Biochem. J. (1973) **136**, 311–319
Printed in Great Britain

Activation of Volatile Fatty Acids in Bovine Liver and Rumen Epithelium

EVIDENCE FOR CONTROL BY AUTOREGULATION

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(*Received* 9 *April* 1973)

1. The total capacities of homogenates of bovine liver and rumen epithelium to activate acetate, propionate and butyrate were determined. 2. Activating capacities were assayed by measuring the rate of formation of the corresponding CoA esters. The methods used for determining the concentrations of the CoA esters allowed the CoA esters of acetate, propionate and butyrate to be distinguished. It was thus possible to investigate the effect of the presence of a second volatile fatty acid on the rate at which a given volatile fatty acid was activated. 3. The propionate-activating capacity in rumen epithelium was decreased by about 87% in the presence of butyrate, the acetate-activating capacity in liver was decreased by about 55% in the presence of either propionate or butyrate, and the butyrate-activating capacity in liver was decreased by about 40–50% in the presence of propionate. 4. All three activating capacities in liver appeared to be located in the mitochondrial matrix and membrane. The three activating capacities had similar locations to each other in rumen epithelium as well, although in this case activity was more evenly divided between the mitochondria and the cytoplasm. 5. The relative activating capacities towards the volatile fatty acids in the two tissues, together with the ability of one volatile fatty acid to inhibit the activation of another volatile fatty acid, appear to ensure that butyrate is mainly metabolized in the rumen epithelium and that propionate is metabolized in the liver.

Over the years several studies have been made of the ability of tissue preparations to utilize the volatile fatty acids, i.e. acetate, propionate and butyrate, *in vitro*. In view of the importance of these acids in ruminant metabolism, most of these studies have been carried out with ruminant tissues, in particular liver and rumen epithelium (see, e.g., Pennington, 1952; Seto *et al.*, 1955, 1959). However, parallel experiments have also been carried out with non-ruminant liver (see, e.g., Quastel & Wheatley, 1933; Masoro *et al.*, 1957). Besides demonstrating that the ability of a given tissue to utilize different volatile fatty acids varies, these studies showed that the rate of uptake and metabolism of one acid can be affected markedly by the presence of another acid. Thus, depending on the circumstances, the presence of the second volatile fatty acid can either decrease or increase the rate at which the first volatile fatty acid is taken up and metabolized (Pennington & Pfander, 1957; Masoro *et al.*, 1957; Pennington & Appleton, 1958; Pritchard & Tove, 1960; Leng & Annison, 1963; Smith, 1971). It is therefore unlikely that one single mechanism is adequate to explain all facets of this autoregulation of the uptake and metabolism of the volatile fatty acids. One suggestion that has been put forward (e.g. Masoro *et al.*, 1957; Pennington & Appleton, 1958) as a partial explanation of the observations is that the rate of activation, i.e. the rate of formation of the corresponding CoA ester, of a given volatile fatty acid might be affected by the presence of other volatile fatty acids. However, it has been difficult to investigate this possibility experimentally, because conventional methods of measuring acyl-CoA synthetase [acid–CoA ligase (AMP), EC 6.2.1.–] activity do not differentiate between the CoA esters of the three volatile fatty acids, which are all liable to activation in the assay medium used.

In the work described in this paper, the activation products arising as a result of acyl-CoA synthetase activity have been analysed by methods (Aas & Bremer, 1968; Huang, 1970) which enable the rate of activation of one volatile fatty acid to be measured in the presence of other volatile fatty acids. By using this technique, the total capacity of homogenates of bovine liver and rumen epithelium to activate each of these acids has been measured in the presence of the appropriate substrate alone and in the presence of both the substrate and one of the other two acids as well. The findings reveal that in several instances the rate of activation of a given volatile

fatty acid is decreased in the presence of a second volatile fatty acid. Evidence is thus provided for the existence of a novel control mechanism that may help to explain some of the phenomena of autoregulation previously observed with tissue preparations *in vitro*, and also contribute to an understanding of the regulation of the utilization of the volatile fatty acids by bovine liver and rumen epithelium *in vivo*.

