Utilization of Volatile Fatty Acids in Ruminants. IV. Relative Activities of Acetyl CoA Synthetase and Acetyl CoA Hydrolase in Mitochondria and Intracellular Localization of Acetyl CoA Synthetase

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The relative activity of acetyl CoA hydrolase and acetyl CoA synthetase was investigated in mitochondria from bovine liver, heart, kidney, lung, brain, mammary gland, and skeletal muscle. Acetyl CoA hydrolase activity is high in liver, mammary gland, kidney, and brain. The enzyme is much less active in heart, lung, and skeletal muscle. Hydrolase activity relative to synthetase activity is high in liver and brain. The synthetase activity is much greater than the hydrolase activity in the other tissues. The intracellular localization of acetyl CoA synthetase was determined in bovine heart, kidney, mammary gland, liver, and lung. Two-thirds of the enzyme is localized in the cytoplasm and one-third in the mitochondria in heart and mammary gland. Acetyl CoA synthetase activity in kidney is equally divided between mitochondria and cytoplasm. The enzyme in lung and liver is localized predominantly in the mitochondria. The relative activities of acetyl CoA synthetase and acetyl CoA hydrolase in the cell no doubt serve an important control function by regulating the cellular concentration of acetyl CoA.

Acetate is produced in the rumen by microbial fermentation of feeds and serves as a major substrate for ruminant tissues. We are interested in studying factors that regulate acetate utilization by ruminants. In a previous publication we proposed that the acetate activation reaction catalyzed by acetyl coenzyme A synthetase [acetate:CoA ligase (AMP) EC 6.2.1.1] is an important control point in acetate utilization by ruminants and we have demonstrated a difference in the activity of this enzyme in mitochondria from several tissues (Cook *et al.*, 1969). A second reaction that may be important in the control of acetate metabolism is the acetate deactivation reaction catalyzed by acetyl CoA hydrolase (EC 3.1.2.1). Acetyl CoA synthetase and acetyl CoA hydrolase can act as antagonists and in this manner regulate the rate acetate is utilized by the cell.

Basic data is needed on the relative activity of the two enzymes in ruminant tissues and on the intracellular localization. In this paper we report the relative activities of the enzymes in the mitochondria from ruminant tissues and the intracellular localization of acetyl CoA synthetase in bovine heart, kidney, mammary gland, liver, and lung.

EXPERIMENTAL PROCEDURES

Source of Ruminant Tissues. The relative activities of acetyl CoA synthetase and acetyl CoA hydrolase were determined in the mitochondria from tissues from a yearling Holstein steer. The intracellular localization of acetyl CoA synthetase was determined in heart, kidney, liver, and lung from a yearling Holstein heifer and in lactating mammary gland from a Holstein cow.

Tissue Preparation. Acetyl CoA synthetase and acetyl CoA hydrolase were assayed in steer mitochondrial extract prepared as previously described (Cook *et al.*, 1969). Acetyl CoA hydrolase from lactating bovine mammary gland mitochondria was partially purified by ammonium sulfate fractionation as described by Hele (1954). The final ammonium sulfate fraction was dissolved in 0.02 *M* $KHCO_3$ buffer pH 8.0, dialyzed, and 2 g of protein were added to the top of a Sephadex G-100 column (2.5 cm × 35 cm). The column was eluted with 0.02 *M* $KHCO_3$ buffer pH 8.5, containing 0.5 m*M* EDTA and 3 m*M* mercaptoethanol. Five-milliliter fractions were collected. The tubes containing acetyl CoA hydrolase activity were combined and rechromatographed on a TEAE cellulose column (2 × 40 cm) using the buffer described above and a KCl gradient of 0.05 *M* KCl to 1.4 *M* KCl. Using this procedure acetyl CoA hydrolase was free of acetyl CoA synthetase activity.

The tissues taken from Holstein heifers and mammary gland from a cow were chilled and then ground in a meat grinder. The tissues were then homogenized in two volumes of 0.13 *M* KCl in a Waring blender for 20 sec at low speed and 20 sec at half-maximal speed. The homogenate was then fractionated according to the scheme shown in Figure 1 using a Sorvall RC2B centrifuge and a Spinco Model L ultracentrifuge. All operations were carried out at 5° C. The material sedimenting at 20,000 × *g* is referred to as the mitochondrial pellet, although it is recognized that this material may contain lysosomes. The activity of acetyl CoA synthetase in the various fractions was assayed as previously described (Cook *et al.*, 1969). Protein determinations were by the method of Lowry *et al.* (1951).

