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Utilization of Volatile Fatty Acids in Ruminants. III. Comparison of Mitochondrial Acyl Coenzyme A Synthetase Activity and Substrate Specificity in Different Tissues*

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ABSTRACT: Studies were undertaken to establish the important rate-limiting steps controlling acetate, propionate, and butyrate metabolism in ruminants. As an initial approach to this problem, the activity of acetyl, of propionyl, and of butyryl coenzyme A synthetase in mitochondria from various ruminant tissues was studied. Mitochondria were frozen and thawed three times to solubilize the enzymes. The acyl coenzyme A synthetase preparation was partially purified and its substrate specificity was studied. The results show that the activity of the enzyme active on acetate is high in highly aerobic tissues such as heart and mammary gland and low in liver, in brain, in aorta, in skeletal muscle, and in rumen muscle. In heart, in mammary gland, in kidney, and in testes, the enzyme preparations were all about equally active on acetate and on propionate. In liver the enzyme preparation was equally active on propionate and on butyrate and least active on acetate. The enzyme from rumen epithelium was most active on butyrate, less active on propionate, and least active on acetate. Lung differs from all the other tissues in that the enzyme is very active on propionate and shows equal but lower activity for acetate and for butyrate. Marginal enzyme activity was found in skeletal muscle, in rumen muscle, in brain, and in aorta. The results offer an explanation for the preferential utilization of acetate by extrahepatic tissues, propionate by the liver, and butyrate by rumen epithelium. Butyrate, if present in peripheral blood, has a pharmacological effect on the pancreas. The ruminant has a unique mechanism for preventing butyrate appearance in peripheral blood, *i.e.*, a high butyryl coenzyme synthetase activity in rumen epithelium ensures rapid activation of butyrate for subsequent oxidation to β-hydroxybutyrate by the epithelium. If some butyrate escapes metabolism by the rumen epithelium, it is readily removed from portal blood by the liver where a high butyryl coenzyme A synthetase activity ensures rapid activation and subsequent metabolism to ketone bodies. Substrate specificity studies show that in addition to the three well-known acyl coenzyme A synthetases active on straight-chain fatty acids (acid:ligase (adenosine monophosphate), EC 6.2.1.1, 6.2.1.2, and 6.2.1.3) there are at least three other acyl coenzyme A synthetases with distinct substrate specificities. The acyl coenzyme A synthetase from sheep liver is specific for C_3 to C_7 straight-chain fatty acids. The acyl coenzyme A synthetase from sheep kidney is specific for C_2 to C_7 straight-chain fatty acids. Sheep lung has an acyl coenzyme A synthetase specific for propionate. This work along with earlier studies by others would suggest that there are at least six acyl coenzyme A synthetases in animal tissues with varying substrate specificities.

This laboratory is engaged in a study of control of acetate, of propionate, and of butyrate utilization in ruminants. These short-chain acids are produced in the rumen by microbial fermentation of carbohydrates and serve as primary metabolic substrates in ruminants. It has been shown that under normal conditions the ruminant liver is the major body organ metabolizing propionate. Most of the acetate present in portal blood is not removed by the liver and is available to extrahepatic tissues (Cook and Miller, 1965; Black *et al.*, 1961). Butyrate is not present in significant amounts in rumen vein or in portal blood (Cook and Miller, 1965; Annison *et al.*, 1957) and is believed to be oxidized to β-hydroxybutyrate on absorption from the rumen (Ramsey and Davis, 1965).

* From the Department of Dairy Science, University of Idaho, Moscow, Idaho 84843. *Received September 30, 1968.* Part of the data in this publication are from a thesis submitted by S.-C. C. L. to the Graduate School in partial fulfillment of the requirements for the Master of Science degree. Some of the data in this paper was presented in a preliminary communication (Cook *et al.*, 1964; Cook and Wiese, 1966).