Experimental

Materials

Most substrates, and the enzyme carnitine acetyltransferase (EC 2.3.1.7), were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. 2-(*p*-Iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride was obtained from Koch–Light, Colnbrook, Bucks., U.K., and the CoA and carnitine esters of propionate and butyrate were obtained from P-L Biochemicals Inc. (International Enzymes Ltd., London S.W.11, U.K.). L-Carnitine hydrochloride was kindly given by Dr. Otsuka of Otsuka Pharmaceuticals, Osaka, Japan. DL-[*methyl*-^{3}H]Carnitine hydrochloride, sodium [1-^{14}C]acetate, sodium [1-^{14}C]-propionate and sodium [1-^{14}C]butyrate were all obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Other chemicals were of analytical grade. Unlabelled acetate, propionate and butyrate were used in the form of their K^+ salts. For other compounds, too, K^+ salts were used in preference to salts containing other cations. Where appropriate, solutions were neutralized before use. Silica gel G (after Stahl; Merck, Darmstadt, W. Germany) was supplied by Anderman and Co. Ltd., London S.E.1, U.K. Gelman chromatography media (I.T.L.C. Type SG, 20cm×20cm; Gelman Instrument Co.) were supplied by Gelman–Hawksley, Lancing, Sussex, U.K. Double-distilled water, the second distillation being from glass, was used throughout.

Preparation of tissue extracts

Acyl-CoA synthetase activities (determined as described below) were measured in homogenates of bovine liver and rumen epithelium. To prepare the homogenates, liver and rumen-wall samples were first obtained from mature non-lactating Friesian ×Ayrshire dairy cows at slaughter and immediately cooled in ice. Before homogenization, the liver was minced with scissors, and the rumen epithelium was stripped off the underlying muscle layer, rinsed with homogenization medium (see below) to remove rumen contents, and finally minced. Liver samples were homogenized at 0°C in a Potter–Elvehjem glass–Teflon homogenizer in homogenization medium consisting of 9 vols. of 0.25M-sucrose containing 10mM-Tris–HCl buffer, pH7.4, and 1mM-2-mercaptoethanol. The homogenization conditions for rumen epithelium samples were the same, except that homogenization was done with a Silverson homogenizer (Silverson Machines Ltd., London S.E.1, U.K.). The homogenates were subsequently sonicated at 0°C by using a Soniprobe Type 1130A (Dawe Instruments Ltd., London W.3, U.K.) at full power (and at 20kHz), for 3×15s for liver and 4×15s for rumen epithelium, and were then ready for use in the assays.

The distribution of acyl-CoA synthetase activities in subcellular fractions of liver and rumen epithelium was also investigated. For this study, the two tissues were each first homogenized in the homogenization medium in a Potter–Elvehjem glass–Teflon homogenizer cooled in an ice bath. For liver, the subsequent fractionation procedure was based on that described by Williamson *et al.* (1968) for rat liver, in which whole homogenate, cytoplasmic and particulate fractions are prepared. Modifications of the procedure were first that the particulate pellet, sedimented at 30000*g* for 30min, was washed once before being sonicated, and secondly that the pellet sedimented by centrifugation of the sonicated particles was resuspended and also assayed for activity. This latter pellet was assumed to consist mainly of sedimentable mitochondrial, and possibly microsomal, membrane fragments, and was termed 'membrane fraction'. For rumen epithelium, the fractionation procedure was similar to that of Baird *et al.* (1970), except that the initial centrifugation was at 100*g* for 10min, rather than 800*g*, and the 100*g* sediment was discarded. The 100*g* supernatant then represented the whole homogenate. Further, a membrane fraction was prepared from the sonicated particles of this tissue, also.

Assay of capacity to activate the volatile fatty acids

The total capacity of liver and rumen epithelium homogenates, and subcellular fractions, to activate each of the three volatile fatty acids was determined by measuring the rate at which each acid was converted into the corresponding CoA ester. For brevity, the activating capacity towards each acid is subsequently termed the acyl-CoA synthetase activity towards that particular acid.