Enzyme Assays. Acetyl CoA synthetase was assayed as described by Cook *et al.* (1969). Acetyl CoA hydrolase was assayed by measuring the appearance of the free sulfhydryl group of coenzyme A using nitroprusside. The reaction mixture for the hydrolase assay contained in a total volume of 0.15 ml, 0.05 *M* tris buffer pH 8.6, Mg^{+2} (2.5 μmol) acetyl CoA (1.0 μmol) and protein (0.05 to 0.5 mg). The tubes were incubated 10 min at 37° C and the reaction was stopped by adding the reagents for the nitroprusside reaction. The

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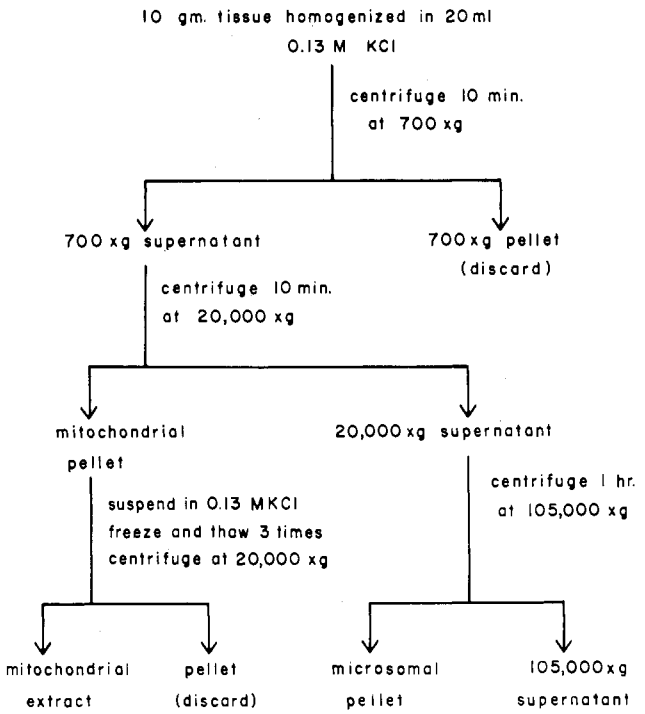

Figure 1. Tissue fractionation scheme

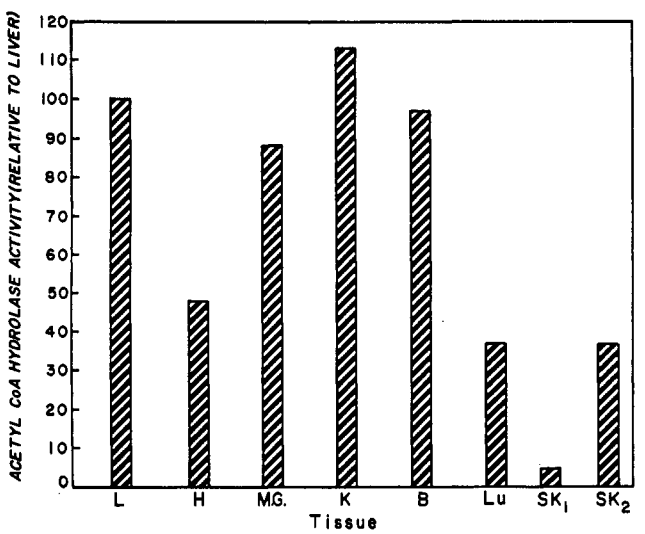

Figure 2. Acetyl CoA hydrolase activity in mitochondria from various tissues relative to liver. Specific enzyme activity in liver was 3.41 units/mg protein. One unit is defined as 1 μmol of substrate reacting per hour. L = liver, H = heart, M.G. = bovine mammary gland, K = kidney, B = brain, LU = lung, SK_1 = skeletal muscle from the shoulder, SK_2 = muscle from the loin

enzyme activities were linear with time and linear over the range of protein concentrations used in the reaction mixture for all tissues studied.

