxymethylglutaryl-CoA synthase was also present in epithelium from the dorsal region of the rumen, from the reticulum and from the omasum, but not from the abomasum.

Ketogenesis in the ruminant is not confined to the liver, but also takes place in the epithelial tissue of the rumen wall (see, e.g., Pennington, 1952; Smith, Goetsch & Jackson, 1961; Hibbitt & Baird, 1967; Jackson, Preston & Carter, 1968). Most studies of hepatic ketogenesis in ruminants have been performed with slice preparations (see, e.g., Smith *et al.* 1961; Baird, 1965), although the value of using liver slices for measuring rates of ketogenesis has now been questioned (Krebs, Wallace, Hems & Freedland, 1969). Recently, however, direct measurements have been made and these show the importance of hepatic ketogenesis in hypoglycaemic ketosis in sheep (Katz & Bergman, 1969). Information on ketogenesis in the rumen epithelium derives from work with pieces of epithelium or rumen wall, and from observations made on the

fate of volatile fatty acids arising within the rumen (Pennington, 1952; Annison, Hill & Lewis, 1957; Katz & Bergman, 1969). The lactating mammary gland of the cow has also been implicated as a site of ketogenesis by Kronfeld and co-workers, who found that there was a net output of acetoacetate by this organ during lactational ketosis (Kronfeld & Kleiber, 1959; Kronfeld, Raggi & Ramberg, 1968).

In the present work, the activities of the enzymes involved in the formation of acetoacetate from acetyl-CoA either via HMG-CoA* or by direct deacylation were measured by the methods of Williamson, Bates & Krebs (1968) in liver, rumen epithelium, mammary gland and kidney, derived from cows at slaughter. This work has the value of

* Abbreviation: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

identifying those tissues that have the necessary enzyme complement to enable them to form acetoacetate. It also provides information on the pathways by which acetoacetate may be formed in the various tissues and on the maximum possible rate of acetoacetate synthesis.

Enzyme activities were measured in tissues derived from healthy cows and from cows suffering from bovine lactational ketosis. No significant differences in enzyme activity were observed in tissues from the two sources. Of note was the finding that appreciable HMG-CoA synthase activity was present in rumen epithelium as well as in liver. It is concluded that, of the four tissues examined, significant ketogenesis is confined to the liver and the rumen epithelium and that acetoacetate formation in these two tissues occurs by the HMG-CoA pathway. A preliminary report of some of this work has been published (Baird & Hibbitt, 1969).

MATERIALS AND METHODS

Materials. Acetoacetyl-CoA was prepared by causing CoA to react with diketene [Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.] in a manner similar to that described by Simon & Shemin (1953) for the preparation of succinyl-CoA from CoA and succinic anhydride. Free acetoacetate was subsequently removed by extraction with diethylether (Williamson *et al.* 1968). The acetoacetyl-CoA in the preparation was measured by the method of Decker (1963). HMG-CoA was prepared from hydroxymethylglutaric acid (Calbiochem, Los Angeles, Calif., U.S.A.) by the method of Hilz, Knappe, Ringelmann & Lynen (1958). D(-)-3-Hydroxybutyrate dehydrogenase (EC 1.1.1.30) was prepared from *Rhodospseudomonas spheroides* by the method of Williamson, Mellanby & Krebs (1962), followed by further purification on DEAE-Sephadex A-50 (Pharmacia, Uppsala, Sweden) as recommended by Dr D. H. Williamson (personal communication). The cells were kindly provided by the Microbiological Research Establishment, Porton, Wilts., U.K. CoA, acetyl phosphate, phosphate acetyltransferase (EC 2.3.1.8) and NADH were all obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.

Animals and tissues. Tissues were obtained from Ayrshire and Friesian cows at slaughter. Up to 10 min elapsed between slaughter and removal of tissue from the animal. Tissue samples were then kept in ice for a maximum period of 60 min before being homogenized. In general the cows were lactating and were producing about 5–10 litres of milk/24 h at slaughter. Occasionally, non-lactating cows were used, although not for enzyme determinations in mammary-gland samples. Enzyme activities measured in the tissues from the non-lactating animals were found to be similar to the activities measured in the corresponding tissues from the lactating animals, so that results from lactating and non-lactating cows were subsequently pooled.