During the work, two different methods were used for determining the amounts of the CoA esters formed, i.e. those of Aas & Bremer (1968) and Huang (1970). Both these methods allow the determination of one CoA ester in the presence of either one or both of the CoA esters of the other two volatile fatty acids. The method of Aas & Bremer (1968) involves conversion of the CoA esters into the corresponding L-[*methyl*-^{3}H]carnitine esters and the subsequent separation of these carnitine compounds by t.l.c. The partition characteristics of propionylcarnitine

and butyrylcarnitine are such that these compounds are well separated from each other and from excess of carnitine. However, acetylcarnitine is less well separated from carnitine. This problem is circumvented by the method of Huang (1970). The latter method involves the assay of acetyl-CoA synthetase by using [1-^{14}C]acetate and subsequently separating acetyl-CoA from unchanged acetate on Gelman media. However, in the present study it was found that this method was equally suitable for separating propionyl-CoA and butyryl-CoA from unchanged propionate and butyrate respectively. Thus, if the method of Huang (1970) is used, the quantity of the CoA ester formed from a given volatile fatty acid can be determined in the presence of the CoA esters of the other two acids, provided that the first acid is radioactively labelled.

When the method of Aas & Bremer (1968) was used, the conditions for formation of the carnitine esters were identical with those of Aas & Bremer (1968), except that the concentration of CoA was 0.8mM. The subsequent separation of the carnitine esters was again identical with that of Aas & Bremer (1968), except that the solvent was allowed to run off the paper to increase the separation of the carnitine esters, the locations of which were determined by co-chromatographing acetyl-, propionyl- and butyryl-carnitine standards dissolved in homogenization medium. When the separation method of Huang (1970) was used, the incubation medium for the formation of the CoA esters was the same as that used by Aas & Bremer (1968) for the formation of the carnitine esters, except that carnitine acetyltransferase, carnitine and KCN were omitted, and the concentration of CoA was 2mM. Control experiments demonstrated that the concentration of CoA used in the assay media did not in fact become rate-limiting at any juncture in the present work.

Whichever separation method was used, production of the CoA ester was followed with time (3–15min) in the presence of a constant quantity of homogenate or subcellular fraction (corresponding to a maximum of 10mg of tissue), and also with quantity of homogenate or fraction (corresponding to 5–15mg of tissue) for a constant time (up to 15min). Linearity was always obtained for both plots, even in the presence of a second volatile fatty acid. A difficulty experienced with the method of Huang (1970) was that the 1-^{14}C-labelled volatile fatty acids that were used each contained a non-volatile radioactive impurity that failed to leave the origin of the chromatogram. The counts due to this impurity had therefore to be allowed for in determining the counts originating from the CoA ester of the 1-^{14}C-labelled volatile fatty acid in each experiment.

Because of the difficulty of separating acetylcarnitine from carnitine in the method of Aas & Bremer (1968), acetyl-CoA synthetase was assayed solely by the method of Huang (1970). Propionyl-CoA synthetase and butyryl-CoA synthetase were assayed by both methods, however. Comparison of the activities found by the two different methods showed that propionyl-CoA synthetase activity determined by the method of Aas & Bremer (1968) was some 40–50% lower than that determined by the method of Huang (1970) in both liver and rumen epithelium, and butyryl-CoA synthetase activity was 50–60% lower. The differences in activity obtained with the two methods may possibly be related to the use of carnitine acetyltransferase in the method of Aas & Bremer (1968), since $V_{max.}$ for the reaction catalysed by this enzyme decreases with increase in chain length of substrate, so that $V_{max.}$ for acetate >propionate>butyrate (cf. Chase, 1967). In spite of the differences in magnitude of activity observed with the two methods, qualitatively similar results were obtained in the inhibition studies (see below).

Assay of marker enzymes

Marker enzymes were used to determine the identity of the subcellular fractions that were prepared in the experiments designed to determine the location of the capacities to activate the volatile fatty acids. The marker enzymes that were assayed were lactate dehydrogenase (EC 1.1.1.27) for cytoplasm, glutamate dehydrogenase (EC 1.4.1.3) for the mitochondrial matrix, and succinate dehydrogenase (EC 1.3.99.1) for the mitochondrial membranes. Lactate dehydrogenase was assayed by the method of Bergmeyer *et al.* (1963), glutamate dehydrogenase by the method of Schmidt (1963) and succinate dehydrogenase by the 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride reductase method of Pennington (1961).