RESULTS

Comparison of Acetyl CoA Synthetase and Hydrolase in Mitochondria. The activity of acetyl CoA hydrolase in mitochondria from various tissues is presented in Figure 2. The activity of this enzyme is high in liver, mammary gland, kidney, and brain. The activity in heart, lung, and loin muscle is 40 to 50% of that found in the liver. Skeletal muscle from the shoulder region showed marginal enzyme activity.

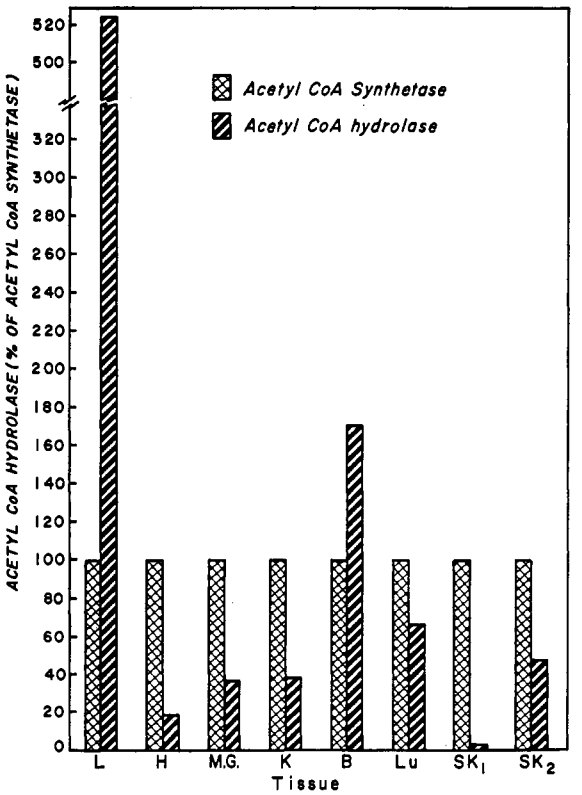

Figure 3. Activity of acetyl CoA hydrolase relative to acetyl CoA synthetase in mitochondria from different tissues

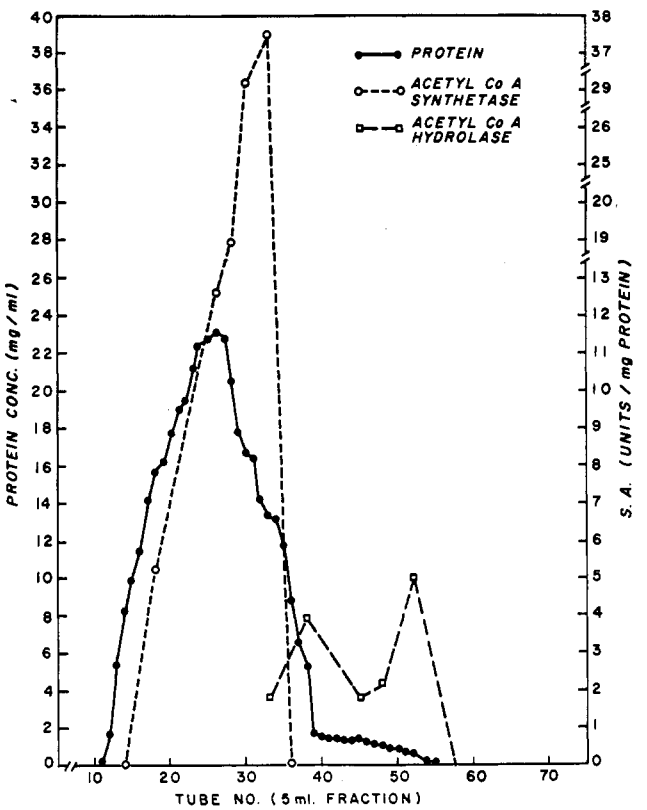

Figure 4. Separation of acetyl CoA synthetase from acetyl CoA hydrolase using Sephadex G-100. 2.2 g of ammonium sulfate fraction 3[2] from bovine mammary gland mitochondria were chromatographed