Cows suffering from untreated primary spontaneous ketosis of lactation were obtained from farms in Berkshire and the neighbouring counties. Diagnosis was based on criteria listed previously (Baird *et al.* 1968). In every in-

stance the blood acetoacetate concentration was measured at slaughter (Williamson *et al.* 1962), and the mean acetoacetate concentration $\pm$ S.D. for the five animals used was found to be $0.89 \pm 0.46 \mu\text{mol/ml}$ of blood.

Preparation of tissue extracts and determination of enzyme activities. Tissue extracts were prepared basically as described by Williamson *et al.* (1968) with the following minor modifications. In the preparation of extracts of 'whole homogenates', liver, lactating mammary gland and kidney were first minced with scissors. Rumen wall, derived from the ventral rumen and possessing long papillae, was thoroughly washed in homogenizing fluid to remove ruminal contents. The epithelium was then stripped off the underlying muscle layer and minced. After the mincing, the tissues were added to homogenization medium in the proportion of 1 g of tissue to 4 ml of medium and then homogenized in a Silverson homogenizer (Silverson Machines Ltd., London S.E.1, U.K.) at 0°C. The remainder of the preparation procedure was identical with that described by Williamson *et al.* (1968) except that the homogenate of rumen epithelium was sonicated for 45 s rather than 30 s.

Samples of epithelium obtained from the dorsal region of the rumen and possessing short papillae, reticulum epithelium, omasum epithelium and abomasum epithelium were also examined for the presence of HMG-CoA synthase. They were treated in the same manner as epithelium derived from the ventral region of the rumen.

For liver and rumen epithelium the intracellular distribution of enzymes of the HMG-CoA pathway was also determined. This involved preparation of soluble and particulate fractions as described by Williamson *et al.* (1968). Homogenization was performed in the Potter-Elvehjem glass-Teflon homogenizer cooled in an ice bath and, for liver, the subsequent procedure was that of Williamson *et al.* (1968). Some difficulty was experienced in homogenizing rumen epithelium by this method. To achieve homogenization it was found necessary to use only the papillae, and these were consequently clipped off the underlying membrane, which is rich in connective tissue. After being minced, the papillae were then homogenized in 9 vol. of homogenization medium. Homogenization required a maximum of 3 min. The homogenate still contained a considerable quantity of readily sedimentable material and this was removed by centrifuging at 800g for 10 min. The supernatant from this centrifugation was then taken to be the equivalent of an initial homogenate and was subsequently fractionated as described by Williamson *et al.* (1968).

Enzyme assays. Measurement of enzyme activity was in every case carried out with fresh tissue. HMG-CoA synthase (EC 4.1.3.5), HMG-CoA lyase (EC 4.1.3.4) acetoacetyl-CoA thiolase (EC 2.3.1.9) and acetoacetyl-CoA deacylase were all assayed basically as described by Williamson *et al.* (1968). For the assay of total HMG-CoA synthase and lyase activity 0.1 ml of liver 'whole-homogenate' extract and 0.2 ml of the corresponding extracts of the other three tissues were taken as a routine in the assay medium of 3.5 ml total volume. For determination of the subcellular distribution of these two enzymes 0.1 ml of each of the liver fractions derived from the 1-in-5 homogenate, and 0.4 ml of each of the rumen epithelium fractions derived from the 1-in-10 homogenate, were taken in 3.5 ml total volume. The volume of the assay

media used for the determination of acetoacetyl-CoA thiolase and deacylase activity was 3.0ml rather than 2.0ml. The volume of 'whole-homogenate' extract taken as a routine in the thiolase assay was then 0.01 ml for liver and kidney and 0.02ml for rumen epithelium and mammary gland. The volume taken in the deacylase assay was 0.1 ml for each tissue. Control assays were carried out to ensure that acetoacetate production and acetoacetyl-CoA consumption were directly proportional to the quantity of extract used and were linear with time, under the conditions employed. The nature of the assay for HMG-CoA synthase is such that the activity of this enzyme in the tissue extract must be less than that of either acetoacetyl-CoA thiolase or HMG-CoA lyase. This requirement was fulfilled for all the tissues examined.