Statistics

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

Results

Propionyl-CoA synthetase activity

The propionyl-CoA synthetase activities in liver and rumen epithelium homogenates are recorded in Table 1. It is clear that, whichever method of assay was used, propionyl-CoA synthetase activity was 3 to 4 times as high in liver as in rumen epithelium. Table 1 also shows that the effect on propionyl-CoA synthetase activity of an equimolar concentration of butyrate was quite different in the two tissues. Thus, whereas butyrate elicited only a small and statistically non-significant decrease in the liver activity, it caused an almost complete inhibition of the rumen epithelium activity. Again, the same qualitative results

Table 1. *Propionyl-CoA synthetase activity in bovine liver and rumen epithelium and the effect of acetate and butyrate*

Enzyme activity is expressed as μmol of propionyl-CoA formed/min per g wet wt. of tissue at 35°C. Each value is the mean±S.D. for the number of animals in parentheses. The concn. of each volatile fatty acid in the assay medium was 10mM (see the Experimental section). $^{**}P<0.01$ for significance of decrease in activity. The percentage decrease in activity is the mean of individual values, which were in each case obtained by comparing enzyme activity in the presence of substrate+second volatile fatty acid with the corresponding activity, in the same animal, in the presence of substrate alone.

		Liver		Rumen epithelium	
Second volatile fatty acid	Method of analysis	Activity	Decrease in activity (%)	Activity	Decrease in activity (%)
—	Aas & Bremer (1968)	2.46±0.16 (6)	—	0.64±0.20 (7)	—
—	Huang (1970)	3.88±0.47 (5)	—	1.32±0.34 (7)	—
Acetate	Huang (1970)	3.5 (1)	Negligible	0.93 (2)	5
Butyrate	Aas & Bremer (1968)	1.99±0.30 (4)	19	0.07±0.05 (4)	89**
Butyrate	Huang (1970)	3.09 (2)	24	0.22±0.14 (4)	84**

Table 2. *Butyryl-CoA synthetase activity in bovine liver and rumen epithelium and the effect of acetate and propionate*

Enzyme activity is expressed as μmol of butyryl-CoA formed/min per g wet wt. of tissue at 35°C. Each value is the mean±S.D. for the number of animals in parentheses. The concn. of each volatile fatty acid in the assay medium was 10mM (see the Experimental section). $^{*}P<0.02$; $^{**}P<0.01$ for significance of decrease in activity. Percentage decrease in activity was determined as in Table 1.

		Liver		Rumen epithelium	
Second volatile fatty acid	Method of analysis	Activity	Decrease in activity (%)	Activity	Decrease in activity (%)
—	Aas & Bremer (1968)	1.15±0.18 (6)	—	1.82±0.33 (7)	—
—	Huang (1970)	2.88±0.30 (4)	—	3.90±0.78 (3)	—
Acetate	Huang (1970)	2.40 (1)	2	3.40 (1)	Negligible
Propionate	Aas & Bremer (1968)	0.56±0.16 (4)	52**	1.56±0.39 (4)	14
Propionate	Huang (1970)	1.80±0.45 (3)	38*	3.40 (1)	Negligible

were obtained whichever method of assay was used. Table 1 finally shows that the presence of acetate, in contrast with that of butyrate, had no effect on propionyl-CoA synthetase activity in either tissue.

Butyryl-CoA synthetase activity

Butyryl-CoA synthetase activities in liver and rumen epithelium are recorded in Table 2. It is evident that, as with propionyl-CoA synthetase activity, both methods of assay gave qualitatively similar results. As Table 2 shows, butyryl-CoA synthetase activity in rumen epithelium was some 3 times that of propionyl-CoA synthetase, whereas, on the other hand, the activity in liver was somewhat less than that of propionyl-CoA synthetase. Table 2 also shows that in the presence of an equimolar concentration of propionate hepatic butyryl-CoA synthetase activity was significantly depressed by 38–52%. By contrast, propionate did not depress butyryl-CoA synthetase activity in rumen epithelium. Further, acetate had no effect on butyryl-CoA synthetase activity from either tissue.