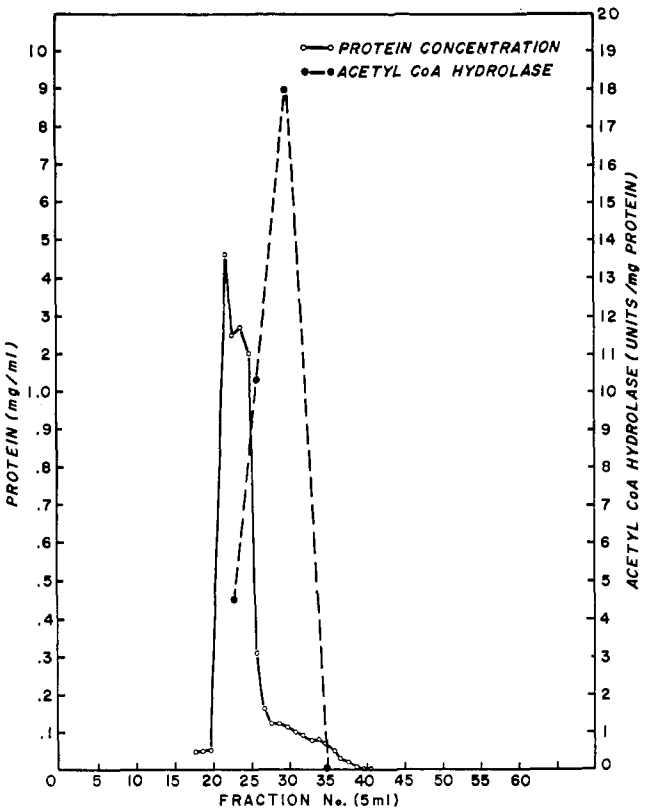

Figure 5. Chromatography of acetyl CoA hydrolase on TEAE cellulose. Tubes containing acetyl CoA hydrolase from the Sephadex G-100 column were pooled and chromatographed

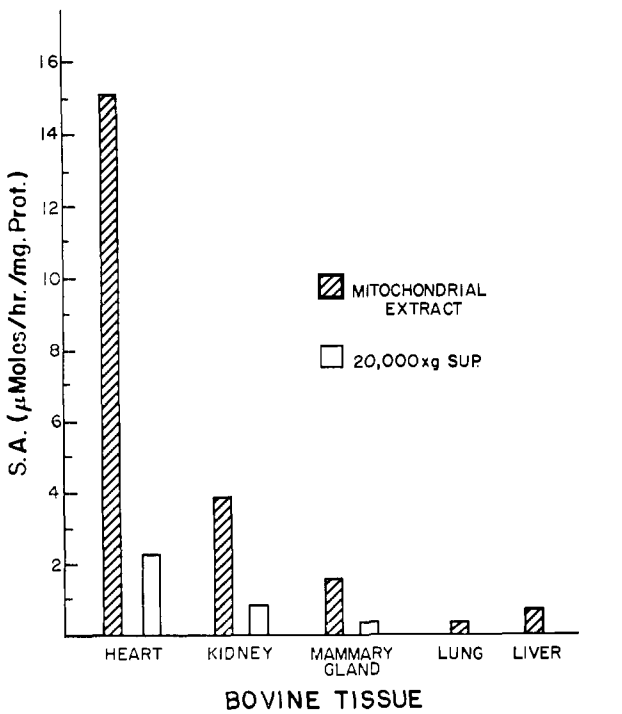

Figure 6. Specific activity (S.A.) of acetyl CoA synthetase in the mitochondrial extract and 20,000 × *g* supernatant

A comparison of the relative activity of acetyl CoA synthetase and acetyl CoA hydrolase in the various tissues is presented in Figure 3. In liver and brain acetyl CoA hydrolase activity is far greater than acetyl CoA synthetase. However, the hydrolase activity relative to the synthetase activity is low in other tissues.

