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Aspects of Carnitine Ester Metabolism in Sheep Liver

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1. Carnitine acetyltransferase (E.C 2.3.1.7) activity in sheep liver mitochondria was 76nmol/min per mg of protein, in contrast with 1.7 for rat liver mitochondria. The activity in bovine liver mitochondria was comparable with that of sheep liver mitochondria. Carnitine palmitoyltransferase activity was the same in both sheep and rat liver mitochondria. 2. The [free carnitine]/[acetylcarnitine] ratio in sheep liver ranged from 6:1 for animals fed *ad libitum* on lucerne to approx. 1:1 for animals grazed on open pastures. This change in ratio appeared to reflect the ratio of propionic acid to acetic acid produced in the rumen of the sheep under the two dietary conditions. 3. In sheep starved for 7 days the [free carnitine]/[acetylcarnitine] ratio in the liver was 0.46:1. The increase in acetylcarnitine on starvation was not at the expense of free carnitine, as the amounts of free carnitine and total acid-soluble carnitine rose approximately fivefold on starvation. An even more dramatic increase in total acid-soluble carnitine of the liver was seen in an alloxan-diabetic sheep. 4. The [free CoA]/[acetyl-CoA] ratio in the liver ranged from 1:1 in the sheep fed on lucerne to 0.34:1 for animals starved for 7 days. 5. The importance of carnitine acetyltransferase in sheep liver and its role in relieving 'acetyl pressure' on the CoA system is discussed.

The oxidation of long-chain fatty acids by isolated rat liver mitochondria may be studied under conditions where the oxidation is completely dependent on the presence of L-carnitine or under conditions where the oxidation is largely independent of carnitine (see Greville & Tubbs, 1968). In the accompanying paper (Koundakjian & Snoswell, 1970) it is shown that the oxidation of long-chain fatty acids (C_{12} to C_{18}, saturated) by sheep liver mitochondria is completely dependent on the presence of L-carnitine under conditions where the oxidation by rat liver mitochondria is largely carnitine-independent. Also, Shepherd, Yates & Garland (1966) have shown that the reaction catalysed by carnitine palmitoyltransferase is the rate-limiting step in the oxidation of palmitate in rat liver mitochondria. Therefore we have examined carnitine acyltransferase activity in sheep liver. Friedman & Fraenkel (1955) have reported that sheep liver showed carnitine acetyltransferase activity, although no quantitative results were given.

The concentrations of free carnitine and of various carnitine esters in rat tissues have been reported by a number of workers (e.g. Marquis & Fritz, 1965; Bøhmer, 1967; Pearson & Tubbs, 1967). However, there are no reports on the amounts of carnitine and carnitine esters in ruminant tissues. In the present paper values for free carnitine and various carnitine esters in sheep liver are presented.

Pearson & Tubbs (1967) showed in experiments with perfused rat heart that, when acetate and more particularly propionate were added to the perfusate, the relative proportions of free carnitine and the various carnitine esters were altered markedly. As acetate and propionate are important metabolites in sheep and other ruminants and the ratio of propionate to acetate produced in the rumen varies considerably with different diets (Hungate, 1966), the effect of different diets on the amounts of various carnitine esters in sheep liver was examined. In particular, the effects of a high lucerne diet, which gives rise to high concentrations of propionate in the rumen, were examined. Some of these results have been reported in a preliminary form (Snoswell, Broadhead & Henderson, 1969). The interrelationships between carnitine and acetylcarnitine, and CoA and acetyl-CoA, and the importance of carnitine acetyltransferase in sheep liver are also discussed.

MATERIALS

Animals. The sheep used were Merino wethers, approx. 4 years old and weighing between 38 and 48kg. In some experiments the animals were used after grazing for at

least 2 weeks on open pastures that consisted mainly of wheaten and oaten stubble. In other experiments the sheep were grazed for 1 week on similar pasture clover but were supplemented *ad libitum* with fresh lucerne and lucerne hay.