Glutamate dehydrogenase (EC 1.4.1.2) was assayed by the method of Schmidt (1963). A 5 μ l portion of the appropriate liver fraction and 0.3 ml of the appropriate rumen epithelium fraction were taken in a total assay volume of 3 ml and read against controls lacking 2-oxoglutarate.

Statistics. The probability values (*P*) were obtained by Student's '*t*' test.

RESULTS

Enzyme activities in extracts of whole homogenates.

The activities of acetoacetyl-CoA thiolase, HMG-CoA synthase and acetoacetyl-CoA deacylase were assayed in extracts of whole homogenates prepared from liver, rumen epithelium, kidney and lactating mammary gland derived from both healthy and ketotic cows. HMG-CoA lyase activity was assayed only in whole homogenates from healthy cows.

Table 1 lists the activities of the four enzymes in whole-homogenate extracts prepared from liver and rumen epithelium. It was observed that, in the deacylase assay, higher activity was obtained if the reaction was started by the addition of tissue extract rather than by the addition of substrate. A possible explanation for this was that iodoacetamide partially inhibited deacylase activity in the extract during preincubation. For this reason the assay was

performed in both ways, i.e. that involving preincubation of extract with iodoacetamide and initiation of the reaction with substrate, and that involving initiation of the reaction with extract, and no preincubation.

Table 1 shows that the activities of the four enzymes in healthy bovine liver were very similar to those reported for rat liver (Williamson *et al.* 1968). As in rat liver, HMG-CoA synthase was present in lower activity than either the thiolase or HMG-CoA lyase and must therefore be the rate-limiting step for acetoacetate formation via HMG-CoA. The activity of the deacylase was at most only one-third of that of HMG-CoA synthase.

Table 1 also shows that all four enzymes were present in healthy rumen epithelium as well, although the activities were in general about one-fifth to one-quarter of those in the liver on a wet weight basis. HMG-CoA lyase was about two-thirds as active as the liver enzyme, however. The presence of HMG-CoA synthase together with the thiolase and the lyase indicates that acetoacetate formation can take place via HMG-CoA as in liver. Once again, HMG-CoA synthase is the rate-limiting enzyme for this pathway. Deacylase activity was very low in rumen epithelium and amounted to no more than one-quarter of the synthase activity.

Finally, it is evident from Table 1 that the activities of the three enzymes measured in liver and rumen epithelium from ketotic cows were very similar to those of the corresponding enzymes from the healthy cows. None of the slight differences in activity observed was found to be statistically significant.

Table 2 lists the activities of the four enzymes in kidney and lactating mammary gland. Kidney tissue was found to have a thiolase activity intermediate between that in rumen epithelium and that in liver. In contrast, HMG-CoA lyase activity, although present, was lower than in either of these two other tissues. However, kidney had the highest

Table 1. *Activities of enzymes involved in acetoacetate formation in liver and rumen epithelium from healthy and ketotic cows*

Enzyme activities represent the whole-homogenate activity (Williamson *et al.* 1968) and are expressed as μ mol of acetoacetate formed or acetoacetyl-CoA consumed/min per g wet wt. at 25°C. The values are means $\pm$ s.d. with the numbers of observations in parentheses.

	Liver		Rumen epithelium	
	Healthy	Ketotic	Healthy	Ketotic
Acetoacetyl-CoA thiolase	26.7 $\pm$ 2.7 (6)	26.6 $\pm$ 4.4 (5)	5.1 $\pm$ 0.9 (6)	4.6 $\pm$ 1.9 (5)
HMG-CoA synthase	1.73 $\pm$ 0.64 (7)	2.34 $\pm$ 0.56 (5)	0.43 $\pm$ 0.06 (5)	0.46 $\pm$ 0.19 (5)
HMG-CoA lyase	6.36 $\pm$ 0.68 (5)	Not assayed	4.46 $\pm$ 1.70 (3)	Not assayed
Acetoacetyl-CoA deacylase				
(i) not preincubated	0.50 $\pm$ 0.11 (4)	Not assayed	0.11 $\pm$ 0.08 (4)	Not assayed
(ii) preincubated	0.28 $\pm$ 0.04 (4)	0.30 $\pm$ 0.06 (3)	0.05 $\pm$ 0.01 (4)	0.05 $\pm$ 0.03 (4)

Table 2. *Activities of enzymes involved in acetoacetate formation in kidney and mammary gland from healthy and ketotic cows*

Enzyme activities represent the whole-homogenate activity (Williamson *et al.* 1968) and are expressed as μmol of acetoacetate formed or acetoacetyl-CoA consumed/min per g wet wt. at 25°C. The values are means $\pm$ s.d. with the numbers of observations in parentheses; when only two values were obtained the mean is given together with the individual values in square brackets.