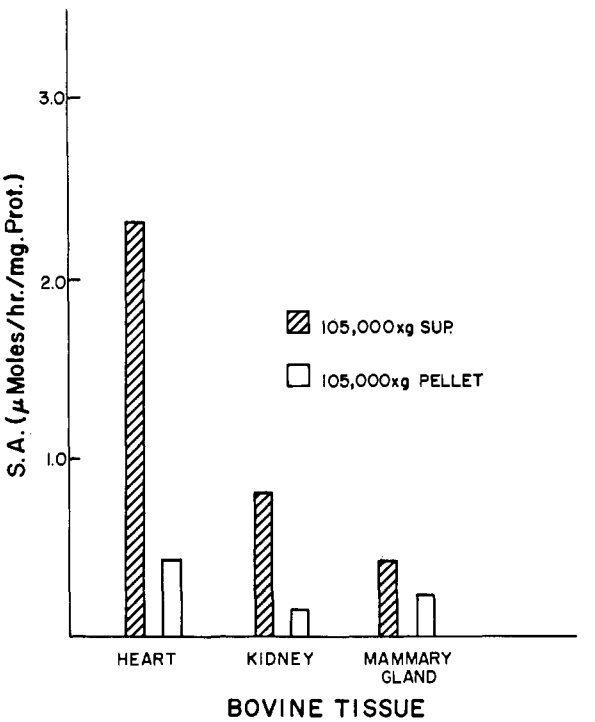

Figure 7. Specific activity of acetyl CoA synthetase in the 105,000 × *g* supernatant and 105,000 × *g* pellet

Table I. Effect of ATP on Acetyl CoA Hydrolase Activity from Two Ruminant Tissues

Incubation mixture	Liver ΔA_{250} nM	Mammary gland ΔA_{250} nM
Complete (ATP, 1.0 μmol)	0.09	0.00
Without ATP	0.16	0.18

The data in Figures 4 and 5 show that acetyl CoA hydrolase can be separated from acetyl CoA synthetase activity using Sephadex G-100. The tubes containing acetyl CoA hydrolase activity from both the Sephadex and TEAE cellulose columns did not contain acetyl CoA synthetase activity.

It was shown that in ruminant tissues ATP (adenosine triphosphate) markedly inhibits the activity of acetyl CoA hydrolase. The data in Table I show this effect for two ruminant tissues, bovine liver, and mammary gland. There is a 60% increase in liver mitochondrial acetyl CoA hydrolase activity when ATP is not present in the incubation mixture. The enzyme from mammary gland is not active in the presence of ATP.

Intracellular Localization of Acetyl CoA Synthetase. Acetyl CoA synthetase activity could not be detected in any of the fractions obtained from skeletal muscle and could be detected only in the mitochondrial extract from lung and liver. Also, enzyme activity could not be measured in intact mitochondria with the assay procedure used. The total enzyme activity measured in the 700, 20,000, and 105,000 × *g* supernatant was in good agreement.

The specific activity of the enzyme in the particulate and soluble fractions is higher in the mitochondrial extract than in the 20,000 or 105,000 × *g* supernatant (Figures 6 and 7). Enzyme activity could be measured in the 105,000 × *g* pellet (Figure 7). However, the activity was very low. There was

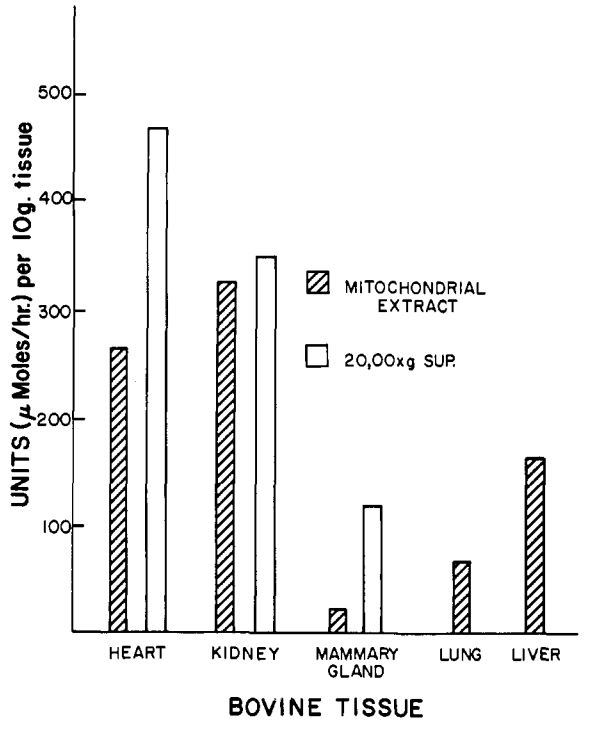

Figure 8. Total acetyl CoA synthetase activity in the mitochondrial extract and 20,000 × *g* supernatant