The rats used were Wistar males weighing 250–300g and were fed on a pelleted rat diet (Barastoc and Co., Melbourne, Vic., Australia).

Tissue preparations. The sheep were killed by severing the necks and the livers were immediately freeze-clamped *in situ* with aluminium-faced tongs previously cooled in liquid N_2 (Wollenberger, Ristau & Schoffa, 1960). The time between the severing of the necks of the animals and the actual freeze-clamping of the liver varied between 10 and 20s. However, analysis of freeze-clamped samples obtained from control sheep with a 'spinal block' (achieved by administering 8ml of Xylocaine into the sacral–lumbar region of the spinal cord) gave results similar to those obtained by the method described above. Other samples of liver were immediately placed in ice-cold 0.25M-sucrose.

The rats were killed by a blow on the head and the livers immediately freeze-clamped *in situ* as described above. Liver samples were also collected into ice-cold 0.25M-sucrose.

The frozen liver samples were powdered in a stainless steel mortar with a heavy stainless steel pestle. Frozen liver powder (5g) was added to 5ml of 30% (w/v) $HClO_4$, previously frozen in liquid N_2, in the mortar of a glass Potter–Elvehjem homogenizer. The homogenizer was then placed in an alcohol bath at −15°C and the powdered liver was homogenized in the $HClO_4$ without the tissue powder thawing (see Lowry, Passonneau, Hasselberger & Schulz, 1964). Ice-cold water (7ml) was subsequently added to facilitate the final homogenization. The homogenate was centrifuged at 10000*g* for 20min and the supernatant used in the assay of the various metabolites after neutralization with 3M-KOH and removal of the $KClO_4$ precipitate by centrifuging.

Mitochondria. These were isolated from both rat and sheep liver by the method of Hogeboom (1955) as described by Meyers & Slater (1957).

METHODS

All assays involved the use of an Eppendorf spectrophotometer fitted with an automatic cell-changer and recorder (Eppendorf Gerätebau, Netheler und Hinz G.m.b.H., Hamburg, Germany) or a Zeiss PMQ II spectrophotometer fitted with an automatic sample-changer and TE converter (Carl Zeiss, Oberkocken, Germany) connected to a Rikadenki model B140 recorder (Rikadenki Kogyo Co. Ltd., Tokyo, Japan). Both instruments were fitted with temperature-controlled cell holders and the temperature was maintained at 20°C for all assays.

Carnitine acetyltransferase. This was measured by the method of Beenakkers & Klingenberg (1964). Rotenone (3μM) was included in the assay mixture to inhibit NADH oxidase.

Carnitine palmitoyltransferase. This was measured spectrophotometrically by the method of Shepherd *et al.* (1966) based on the measurement of CoA released with α-oxoglutarate oxidase (Garland, Shepherd & Yates, 1965). α-Oxoglutarate oxidase was isolated from bovine heart by the method of Sanadi, Littlefield & Bock (1952 as modified by Hirashima, Hayakawa & Koike (1967).

Both carnitine acetyl- and palmitoyl-transferase were measured in mitochondrial preparations after sonication. The mitochondrial pellets were suspended in cold 0.1M-sodium–potassium phosphate buffer, pH7.4, to give a concentration of approx. 40mg of mitochondrial protein/ml. The suspensions were disrupted in an MSE sonic disintegrator (Measuring and Scientific Equipment Ltd., London S.W.1, U.K.) at 20kHz for 20s. Enzyme activities were determined in the supernatants obtained after centrifuging the sonicated mitochondrial suspensions at 14000*g* for 10min.