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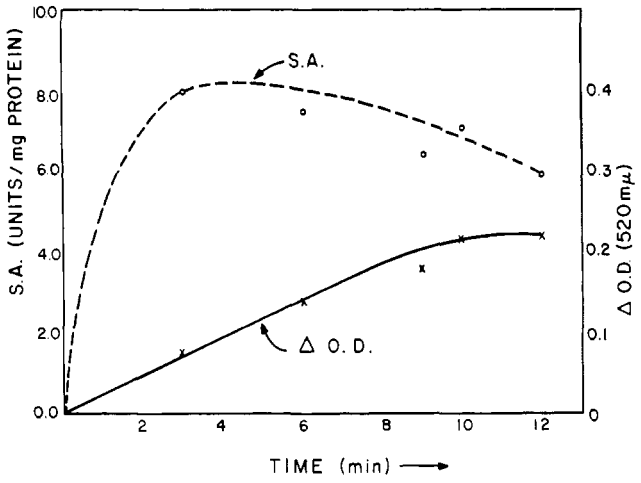

FIGURE 1: Effect of incubation time on the linearity of the acetyl-CoA synthetase reaction. The purified enzyme preparation from goat testicle was used for this study.

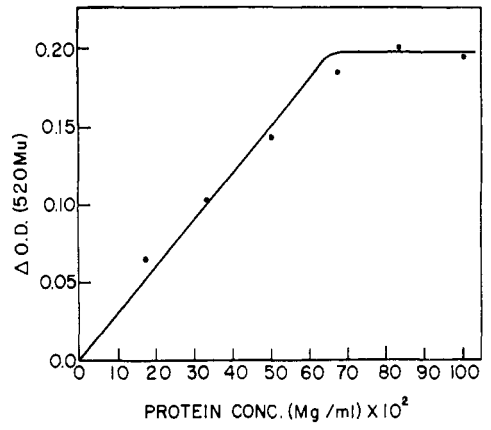

FIGURE 2: Effect of protein concentration on the linearity of the acetyl-CoA synthetase reaction. The enzyme from mammary gland was used for this study.

Preferential utilization of volatile fatty acids by ruminant tissues may be related to the tissue levels of fatty acid activating enzymes. Also, acyl-CoA synthetases are generally considered to be nonequilibrium enzymes and, therefore, can play a role in control of over-all cell processes. Consequently, the acetate, the propionate, and the butyrate activation reactions can function as important regulatory steps in short-chain fatty acid metabolism.

The purpose of the experiments reported in this communication was to study the activity of acetyl-, of propionyl- and of butyryl-CoA synthetase in ruminant tissues as a initial approach to the problem of elucidating control mechanisms regulating short-chain fatty acid metabolism.

Experimental Section

Enzyme Assay. The acyl-CoA synthetases were assayed by measuring the disappearance of the free sulfhydryl group of coenzyme A according to the procedure of Mahler *et al.* (1953). One enzyme unit is defined as 1 μmole of CoA reacting in 60 min. Specific activity is in units per milligram of protein. Protein was determined by the method of Lowry *et al.* (1951). A 10-min incubation time was chosen in order to ensure an optimum change in optical density between control and experimental tubes (Figure 1). Enzyme activity was linear over the range of protein concentrations used in the reaction mixture for all tissues studied (an example is shown in Figure 2).

Purification of Acetyl-CoA Synthetase. The method for isolation of mitochondria was similar to that reported by Hele (1954) for isolation of heart muscle mitochondria. The material sedimenting in the Sharples centrifuge is defined as the mitochondrial pellet. Although the purity of the mitochondrial pellet was not rigorously established, all ruminant tissues studied possess mitochondria including rumen epithelium where electron micrographs have demonstrated an abundant supply (Hyden and Sperber, 1965). The enzymes were liberated from the mitochondrial pellet by freezing and thawing and partially purified according to the procedure of Hele (1954).

Results and Discussion

The activity of acetyl-, of propionyl-, and of butyryl-CoA synthetase was measured in the mitochondrial extract and purified preparations. Also, both the crude mitochondrial extracts and purified preparations were used for substrate specificity studies. The relative enzyme activities from both