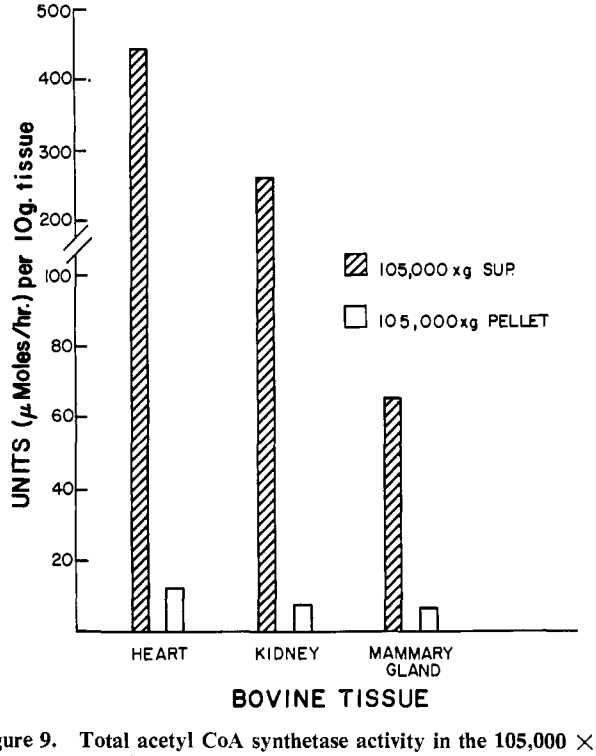

Figure 9. Total acetyl CoA synthetase activity in the 105,000 × *g* supernatant and 105,000 × *g* pellet

no enzyme activity in the soluble or microsomal fractions from lung and liver.

When the enzyme activity is expressed as units per 10 g of tissue, it can be seen that about 60% of the total enzyme activity in heart is in the 20,000 × *g* supernatant and 40% is in the mitochondria (Figure 8). In kidney, the enzyme activity is about equally divided between the mitochondria and supernatant. All of the enzyme activity in lung and liver was found in the mitochondria (Figure 8).

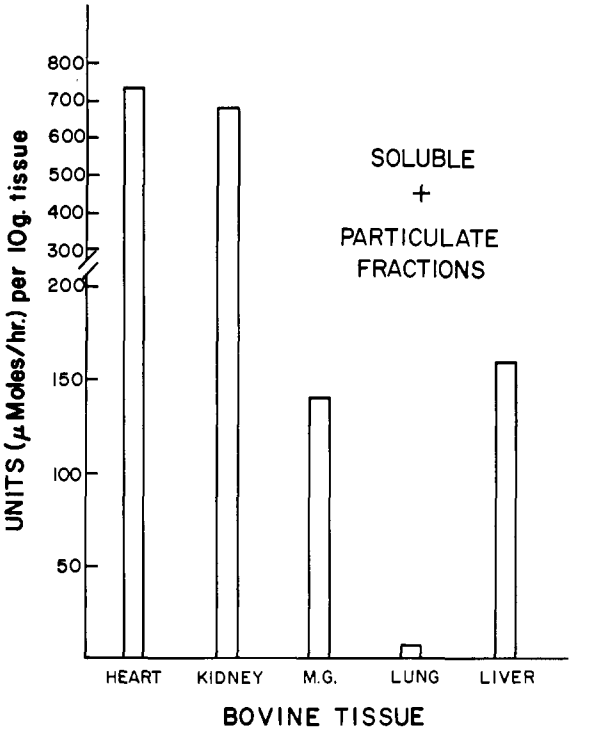

Figure 10. Summary of the activity of acetyl CoA synthetase in the soluble and particulate fractions of the cell

The data in Figure 9 show that the enzyme activity in the microsomes (105,000 × *g* pellet) is very low compared to the activity in the mitochondrial extract or the other fractions. The exception is lung where the activity found in the mitochondrial extract is similar to that found in the microsomes from other tissues.