Carnitine compounds. Acetylcarnitine was measured by the method of Pearson & Tubbs (1964) and free carnitine by the method of Marquis & Fritz (1964). High blank values in the latter assay, due to endogenous free thiol groups, were decreased to quite acceptable values by adjusting the extracts to pH8.5 and heating at 90°C for 5min before assay, as suggested by Marquis & Fritz (1964). Pearson, Chase & Tubbs (1969) suggest that significant hydrolysis of short-chain carnitine esters may occur under these conditions but we could detect no breakdown of a 0.2mM solution of *O*-acetyl-L-carnitine at pH8.5 and 90°C even after 15min heating. Total acid-soluble carnitine and acid-insoluble carnitine were measured by the method of Pearson & Tubbs (1967).

Acetyl-CoA and CoA. Acetyl-CoA and acetyl-CoA plus CoA were measured by the kinetic method of Allred & Guy (1969). Free CoA was determined by taking the difference between acetyl-CoA plus CoA and acetyl-CoA. In these kinetic determinations a standard curve was prepared on each occasion by using a CoA standard solution, the concentration of which was determined with phosphotransacetylase (EC 2.3.1.8) by the method of Michal & Bergmeyer (1963). However, as this method only assays reduced CoA the standards were preincubated for 10min at 25°C with 2μl of 0.2M-dithiothreitol to ensure that all the CoA was in the reduced form.

Protein. This was measured by the biuret method (Gornall, Bardawill & David, 1949).

Chemicals. D- and L-Carnitine hydrochlorides and *O*-acetyl-L-carnitine chloride were generously supplied by Dr Otsuka of Otsuka Pharmaceuticals, Osaka, Japan. All products were recrystallized from ethanol. Other biochemicals were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A., and enzymes from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Palmitoyl-CoA was prepared by the method of Seubert (1960) and propionyl- and butyryl-CoA from free CoASH and the appropriate anhydrides (redistilled) by the method of Stadtman (1957).

RESULTS

Carnitine acyltransferase activities in sheep liver mitochondria. The results in Table 1 indicate that the activity of carnitine palmitoyltransferase of sheep liver mitochondria is the same as that of rat liver mitochondria. However, the activity of carnitine acetyltransferase is some 40–50 times that of rat liver mitochondria. The high activity of carnitine acetyltransferase appears to be an

Table 1. *Carnitine acyltransferase activities in sheep liver mitochondria*

The mitochondria were suspended in 0.1 M-sodium–potassium phosphate buffer to give a concentration of 40 mg of protein/ml and were sonicated at 20 kHz for 20 s. The sonicated suspensions were centrifuged at 14000*g* for 10 min and the supernatants used for assay. The enzyme activities were measured as described in the text. The results, expressed as nmol/min per mg of protein, are means ± S.E.M. for five individual preparations in each case.

Species	Carnitine acetyltransferase	Carnitine palmitoyltransferase
Rat	1.7 ± 0.4	1.1 ± 0.1
Sheep	76.0 ± 6.9	1.1 ± 0.1

Table 2. *Free carnitine and various carnitine fractions in the liver of sheep under different dietary conditions*

One group of Merino wethers was grazed on open pasture consisting mainly of wheaten and oaten stubble and dried rye grass, and the diet of the second group was supplemented with lucerne hay *ad libitum*. The third group was starved for 7 days. The animals were killed by severing the necks and samples of liver were immediately frozen *in situ* with aluminium-faced tongs previously cooled in liquid N_2. The frozen liver powders were extracted with $HClO_4$ and assayed as described in the text. Values are means ± S.E.M. for four animals in each case. N.D. indicates not detectable, i.e. <0.5 nmol/g wet wt. under the conditions of assay.

Dietary condition	Acetylcarnitine (nmol/g wet wt.)	Free carnitine (nmol/g wet wt.)	Total acid-soluble carnitine (nmol/g wet wt.)	Acid-insoluble carnitine (nmol/g wet wt.)
Grazed plus lucerne *ad libitum*	15 ± 2	82 ± 7	143 ± 15	N.D.
Grazed on open pasture	80 ± 23	55 ± 18	148 ± 41	15 ± 8
Starved for 7 days	442 ± 46	192 ± 15	695 ± 43	30 ± 5

intrinsic feature of the species and is not associated with active rumen fermentation as young lambs (2 weeks old) had enzyme activities of 79 ± 6 (3) nmol/min per mg of protein in liver mitochondria, which are similar to those of adult sheep (Table 1). Mitochondria isolated from bovine liver and assayed in the same manner showed carnitine acetyltransferase activity of 69 ± 5 (2) nmol/min per mg, which again is similar to the activity in sheep liver mitochondria.