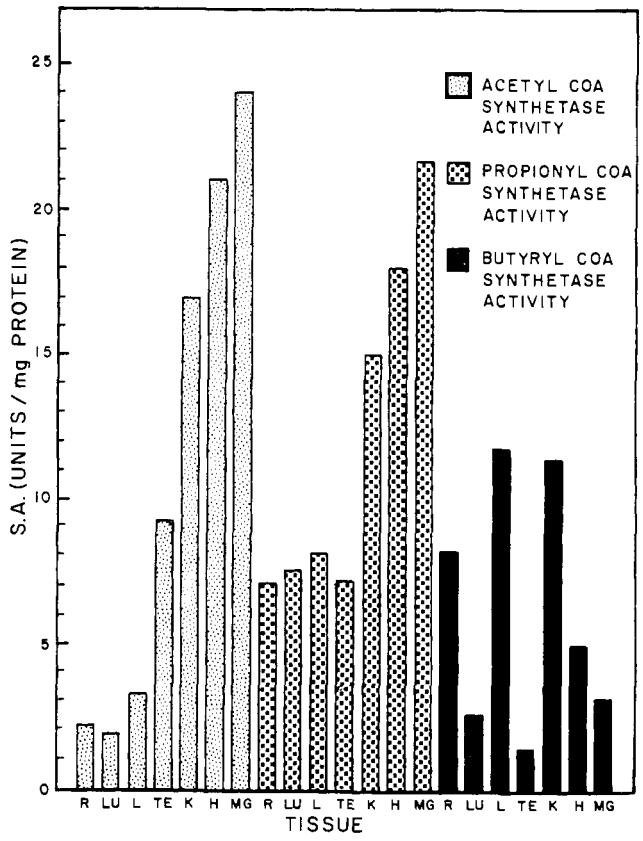

FIGURE 3: Summary of the activity of acetyl-, of propionyl-, and of butyryl-CoA synthetase from mitochondria of various ruminant tissues (R = rumen, LU = lung, L = liver, TE = testicle, K = kidney, H = heart, and MG = mammary gland).

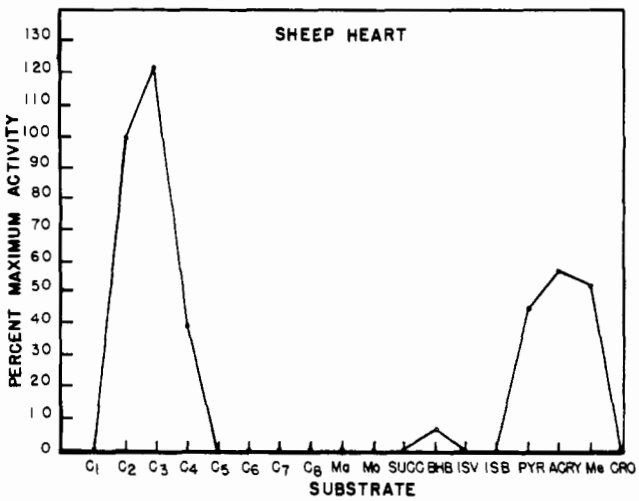

FIGURE 4: Substrate specificity of the enzyme preparation from sheep heart. The purest preparations from sheep heart as well as from mammary gland, from liver, from lung, from testes, and from kidney were used in the studies (Ma = malate, Mo = malonate, SUCC = succinate, BHB = betahydroxybutyrate, ISV = isovalerate, ISB = isobutyrate, PYR = pyruvate, ACRY = acrylate, Me = maleate, and CRO = crotonate).

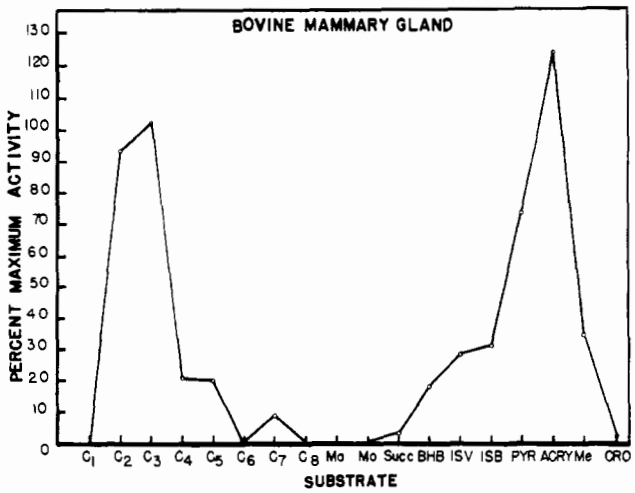

FIGURE 6: Substrate specificity of the enzyme preparation from bovine mammary gland.

fractions were similar. Studies using the purified enzyme preparations are reported.