Total enzyme activity (soluble plus particulate fractions) is summarized in Figure 10. Heart and kidney have about the same total enzyme activity per unit weight of tissue. Mammary gland and liver have approximately the same total enzyme activity and this activity is about one-half that found in heart and kidney. However, the activity present in the mammary gland depends on the stage of lactation. Our recent work (Cook, 1971) shows that the activity is very high at the beginning of lactation and declines to almost undetectable amounts at the end of lactation. The mammary gland sample analyzed here was taken from a cow in late lactation. The total cellular activity is approximately five times higher in early lactation. Consequently, in early lactation there could be as much activity in the mammary gland as in the other tissues studied. There is no evidence that the relative proportion of acetyl CoA synthetase in the mitochondria and cytoplasm changes with advancing lactation.

DISCUSSION

Acetyl CoA hydrolase and several other acyl CoA hydrolases have been demonstrated in mammalian tissues (Dekker *et al.*, 1958; Gergely *et al.*, 1952; Porter and Long, 1958; Redina and Coon, 1957; Srere *et al.*, 1959). The probable role of these hydrolytic enzymes in metabolism has been discussed by Srere *et al.* (1959). Acetyl CoA hydrolase could function to transfer acetate or CoA to an acceptor molecule such as carnitine or another fatty acid to form an intermediate in a metabolic pathway. Another function for the enzyme would be to regulate the concentration of acetate of CoA in the cell. The enzyme could have different functions in differ-

ent tissues. In liver where acyl carnitine derivatives are important in metabolism, the hydrolase could be involved in transfer reactions. On the other hand, acetyl CoA hydrolase in brain may function to maintain an optimum level of CoA needed for glucose oxidation.

Acetyl CoA hydrolase activity relative to the synthetase activity is low in heart, kidney, mammary gland, and skeletal muscle. However, the enzyme could have a regulatory role in these tissues. Porter and Long (1958) have shown that palmityl CoA hydrolase can have a regulatory role in fatty acid synthesis. Acetyl CoA hydrolase may play an important regulatory role in fatty acid synthesis in ruminant mammary glands.

In addition, ATP may be involved in some way in the control of acetyl CoA hydrolase activity and in this manner influence the utilization of acetate and fatty acid synthesis.

Acetyl CoA synthetase is localized predominantly in the cytoplasm in heart and mammary gland and is approximately equally divided between mitochondria and cytoplasm in kidney. The enzyme is localized predominantly in the mitochondria in lung and in liver. The presence of acetyl CoA synthetase in the cytoplasm in ruminants is of biological significance. This provides a direct source of acetyl CoA in the cytoplasm for synthetic reactions. Consequently, the need for a source of acetyl CoA from the mitochondria *via* the citrate cleavage pathway is eliminated. This is particularly important in the ruminant mammary gland where the activity of the citrate cleavage enzyme is low. In this case, acetate, activated in the cytoplasm, is a major precursor for the synthesis of fat.

The absence of an active acetyl CoA synthetase in the liver cytoplasm suggests that if this tissue is active in lipid synthesis, substrates other than acetate must serve as precursors. It has been established that ruminant liver is the major site of propionate metabolism. Also, butyrate can be metabolized by the liver (Cook and Miller, 1965; Cook *et al.*, 1969; Cook, 1970). Consequently, propionate and butyrate may be important precursors for lipid synthesis in ruminant liver.

Acetyl CoA hydrolase was not measured in the cytosol in the studies reported here. However, a study has been completed in which the enzyme was found in the cytosol of 13 different goat tissues. These studies will be reported later. Acetyl CoA hydrolase has recently been found in the cytosol of several bovine tissues (Cook, 1971).

There is no doubt that the relative activity of acetyl CoA synthetase and acetyl CoA hydrolase can play a major role in the control of metabolism by regulating the cellular concentrations of acetyl CoA and acetate. Acetyl CoA regulates carbohydrate metabolism by activating pyruvate carboxylase and by inhibiting pyruvate dehydrogenase (Garland *et al.*, 1968; Williamson *et al.*, 1968). It is well known that acetyl CoA carboxylase and the citrate cleavage enzyme are regulatory enzymes in lipid synthesis. The relative activities of acetyl CoA synthetase and acetyl CoA hydrolase offer an additional regulatory mechanism that may be more significant.

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