The specificity of the carnitine acetyltransferase reaction of sheep liver was examined by using various CoA esters as substrates and by using the carnitine palmitoyltransferase assay system. If the activity with the acetyl ester was expressed as 100, then the activities found with the propionyl and butyryl esters were 121 and 95 respectively. This pattern of specificity of carnitine acetyltransferase from sheep liver is very similar to that reported by Bøhmer & Bremer (1968) for the commercially available enzyme, which is isolated from pigeon breast muscle.

Free carnitine and carnitine esters in sheep liver and the effect of diet. The results presented in the previous section revealed a relatively high activity of carnitine acetyltransferase in sheep liver. It was therefore considered important to determine the amounts of the various carnitine esters in sheep liver.

The results in Table 2 for freeze-clamped liver samples show that when sheep were grazed on pastures consisting of wheaten and oaten stubble and dried grass the amount of acetylcarnitine was approximately the same as the amount of free carnitine. In contrast, in the animals that grazed on pastures and whose diet was supplemented *ad libitum* with fresh lucerne and lucerne hay the [free carnitine]/[acetylcarnitine] ratio was markedly ($P<0.001$) different, being approx. 6:1, although the amount of total acid-soluble carnitine was similar in both cases (Table 2). In the former group of sheep the sum of the free carnitine plus acetylcarnitine is not significantly different from the total acid-soluble carnitine, whereas in the latter group free carnitine plus acetylcarnitine represents only 74% of the total acid-soluble carnitine. Samples of neutralized perchloric acid extracts of powdered liver from the sheep whose diet was supplemented with lucerne were incubated with 10 μmol of CoA and 20 units of carnitine acetyltransferase at pH 8.0 for 30 min. Under these conditions, due to the specificity of this enzyme (see Bøhmer & Bremer, 1968), both acetylcarnitine and propionylcarnitine would be converted into the

Table 3. *Free carnitine, acetylcarnitine, free CoA and acetyl-CoA in the liver of sheep under different dietary conditions*

The various groups of sheep, the dietary conditions and the removal of liver samples and their subsequent treatment were as described in Table 2. The liver samples were extracted and assayed as described in the text. Results are means ± S.E.M. for four animals in each case.

Dietary condition	Acetyl carnitine (nmol/g wet wt.)	Free carnitine (nmol/g wet wt.)	Acetyl-CoA (nmol/g wet wt.)	Free CoA (nmol/g wet wt.)	[Free carnitine]/[acetyl-carnitine] ratio	[Free CoA]/[acetyl-CoA] ratio
Grazed plus lucerne *ad libitum*	15 ± 2	82 ± 7	51 ± 6	52 ± 5	6.1 ± 0.8	1.04 ± 0.10
Grazed on open pasture	80 ± 23	55 ± 15	77 ± 7	29 ± 1	0.77 ± 0.22	0.38 ± 0.04
Starved for 7 days	442 ± 46	192 ± 15	70 ± 7	23 ± 1	0.46 ± 0.09	0.34 ± 0.08

corresponding CoA derivatives and an equivalent amount of free L-carnitine would be released. Any butyrylcarnitine would also be utilized in a similar manner. However, most of the butyrate produced in the rumen of sheep is converted into ketone bodies in the rumen epithelium (Pennington, 1952). After the incubation described above, total carnitine was measured in these mixtures. The values for acetylcarnitine and free carnitine were deducted from these total carnitine values. The remaining portion was tentatively considered to have been derived from propionylcarnitine and the amount of extra carnitine liberated by the procedure outlined above was found to be equal to approx. 80% of the total acid-soluble carnitine minus free carnitine plus acetylcarnitine. This experiment, however, only gives a qualitative indication that the unaccounted fraction of total acid-soluble carnitine is propionylcarnitine, as the carnitine transferase reaction may not have gone to completion and the presence of unchanged CoA in the carnitine assay gave very high blank values.