	Kidney		Mammary gland	
	Healthy	Ketotic	Healthy	Ketotic
Acetoacetyl-CoA thiolase	10.4 $\pm$ 1.6 (5)	13.6 $\pm$ 2.8 (5)	1.2 $\pm$ 0.5 (4)	0.9 $\pm$ 0.3 (5)
HMG-CoA synthase	0.03 $\pm$ 0.03 (3)	0.05 (2) [0.04; 0.05]	None (2)	0.02 $\pm$ 0.01 (3)
HMG-CoA lyase	1.38 (2) [1.23; 1.53]	Not assayed	0.85 (2) [0.74; 0.96]	Not assayed
Acetoacetyl-CoA deacylase (i) not preincubated	0.88 $\pm$ 0.14 (4)	Not assayed	0.08 (2) [0.08; 0.08]	Not assayed
(ii) preincubated	0.28 $\pm$ 0.03 (4)	0.28 $\pm$ 0.04 (4)	0.08 $\pm$ 0.03 (4)	0.07 $\pm$ 0.03 (4)

Table 3. *Intracellular distribution of HMG-CoA synthase and lyase in liver and rumen epithelium of healthy cows*

Activities are expressed as μmol of acetoacetate formed or oxoglutarate reduced/min per g wet wt. at 25°C. The numbers of observations are given in parentheses; where two observations were made, the mean is given together with the individual values in square brackets.

	Liver			Rumen epithelium					
	Whole Homogenate	Particles (P)	Cytoplasm (C)	Percentage of (P + C) in cytoplasm sediment	800g supernatant	800g supernatant	Particles (P)	Cytoplasm (C)	Percentage of (P + C) in cytoplasm
HMG-CoA synthase	1.31 (2) [1.29; 1.33]	0.94 (2) [0.80; 1.08]	0.23 (2) [0.29; 0.17]	19.7	0.16 (1)	0.65 (2) [0.49; 0.81]	0.18 (2) [0.16; 0.20]	0.45 (2) [0.31; 0.58]	73.5
HMG-CoA lyase	5.85 (2) [5.08; 6.22]	4.65 (2) [3.8; 5.5]	0.65 (2) [0.64; 0.65]	12.3	0.95 (1)	4.96 (1)	1.11 (1)	3.18 (1)	74
Glutamate dehydrogenase (i) lactating cow	—	49.2 (1)	1.45 (1)	2.9	—	—	0.16 (2) [0.13; 0.19]	0.22 (2) [0.25; 0.18]	58
(ii) non-lactating cow	—	27.0 (1)	1.45 (1)	5.1	—	—	—	—	—

deacylase activity of all the tissues when activity was measured by the assay in which tissue extract was not preincubated with iodoacetamide. In contrast with liver and rumen epithelium, HMG-CoA synthase activity was very low in kidney tissue and the values obtained were virtually within the limits of experimental error. It therefore seems unlikely that acetoacetate formation can occur via HMG-CoA in this tissue.

Mammary gland had some thiolase and lyase activity and also a very low deacylase activity. HMG-CoA synthase activity seemed to be entirely absent from this tissue, however, again indicating that acetoacetate formation via HMG-CoA cannot take place.

As was the case with liver and rumen epithelium, the enzyme activities measured in kidney and lactating mammary gland from ketotic cows were very similar to those found in the healthy animals (Table 2). The very low values for HMG-CoA

synthase were not compared statistically. However, in contrast with the apparent total inactivity of the healthy tissue, there appeared to be trace amounts of HMG-CoA synthase in the ketotic mammary gland.

One may conclude from these studies on extracts of whole homogenates that, whereas all four tissues possess thiolase and lyase activity, the presence of appreciable HMG-CoA synthase is restricted to those tissues that have previously been assigned a ketogenic function, i.e. liver and rumen epithelium.