Acetyl-CoA synthetase activity

In spite of the fact that acetyl-CoA synthetase activity in the liver was the lowest of the three activating capacities, it was still appreciable, being 0.83μmol/min per g at 35°C (Table 3). However, in the presence of equimolar concentrations of either propionate or butyrate the activity was decreased by

about 55%. Acetyl-CoA synthetase activity in rumen epithelium was considerably less than that in liver and was again the lowest of the three activating capacities (Table 3), in confirmation of the results of Cook *et al.* (1969). Nevertheless, the acetyl-CoA synthetase activity that was present in the rumen epithelium appeared to be much less sensitive to inhibition by either propionate or butyrate than was the liver activity. As Table 3 shows, equimolar concentrations of either propionate or butyrate produced a depression of rumen epithelium acetyl-CoA synthetase activity that was not statistically significant.

Localization studies

The possibility existed that the inhibitory effect of the second volatile fatty acid in the several instances listed above had no counterpart *in vivo*. Thus, if the distributions of the three synthetase activities in a given tissue were dissimilar, then one or more of the synthetases might never be exposed to a foreign volatile fatty acid, when the latter was in the non-activated form. To place the findings on a firmer basis it therefore seemed necessary to determine the subcellular distribution of the three synthetase activities in liver and rumen epithelium. This was done by fractionating each of the two tissues and

Table 3. *Acetyl-CoA synthetase activity in bovine liver and rumen epithelium and the effect of propionate and butyrate*

Enzyme activity is expressed as μmol of acetyl-CoA formed/min per g wet wt. of tissue at 35°C. Each value is the mean±S.D. for the number of animals in parentheses. The concn. of each volatile fatty acid in the assay medium was 10mM (see the Experimental section). $^{**}P<0.01$ for significance of decrease in activity. Percentage decrease in activity was determined as in Table 1.

		Liver		Rumen epithelium	
Second volatile fatty acid	Method of analysis	Activity	Decrease in activity (%)	Activity	Decrease in activity (%)
—	Huang (1970)	0.83±0.18 (11)	—	0.23±0.07 (7)	—
Propionate	Huang (1970)	0.41±0.12 (4)	55**	0.21 (2)	14
Butyrate	Huang (1970)	0.40±0.18 (4)	56**	0.24±0.04 (3)	18

Table 4. *Subcellular distribution of capacity to activate the three volatile fatty acids, and of marker enzyme activities, in bovine liver*

The subcellular fractions were prepared as described in the Experimental section. The values for the synthetase activities are in each case the means for two different animals. Marker enzyme activities were determined on each occasion to confirm the identity of the fractions, and the values given are the means for four separate animals. Synthetase activities are expressed as μmol of activated volatile fatty acid formed/min per g wet wt. at 35°C, lactate dehydrogenase activity as μmol of NAD^+ formed/min per g wet wt. at 25°C, glutamate dehydrogenase activity as μmol of NADH formed/min per g wet wt. at 25°C and succinate dehydrogenase as ΔE_{490}/min per 0.1g wet wt. at 37°C. Enzyme activities in the fractions are expressed as percentages of the whole homogenate activity, except for succinate dehydrogenase, when activity is expressed as a percentage of the membrane fraction activity.

		Percentage of activity in each fraction			
Enzyme activity	Whole homogenate activity	Whole homogenate	Cytoplasmic fraction	Particulate fraction	Membrane fraction
Acetyl-CoA synthetase	0.52	100	3	76	29
Propionyl-CoA synthetase	3.60	100	18	73	41
Butyryl-CoA synthetase	2.24	100	13	81	22
Lactate dehydrogenase	123	100	105	7	0.4
Glutamate dehydrogenase	37	100	6	98	6
Succinate dehydrogenase	0.35	61	7	79	100

Table 5. *Subcellular distribution of capacity to activate the three volatile fatty acids, and of marker enzyme activities, in bovine rumen epithelium*

The subcellular fractions were prepared as described in the Experimental section. The values for the synthetase activities are in each case the means for two different animals. Marker enzyme activities were determined on each occasion to confirm the identity of the fractions, and the values given are the means for three separate animals. For further information, see Table 4.