The results in Table 3 also indicate that acid-insoluble carnitine was not detectable in the livers of the sheep on diets supplemented with lucerne, whereas in the non-supplemented animals there was 15nmol of this acid-insoluble fraction/g wet wt. This fraction is considered by Pearson & Tubbs (1967) to represent long-chain fatty-acylcarnitine esters.

Effects of starvation on the amounts of carnitine and carnitine esters in sheep liver. The results presented in Table 2 indicate that the amount of acetylcarnitine in the liver of the starved sheep increased fivefold over the amount found in the livers of sheep grazed on open pastures. This was a highly significant increase ($P<0.001$) but it did not occur at the expense of free carnitine, for the total acid-soluble carnitine also increased some four- to five-fold ($P<0.001$) on starvation. This is in marked contrast with the situation in rat liver, where the amount of acetylcarnitine does increase on starvation (about twofold) but at the expense of carnitine (Pearson & Tubbs, 1967). If the amounts of acetylcarnitine in liver of the starved sheep are contrasted with the amounts present in the livers of the grazing animals that were supplemented with lucerne (Table 2) then the increase of acetylcarnitine on starvation is even more marked, being some thirty-fold.

The acid-insoluble carnitine fraction in the liver only increased twofold on starvation (when compared with the sheep on open grazing) and in the starved sheep only constitutes about 5% of the total carnitine (Table 3). Again this is in marked contrast with the situation in rat liver, where this fraction increases sevenfold on starvation and constitutes some 20% of the total carnitine in the starved state (Pearson & Tubbs, 1967).

Striking increases in free carnitine, acetylcarnitine and total acid-soluble carnitine concentrations have also been observed under other conditions. In an alloxan-diabetic sheep (60mg of alloxan/kg body wt. given intravenously 60h before slaughter) the total acid-soluble carnitine had reached 824nmol/g wet wt. compared with a normal average of 148nmol/g (Table 2). Also, in one sheep that was in rather poor condition initially (weight 36kg) the total acid-soluble carnitine in the liver was found to be 1550nmol/g wet wt. after 7 days starvation.

CoA, acetyl-CoA, carnitine and acetylcarnitine in the livers of normal and starved sheep. In the previous section the amounts of various carnitine fractions in sheep liver were reported. The proportions of these various fractions were found to be markedly different from those found in rat liver. In view of this fact and because carnitine acetyltransferase allows equilibration between acetyl-CoA and acetylcarnitine, at least in rat tissues (Pearson &

Tubbs, 1967), the relationship between acetyl-CoA and acetylcarnitine was examined in sheep liver.

The results presented in Table 3 show that in the sheep that received lucerne the [free carnitine]/[acetylcarnitine] ratio in the liver was 6:1 and the [free CoA]/[acetyl-CoA] ratio was 1:1. In contrast, in the sheep that were grazed with no lucerne, the [free carnitine]/[acetylcarnitine] ratio was approx. 1:1 and the [free CoA]/[acetyl-CoA] ratio was 0.38:1 (Table 3). On starvation the [free carnitine]/[acetylcarnitine] ratio in a sheep liver was further lowered to 0.46:1, although the total amounts of both fractions increased markedly (Table 3), and the [free CoA]/[acetyl-CoA] ratio was 0.34:1. The total content of CoA plus acetyl-CoA was not significantly different in any of the three groups of sheep and the overall mean was 101nmol/g wet wt. compared with a mean value of 173nmol/g for rat liver as reported by Allred & Guy (1969). Jarrett & Filsell (1964) have reported that sheep liver contains less CoA than rat liver.