Subcellular distribution of HMG-CoA synthase and lyase. Studies aimed at elucidating the subcellular distribution of these two enzymes were carried out in the liver and rumen epithelium obtained from healthy cows (Table 3).

Table 3 shows that the distribution of hepatic HMG-CoA synthase and lyase between particulate and cytoplasmic fractions was similar to that observed in rat liver in that the major portion of both

Table 4. *Activities of HMG-CoA synthase in different regions of the bovine stomach*

Enzyme activities represent the whole-homogenate activity (Williamson *et al.* 1968) and are expressed as μmol of acetoacetate formed/min per g wet wt. at 25°C. The values are means $\pm$ s.d. with the numbers of observations in parentheses. * $P < 0.02$ and † $P < 0.01$, compared with the routine preparation.

Routine rumen epithelium preparation (long papillae)	Rumen epithelium from dorsal region (short papillae)	Reticulum epithelium	Omasum epithelium	Abomasum epithelium
0.39 $\pm$ 0.13 (5)	0.16 $\pm$ 0.05 (4)*	0.32 $\pm$ 0.08 (4)	0.27 $\pm$ 0.01 (3)	0.03 $\pm$ 0.01 (3)†

enzymes was present in the particulate fraction (Williamson *et al.* 1968). As in the rat, there was definite activity in the cytoplasmic fraction. This activity may not be due solely to mitochondrial damage because at least 95% of the glutamate dehydrogenase activity was associated with the particulate fraction.

With rumen epithelium, activity determinations were carried out on the 800g sediment, the 800g supernatant and on the particulate and cytoplasmic fractions derived from the 800g supernatant. The greater part of the total enzymic activity was still present after removal of the 800g sediment. Further, of the activity in the 800g supernatant, about 74% remained in the cytoplasmic fraction after centrifugation at 30000g. However, this distribution may have arisen as a result of mitochondrial damage. The use of glutamate dehydrogenase as a marker for mitochondrial sap is not satisfactory in this instance owing to the much lower activity of this enzyme in rumen epithelium than in liver. In addition, nothing is known of the distribution of glutamate dehydrogenase in rumen epithelium. Nevertheless, values for the trace amounts of glutamate dehydrogenase present in the particulate and cytoplasmic fractions given in Table 3 clearly show that over 50% of the activity is in the cytoplasmic fraction.

Presence of HMG-CoA synthase in other regions of the bovine stomach. In conjunction with the finding that rumen epithelium could form ketone bodies from butyrate, Pennington (1952) observed that the epithelia of the reticulum and the omasum also had this property, whereas that of the abomasum did not. It was therefore decided to determine whether the possession of HMG-CoA synthase by the various regions of the bovine stomach corresponded to their ketogenic activity. The results of this series of experiments are recorded in Table 4.

As Table 4 shows, abomasum epithelium had only very slight HMG-CoA synthase activity. Appreciable activity was, however, present in epithelia from the other regions of the stomach, although the activity in the epithelium from the dorsal region of the rumen was also significantly lower than in the routine preparation. It is therefore evident that

there is a close correlation between the possession of appreciable HMG-CoA synthase activity and the ability to perform ketogenesis, as reported by Pennington (1952).

DISCUSSION

HMG-CoA synthase and ketogenesis. This work clearly shows that significant quantities of HMG-CoA synthase are present in the epithelia of the rumen, reticulum and omasum of the cow. This is the first demonstration in any mammalian species of the presence of this enzyme at such a high activity in an extrahepatic tissue.

The assay for deacylase activity involves the use of acetoacetyl-CoA concentrations that are grossly higher than those calculated to be present in rat liver, at least (Greville & Tubbs, 1968), and probably in other tissues as well. This fact, taken together with the rather low deacylase activities measured in the four tissues, must cast doubt on the physiological significance of direct deacylation as a means of acetoacetate formation (see also Williamson *et al.* 1968). It may, in fact, be concluded that the major, if not the only, pathway of physiological ketone-body formation is via HMG-CoA. Of the four tissues examined, only liver and rumen epithelium possessed appreciable quantities of HMG-CoA synthase. It therefore seems likely that true ketogenesis is restricted to these two tissues *in vivo*. This conclusion correlates satisfactorily with the considerable volume of evidence in support of a ketogenic role for both liver and rumen epithelium in ruminants.