Acetyl-CoA synthetase (Figure 3) is high in kidney, in heart, in mammary gland, and in testes but low in liver, in rumen epithelium, and in lung. However, all tissues have a very active propionyl-CoA synthetase. Butyryl-CoA synthetase activity is high in rumen epithelium, in liver, and in kidney and is low in heart, in lung, in mammary gland, and in testes. With the exception of the enzyme isolated from lung, the enzyme preparations were either about equally active on acetate and on propionate and least active on butyrate or equally active on propionate and on butyrate and least active on acetate. Lung differs from all the other tissues in that the enzyme is specific for propionate.

Acetyl-, propionyl-, and butyryl-CoA synthetase activity was studied in brain, in skeletal muscle, in aorta, and in rumen muscle. Low levels of activity were found in aorta and in rumen muscle. Brain and skeletal muscle showed marginal enzyme activity.

The active butyryl-CoA synthetase in rumen epithelium is consistent with studies showing that butyrate is readily metabolized by rumen epithelium (Sutton *et al.*, 1963a,b; Ramsey and Davis, 1965). The oxidation of butyrate to β-hydroxybutyrate by rumen epithelium provides a mechanism for preventing the appearance of butyrate in peripheral blood. As shown by Phillips and Black (1966), butyrate in peripheral blood causes hyperglycemia by stimulating the release of glucagon from the pancreas. This pharmacological effect of butyrate is not observed with acetate, with propionate, or β-hydroxybutyrate (R. W. Phillips, 1968, personal communication).

The activity of acetyl-CoA synthetase is consistent with *in vivo* studies showing that acetate is metabolized primarily by extrahepatic tissues. The absence of acetyl-CoA synthetase activity in skeletal muscle and the high activity in heart and in mammary gland suggests that acetate is metabolized

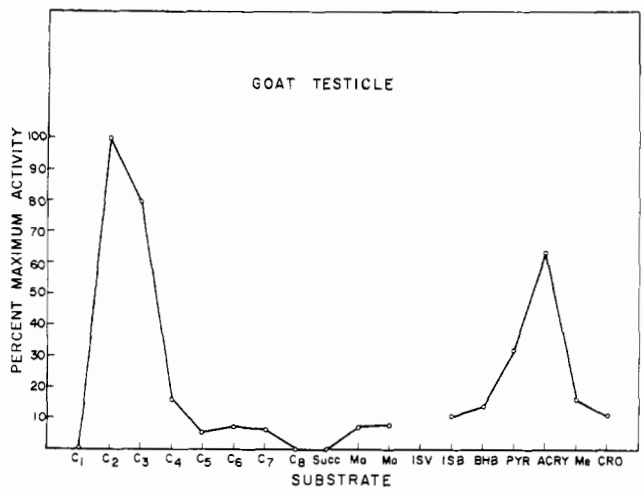

FIGURE 5: Substrate specificity of the enzyme preparation from goat testicle.

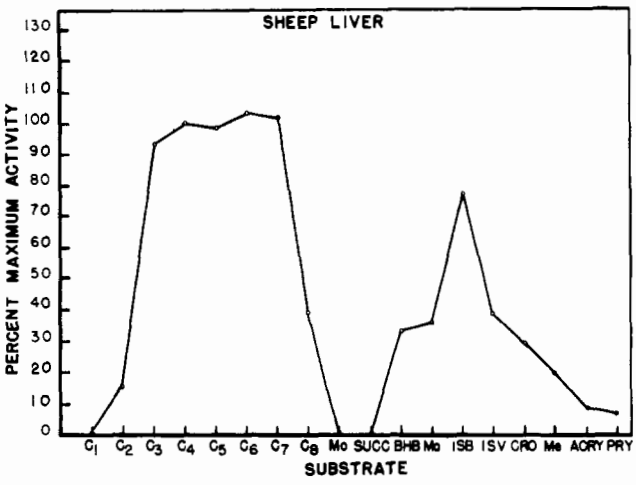

FIGURE 7: Substrate specificity of the enzyme preparation from sheep liver.