DISCUSSION

The most noteworthy results presented in this paper are the remarkable changes in the [free carnitine]/[acetylcarnitine] ratio in the liver of the sheep when the animals were fed on a diet of mainly lucerne, in contrast with animals grazed on open pastures, and the striking increase in total acid-soluble carnitine in the sheep liver on starvation. These two findings are discussed in turn.

In the sheep that were fed *ad libitum* on lucerne, the [free carnitine]/[acetylcarnitine] ratio in the liver was 6:1. In the rat, the [free carnitine]/[acetylcarnitine] ratio in the liver of animals fed on a normal diet is reported to be 3:1 by Bøhmer (1967) and 4:1 by Pearson & Tubbs (1967). Thus the ratio in the liver of these sheep indicated an even greater proportion of free carnitine than is observed in the rat. In these particular sheep the free carnitine plus acetylcarnitine was found to constitute 74% of the total acid-soluble carnitine in the liver. This is comparable with the situation in the rat (Pearson & Tubbs, 1967). Bøhmer & Bremer (1968) have shown a considerable proportion of the remaining acid-soluble carnitine (i.e. total minus free plus acetyl) in rat liver is propionylcarnitine and the results presented here tentatively suggest that most of the unspecified acid-soluble carnitine is also propionylcarnitine. It should be noted that the crude carnitine acetyltransferase of sheep liver, as reported here, shows a greater activity with the propionyl ester than the acetyl ester, as does the purified enzyme from pigeon breast muscle (Bøhmer & Bremer, 1968).

In the sheep grazed on open pastures the free carnitine plus acetylcarnitine in the liver is approximately equal to the total acid-soluble carnitine, whereas in the animals fed on lucerne *ad libitum* free carnitine plus acetylcarnitine represents only 74% of the total acid-soluble carnitine. Also, in the former group of sheep the [free carnitine]/[acetylcarnitine] ratio is approx. 1:1, in contrast with the value of 6:1 for the latter group of sheep. These changes are undoubtedly due to the different diets affecting the proportions of the various volatile fatty acids produced in the rumen of the sheep. A high rate of lucerne intake by sheep under the conditions used here results in large amounts of propionate being produced in the rumen (A. R. Egan, unpublished work). Thus the dramatic changes in the [free carnitine]/[acetylcarnitine] ratio reported here appear due to changes in the relative proportions of propionate and acetate reaching the liver from the rumen. Pearson & Tubbs (1967) have shown that perfusion of rat hearts with propionate induces an almost complete disappearance of acetylcarnitine from the heart-muscle tissue. A similar situation is observed here in the sheep liver but is undoubtedly of considerable physiological significance as far as the overall metabolism of the sheep is concerned.

The results presented here indicate that the metabolism of sheep liver is under much more 'acetyl pressure' than is the system in the rat liver, as even in the starved rat the [free carnitine]/[acetylcarnitine] ratio is never less than 1:1 (Pearson & Tubbs, 1967; Bøhmer, 1967). The effects of this 'acetyl pressure' in the sheep liver on the CoA system are probably of considerable physiological significance. Even in the sheep fed on lucerne the [free CoA]/[acetyl-CoA] ratio in the liver was only slightly greater than 1:1 and in the starved sheep the [free CoA]/[acetyl-CoA] ratio is 0.34, whereas in normal rat liver this ratio is approx. 4:1 in favour of free CoA (Allred & Guy, 1969). As the [free CoA]/[acetyl-CoA] ratio has an important controlling effect on metabolism generally (Tubbs & Garland, 1964; Wieland & Weiss, 1963; Garland *et al.* 1965), and the CoA system is under considerable 'acetyl pressure' in sheep liver, the buffering role of the carnitine system would appear to be of particular importance in the starved sheep. Under these conditions the amount of acetyl-CoA in the liver is the same as in the normal sheep, but the content of acetylcarnitine is five times greater after starvation. Also, in the starved sheep an apparent equilibrium constant for the reaction:

$$\text{Acetyl-L-carnitine} + \text{CoA} \leftrightarrow \text{acetyl-CoA} + \text{L-carnitine}$$

may be calculated from the data in Table 3 to be 1.3. Fritz, Schultz & Srere (1963) determined the apparent equilibrium constant for carnitine acetyltransferase, that has been partially purified from

pig heart, to be 0.6 at pH7.0. If one assumes a similar equilibrium constant for the sheep liver enzyme, then the system in the starved sheep liver would appear to be close to equilibrium. The situation in the normal sheep liver is less clear and is complicated by the fact that the carnitine acetyltransferase also reacts quite strongly with the propionyl ester.

The results discussed above thus seem to point to an important role for the relatively high activity of the carnitine acetyltransferase in sheep liver. The physiological function of this enzyme is uncertain (see Fritz, 1967). However, the results presented here for the starved sheep strongly suggest that the presence of carnitine acetyltransferase in large amounts in sheep liver allows the 'acetyl pressure' in the starved condition to be shifted from the vital CoA system to the carnitine system. This is probably true in other ruminant species. A similar 'buffering' role for carnitine acetyltransferase has been suggested by Pearson and Tubbs (1967) on the basis of studies in perfused rat heart.

It is difficult to envisage any other function than that of an 'acetyl buffer' system as suggested here for the high activity of carnitine acetyltransferase. Very little synthesis of fatty acids takes place in ruminant liver compared with rat liver (Hanson & Ballard, 1967), and fatty acid synthesis occurs in the cytoplasm, mainly from acetate, whereas carnitine acetyltransferase is associated with the inner mitochondrial membrane in sheep liver mitochondria (G. D. Henderson, unpublished work). Homogenates of sheep liver are capable of oxidizing acetate (Mayfield, Smith & Johnson, 1965) and the results presented above are certainly indicative of acetate utilization, although the main oxidation of acetate in the whole animal would appear to be by extrahepatic tissues (Mayfield, Bensadoun & Johnson, 1966). The oxidation of short-chain fatty acids is considered to be carnitine-independent, at least in the rat (Bremer, 1967).

However, carnitine does increase acetate oxidation in sheep liver homogenates (Mayfield *et al.* 1966) and we find isolated sheep liver mitochondria do not oxidize acetate but oxidize acetylcarnitine very slowly (Koundakjian & Snoswell, 1970). Thus carnitine acetyltransferase may have a minor role in acetate oxidation.

The other particularly striking feature of the results presented here is the remarkable increase in total acid-soluble carnitine that is seen in the sheep liver on starvation (nearly fivefold). This is in marked contrast with the rat, where only a 10–20% increase is seen on starvation (Pearson & Tubbs, 1967). As suggested above, this increase in total acid-soluble carnitine on starvation is probably related to the role of carnitine acetyltransferase in relieving 'acetyl pressure' on the CoA system in the sheep liver. Free fatty acid turnover, giving rise to acetyl-CoA, increases markedly on starvation (Katz & Bergman, 1969) and acetylcarnitine is the largest fraction of the total acid-soluble carnitine. However, free carnitine also increases in starvation in the sheep liver. Also the amount of total acid-soluble carnitine was found to increase similarly or even to greater extent in an alloxan-diabetic sheep.

The striking increases in total acid-soluble carnitine in the sheep liver on starvation imply that there is synthesis of carnitine under these conditions. The values presented here are given in nmol/g wet wt. of tissue; and, although the weight of sheep liver increases by approx. 13% after 5 days starvation (O. H. Filsell & I. G. Jarrett, unpublished work), the increase in total acid-soluble carnitine on starvation is still over fourfold, even allowing for the slight increase in liver weight.

Little is known of the biosynthesis of carnitine, apart from the source of the methyl groups (Bremer, 1962), and it would appear that these remarkable changes in the amounts of carnitine in sheep liver indicate an important system for the study of the biosynthesis of carnitine and its control.

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