McGarry & Foster (1969a) have reported the presence of significant quantities of HMG-CoA synthase activity in several extrahepatic tissues in the rat. These tissues included primarily kidney and heart. Expressed in the same terms as those used in the present study, the mean activity of HMG-CoA synthase found by these authors in rat kidney amounted to about 0.12 $\mu\text{mol}/\text{min}$ per g at 25°C, assuming Q_{10} 2. No activity of such magnitude was ever observed for bovine kidney, suggesting a possible difference in the precise nature of the assay used in the two instances. One explanation is that a portion of the acetoacetate production attributed

by McGarry & Foster (1969a) to kidney and heart HMG-CoA synthase may in fact have arisen by the reversal of acetoacetate activation via the thiolase and 3-oxo acid CoA-transferase (EC 2.8.3.5) reactions. This possibility was in fact discussed by McGarry & Foster (1969a).

Liver enzymes. It is of note that the activities *in vitro* of the four enzymes involved in acetoacetate formation are very similar in both cow and rat liver. There is also close similarity in the distribution of HMG-CoA synthase and HMG-CoA lyase between mitochondria and cytoplasm in the two species (cf. Williamson *et al.* 1968). These facts taken together suggest that the circumstances under which ketogenesis occurs may also be comparable. The main source of ketone bodies in the rat is plasma non-esterified fatty acids (see Greville & Tubbs, 1968). Work by Katz & Bergman (1969) has shown that plasma non-esterified fatty acids are probably the main substrate for hepatic ketogenesis in ruminants as well. Thus it appears that in the sheep only limited hepatic ketogenesis takes place in the fed animal. The main source of ketone bodies under these circumstances is the rumen epithelium and here the substrate is butyrate. Little butyrate ever reaches the liver, however (e.g. Bergman, Roe & Kon, 1966). Only during hypoglycaemic ketosis, induced by starvation, does hepatic ketogenesis assume major importance and in this case the principal substrate is plasma non-esterified fatty acids.

Rumen epithelium enzymes. HMG-CoA synthase was the least active of the enzymes involved in the formation of acetoacetate by the rumen epithelium via the HMG-CoA pathway. It may therefore be concluded that this enzyme is rate-controlling for physiological ketogenesis in rumen epithelium as it is in liver. On the basis of the distribution of label in the acetoacetate formed after administration of butyrate samples labelled in different carbon atoms, Hird & Symons (1961) deduced that acetoacetate synthesis in rumen epithelium probably took place mainly via HMG-CoA. The demonstration of the presence of the enzymes of the HMG-CoA pathway confirms this conclusion.

Mammary-gland enzymes. Kronfeld *et al.* (1968) have found that the lactating mammary gland of the cow is a source of net acetoacetate formation during bovine ketosis. However, the output of acetoacetate is approximately matched by an increase in the uptake of hydroxybutyrate. There is therefore no net formation of total ketone bodies and it seems most likely that net output of acetoacetate under these circumstances is due to a shift in the redox state rather than to active ketogenesis.

Slices of lactating mammary gland derived from either healthy or ketotic cows produce only trace amounts of ketone bodies from endogenous sub-

strates. Nevertheless, addition of malonate causes an increased production of acetoacetate and when acetate is added as well the amount of acetoacetate formed is even greater (Baird, 1965, and unpublished work). The extent of the ketogenesis is similar for both healthy and ketotic tissues, however, and it seems that ketogenesis in this case is due to the reversal of acetoacetate activation (McGarry & Foster, 1969a; Weidemann & Krebs, 1969).

Bovine ketosis. Direct measurements have shown that in the starved pregnant sheep hepatic ketogenesis increases and becomes of major importance (Katz & Bergman, 1969). On the basis of this observation it is reasonable to assume that hepatic ketogenesis also increases during bovine lactational ketosis. Metabolic changes occurring in the liver during the disease also support this view (Baird *et al.* 1968). It is less easy to speculate about changes in the rate of ketogenesis from bovine rumen epithelium during ketosis. However, it is evident from the results that any changes that do occur are not accompanied in either tissue by significant changes in the total activities *in vitro* of the enzymes involved. Similar observations have been made previously with rat liver (Williamson *et al.* 1968; McGarry & Foster, 1969b).

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