		Percentage of activity in each fraction			
Enzyme activity	Whole homogenate activity	Whole homogenate	Cytoplasmic fraction	Particulate fraction	Membrane fraction
Acetyl-CoA synthetase	0.20	100	56	43	17
Propionyl-CoA synthetase	0.77	100	69	37	9
Butyryl-CoA synthetase	2.60	100	67	34	3
Lactate dehydrogenase	53	100	110	4	2
Succinate dehydrogenase	0.04	40	18	30	100

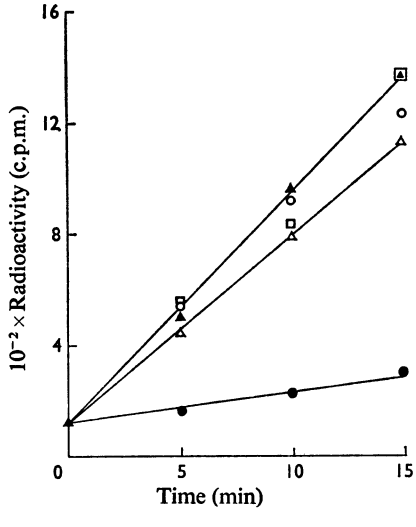

Fig. 1. *Effect of butyrate* (●), *butyryl-CoA* (□), *AMP* (○) *and potassium pyrophosphate* (△) *on total propionyl-CoA synthetase activity* (▲) *in bovine rumen epithelium*

To determine each point, 10mg of tissue was incubated in 1.0ml total assay volume (see the Experimental section). Deproteinization at the end of the reaction increased this volume to 1.2ml. A 10μl sample of this 1.2ml volume was then taken for chromatographic separation by the method of Huang (1970) and subsequent radioactivity counting. The concentration of butyrate in the assay was 10 mM and that of pyrophosphate, 0.6mM. The concentrations of butyryl-CoA and AMP were 0.2, 0.4 and 0.6mM for the 5, 10 and 15min incubations respectively.

determining synthetase activity in the various fractions, as described in the Experimental section. The nature and content of each fraction was checked as far as possible with the use of marker enzymes (see the Experimental section).

The localization of the synthetase activities in the fractions derived from liver is recorded in Table 4, and that of synthetase activities in fractions of rumen epithelium in Table 5. Table 4 shows that the subcellular distribution of the three synthetase activities in liver was very similar. In each case, the major portion of the activity was found in the particulate fraction and in the membranes derived from this fraction, and there was only slight activity in the cytoplasmic fraction. Table 5 shows that in rumen epithelium, too, the subcellular distributions of the three synthetase activities appeared to be very similar to each other, although in this case activity appeared to be about equally distributed between the cytoplasmic fraction on the one hand and the particulate and membrane fractions on the other. Rumen epithelium is, however, difficult to homogenize and conventional methods of homogenization may result in damage to subcellular organelles. At least a portion of the synthetase activity in the cytoplasmic fraction may therefore be due to contamination with mitochondrial contents. Unfortunately, this possibility cannot be checked by using glutamate dehydrogenase, because the activity of this enzyme is very low in rumen epithelium (Baird *et al.*, 1970). The findings with liver suggest that the three synthetase activities in this tissue are, in fact, located in the mitochondrial matrix and membrane. Quraishi & Cook (1972) have previously reported that acetyl-CoA synthetase is located in the mitochondria in bovine liver. The report of Ballard (1972) that 63% of acetyl-CoA synthetase activity in sheep liver is found in the cyto-

plasm suggests that there may be a species difference in this respect.

Nature of the inhibitory agent

The production of CoA ester in the synthetase assays was always linear with time and with quantity of tissue used. Even when the reaction was partially inhibited by the presence of a second volatile fatty acid, the production of CoA ester that still took place was also linear. These observations suggested that inhibition in the presence of the second volatile fatty acid was due to that acid itself and not to a product of the combined activation of the two volatile fatty acids. Thus, if the inhibition were due to an activation product, it might be expected that the rate of production of CoA ester would decrease as the inhibitory product accumulated.

In spite of these considerations, it seemed pertinent to determine the effect of activation products on synthetase activity. The results of an experiment carried out with this end in view are given in Fig. 1, which depicts the effects of butyrate, butyryl-CoA, AMP and potassium pyrophosphate on propionyl-CoA synthetase activity in rumen epithelium. The linearity of propionyl-CoA production with time in the presence of propionate alone, and in the presence of both propionate and butyrate, is apparent. Fig. 1 also shows that neither butyryl-CoA, AMP or pyrophosphate, when present at concentrations that would be expected to develop in the presence of equimolar concentrations of propionate and butyrate, cause an inhibition of propionyl-CoA synthetase activity that would be sufficient to account for the inhibition observed in the presence of butyrate.