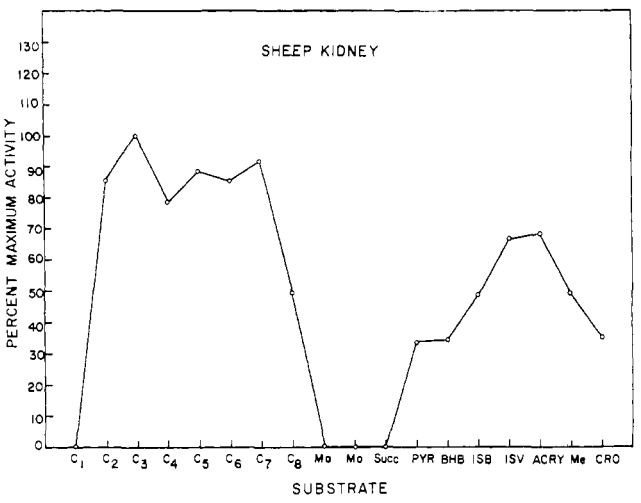

FIGURE 8: Substrate specificity of the enzyme preparation from sheep kidney.

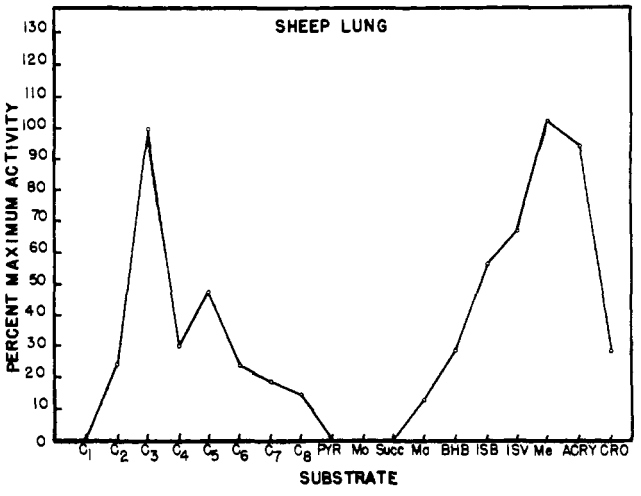

FIGURE 9: Substrate specificity of the enzyme preparation from sheep lung.

mainly by those extrahepatic tissues that are highly aerobic. *In vivo* studies have shown that propionate is removed from portal blood by the liver under normal conditions (Cook and Miller, 1965). However, when a high propionate fermentation is produced in the rumen, propionate is found in peripheral blood and can be activated and metabolized by several tissues including kidney, lung, heart, and mammary gland.

Substrate specificity studies show the enzyme preparations from heart, from mammary gland, and from testes to be specific for acetate, propionate, and acrylate. The enzyme preparation from lung is specific for propionate and for acrylate. The preparation from liver is specific for C_3 to C_7 straight-chain fatty acids, but the preparation from kidney is specific for C_2 to C_7 fatty acids (Figures 4–9). It is commonly accepted that there are three acyl-CoA synthetases in mammalian liver or heart. These enzymes are acetate:CoA ligase (AMP) (EC 6.2.1.1) active on acetate propionate and acrylate; acid:CoA ligase (AMP) (EC 6.2.1.2) active on C_4 to C_{11} acids; acid:CoA ligase (AMP) (EC 6.2.1.3) active on C_6 to C_{20} acids. The acyl-CoA synthetases we have found in ruminant heart, in mammary gland, and in testes can be classified as acetate:CoA ligase (AMP) (EC 6.2.1.1). However the acyl-CoA synthetase preparations from ruminant kidney, from lung, and from liver have not been previously reported. The liver, the lung, and the kidney enzymes are three different enzymes and all three are different from the heart, from the mammary gland, and from the testes enzymes.

The data in this communication clearly show a genetic difference between ruminant tissues in the activity of acyl-CoA synthetases active on short-chain fatty acids. Although it is recognized that many factors may be involved in the control of acetate metabolism, these results support our hypothesis that the activation reaction is an important control point in the regulation of short-chain fatty acid metabolism in ruminant tissues.

Acknowledgments

We wish to thank Mr. Lee Wong and Mrs. Helen Maxson for their valuable technical assistance. Also, we are grateful to Dr. A. C. Weise, Head of the Department of Agricultural Biochemistry and Soils, for kindly permitting us to use the Sharples supercentrifuge.

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