Discussion

Activation represents the first step in the metabolism of acetate, propionate and butyrate, and the activities of the enzymes catalysing this step are relatively low. Control at this point is therefore likely to play a key role in regulating utilization of the volatile fatty acids. For this reason, the ability of one volatile fatty acid to decrease the rate at which another is activated must be regarded as a potentially important mechanism for regulating the rate of uptake of the individual volatile fatty acids by liver and rumen epithelium. Support for the contention that this control mechanism is likely to operate *in vivo* as well as in homogenates *in vitro* is provided by the observation that in both liver and rumen epithelium the subcellular distributions of the three synthetase activities are very similar to each other. The possibility is therefore excluded that homogenization destroys intracellular barriers which otherwise ensure that the volatile fatty acids are activated in separate compartments.

Since, in each case, the free acid appears to be the inhibitor, it seems possible that inhibition may be due to competition between the two acids for an active site. If this is so, then the findings could be explained by assuming that, although there are only the two common enzymes for activating volatile fatty acids in rumen epithelium, i.e. those for which the preferred substrates are acetate and butyrate (EC 6.2.1.1 and EC 6.2.1.2 respectively), there are three such enzymes in bovine liver, i.e. these same two and also a third enzyme for which propionate is the preferred substrate (cf. Smith & Russell, 1967). In rumen epithelium, the observed propionyl-CoA synthetase activity would then be due mainly to the acyl-CoA synthetase for which butyrate was the preferred substrate. In the presence of both propionate and butyrate, propionate activation would be largely suppressed. In liver, the partial suppression of butyryl-CoA synthetase by propionate could be similarly explained if a portion of the butyrate activation were due to the synthetase enzyme for which propionate was the preferred substrate. Again, a portion of the acetate activation in liver would also have to be due to this same enzyme.

The overall purpose of the disposition of synthetase activities in the two tissues, and of the inhibition of the activation of one volatile fatty acid by a second volatile fatty acid, appears to be to ensure that in the cow butyrate metabolism occurs mainly in the rumen epithelium and propionate metabolism in the liver, and acetate passes mainly into the peripheral bloodstream. When any one acid is present in excess, however, the potential appears to exist for its metabolism at another location as well. Thus, under these circumstances propionate might also be metabolized extensively in the rumen epithelium and butyrate and acetate might be similarly metabolized in the liver.

In the following paragraphs the findings reported in the present paper are discussed in relation to observations that have previously been made on the uptake and utilization of the volatile fatty acids by liver and rumen epithelium *in vitro* and *in vivo*.

Propionyl-CoA synthetase activity

In general confirmation of the present results, Scholte *et al.* (1971) have previously reported that propionyl-CoA synthetase is the most active of the enzymes activating volatile fatty acids in ox liver mitochondria. The findings in the present work are consistent with the view that the liver is the main site of propionate metabolism in the cow and that little propionate ever reaches the hepatic venous blood (cf. Bergman & Wolff, 1971). The findings also suggest that the maximum rate of uptake of propionate is unlikely to be affected by variation in the composition of the volatile fatty acids presented to the

liver. Studies with liver preparations *in vitro* have shown that addition of either acetate or butyrate actually increases propionate uptake (e.g. Leng & Annison, 1963; Smith, 1971). This effect is probably due to the provision by these compounds of acetyl-CoA, which will combine with oxaloacetate, derived from propionate, to form citrate. The observed propionyl-CoA synthetase activity in the current work is also consistent with the concept that propionate is a major precursor of glucose in bovine liver, since this activity would allow a potential rate of glucose synthesis of more than 1 μmol/min per g. Studies by Bergman & Wolff (1971) have demonstrated that the rate of uptake of propionate by the liver of sheep fed on a normal diet is about 0.6 μmol/min per g.

The existence of significant propionyl-CoA synthetase activity in rumen epithelium indicates that this tissue is potentially capable of metabolizing propionate. However, because an equimolar concentration of butyrate decreases the activity of the enzyme substantially, it seems unlikely that there can be any extensive metabolism of propionate by rumen epithelium *in vivo* whenever the rate of butyrate absorption from the rumen is of a similar order to that of propionate.

Previous studies designed to elucidate whether or not propionate is metabolized by the rumen epithelium during absorption appear to have yielded conflicting results. With regard to experiments *in vivo*, Leng *et al.* (1967) concluded that in sheep up to 70% of the propionate that was incorporated into glucose was first converted into lactate in the rumen epithelium. To achieve this degree of conversion into lactate, the rate of activation of propionate by rumen epithelium would have been about 0.6 μmol/min per g. By contrast, Weigand *et al.* (1972) found that in the cow conversion of propionate into lactate in rumen epithelium was virtually negligible and they suggested that lactate measured in the studies of Leng *et al.* (1967) was derived from glucose rather than propionate (see also Weekes, 1972). Nevertheless, the results of Bergman & Wolff (1971) appear to indicate that propionate utilization by sheep rumen epithelium may be as high as 0.8 μmol/min per g.

As far as work *in vitro* is concerned, Pennington & Pfander (1957) first found that butyrate decreased propionate uptake into rumen epithelium pieces. Stevens & Stettler (1966*a*) reported that 60–70% of propionate was metabolized during passage through the rumen epithelium. This experiment was carried out in the absence of butyrate and acetate, however. When the work was repeated in the presence of all three volatile fatty acids it was found that propionate transport increased (Stevens & Stettler, 1966*b*). Metabolism was not measured on this occasion, but the increase in transport was probably due to a corresponding decrease in metabolism in the presence of butyrate.

Butyryl-CoA synthetase activity

Even in the presence of propionate, butyryl-CoA synthetase activity in bovine liver is still substantial and more than adequate to cope with rates of butyrate uptake similar to the value of about 0.07 μmol/min per g reported for the fed sheep by Bergman & Wolff (1971). This would explain why under normal conditions little butyrate escapes metabolism in the liver (Bergman & Wolff, 1971). It must be remembered, however, that in the sheep, at least, the molar proportions of propionate to butyrate are 9:1 on arrival at the liver (Bergman & Wolff, 1971) and the possibility exists that under these conditions propionate might suppress butyryl-CoA synthetase activity still further.

The work of Smith (1971) provides some evidence that propionate can inhibit butyrate uptake into liver and liver mitochondria *in vitro*. However, the situation is complicated by the fact that under different experimental conditions propionate will also stimulate butyrate metabolism by providing oxaloacetate.

From Table 2 it would seem that butyryl-CoA synthetase in bovine rumen epithelium is sufficiently active to ensure rates of butyrate metabolism that are at least as high as the rate reported to occur in fed sheep, i.e. about 1.0 μmol/min per g (Bergman & Wolff, 1971).

Acetyl-CoA synthetase activity

The finding that both propionate and butyrate partially inhibit hepatic acetyl-CoA synthetase activity is in agreement with earlier observations *in vitro*, which have shown that propionate and butyrate decrease acetate uptake by liver slices and liver mitochondria (Leng & Annison, 1963; Smith, 1971). The fact that some acetyl-CoA synthetase activity remains in the presence of propionate or butyrate suggests that liver can still metabolize acetate to a limited extent *in vivo* (cf. Holdsworth *et al.*, 1964; Cook & Miller, 1965). Bergman & Wolff (1971) have calculated that acetate uptake by the liver can amount to about 0.20 μmol/min per g in fed sheep, when allowance has been made for endogenous acetate production. Also, a rate of acetate uptake of about 0.6 μmol/min per g was observed in the same study when exogenous acetate was infused.

Acetyl-CoA synthetase activity in bovine rumen epithelium, although essentially unaffected by propionate or butyrate, is still clearly quite insufficient to allow rates of acetate utilization of the order of 1.7 μmol/min per g, which is the rate at which Bergman & Wolff (1971) suggest acetate may be utilized by the epithelium of the fed sheep.

We thank Mr. R. Collins for valuable help in obtaining tissue samples. R. A. is in receipt of an A.R.C. Research Studentship.

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