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Tissue Metabolism of Methionine in Sheep

N. J. Benevenga,[A,B] *B. C. Radcliffe,*[A] *and A. R. Egan*[A,C]

[A] Agronomy Department, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, S.A., 5064.
[B] Present address: Department of Meat and Animal Science, and Department of Nutritional Sciences, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.
[C] Present address: School of Agriculture and Forestry, University of Melbourne, Parkville, Vic. 3052.

Abstract

The rate of oxidization of the carboxyl and methyl carbons of [^{14}C]methionine to CO_2 by homogenates of liver, kidney cortex, pancreas, muscle and small intestinal mucosa was studied in two breeds of sheep (Merino and Poll Dorset Horn) at three ages (2 weeks, 3 months, 4 years). Sodium α-keto-γ-methiolbutyrate (0·4 mM) stimulated production of CO_2 from the carboxyl carbon of methionine, but not from the methyl carbon. Sodium pyruvate did not affect the recovery of CO_2 from either carboxyl or methyl of methionine. Sodium formate (15 mM) suppressed the conversion of the methyl carbon of methionine to CO_2 by liver and kidney homogenates to 4 and 50%, respectively, of control values, but did not affect the percentage of carboxyl carbon of methionine recovered in CO_2 with either tissue. With addition of *S*-methyl-L-cysteine (40 mM) and 3-methylthiopropionate (10 mM) the percentage of methyl and carboxyl carbons recovered in CO_2 was reduced to about 20% of control values in homogenates of both tissues.

Activity per gram of tissue was higher in liver and kidney cortex than in pancreas, intestinal mucosa, or muscle, with no significant differences due to breed (Merino or Poll Dorset Horn) or sex (ewe, ram or wether) of sheep. Conversion of both the carboxyl and methyl carbons to CO_2 by liver was significantly lower in 2-week-old lambs than in older animals ($P<0·01$). The activity of other tissues was not markedly affected by age.

Results are discussed in relation to evidence of alternative pathways of methionine catabolism, and capacities of the tissues of the sheep to catabolize methionine by alternative pathways.

Introduction

When given supplements of methionine or cyst(e)ine post-ruminally, sheep fed roughage diets often increase rate of wool growth (Reis and Schinkel 1963, 1964; Downes *et al.* 1975; Reis 1979). Methionine infused post-ruminally can increase nitrogen retention or growth rate (Bird and Moir 1972; Fennessy 1976; Wheeler *et al.* 1979). These observations have been related to the relatively small amounts of *S*-containing amino acids present in the proteins normally digested in the sheep intestine (Hogan 1975; Egan and Walker 1975), and to the high cystine content of wool. Methods for provision of more *S*-amino acids for intestinal absorption have centred on the use of methionine and its derivatives (Ferguson 1975), presumably because methionine is the indispensable *S*-amino acid, and in the animal it can provide the sulfur for synthesis of cyst(e)ine through the transsulfuration pathway (Finklestein 1970). However, methionine absorbed in excess (e.g. 6 g/day) results in decreases in feed intake, nitrogen retention, growth, and wool production (Fennessy 1976; Reis 1979; Hoey 1981). When methionine is provided at three to four times the accepted daily requirement, adverse effects have been reported in many studies with several animal species (Harper *et al.* 1970; Benevenga 1974). The tissue damage described for sheep subjected to very high methionine provision in the *diet* (Doyle and Adams 1980)

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is similar to that described in rats and chickens (Harper *et al.* 1970; Harter and Baker 1978). The methionine requirement of sheep is not defined, nor has the ability of the sheep to convert methionine sulfur to cysteine sulfur at different levels of methionine supply been established. The distribution of two enzymes of the transsulfuration pathway among the tissues of the sheep has been described (Radcliffe and Egan 1974, 1978), but tissue capacity to catabolize methionine by this or other pathways has not been assessed.

Alternative pathways of degradation of methionine, which involves transamination, have been reported in the rat, monkey and pig (Case and Benevenga 1976; Mitchell and Benevenga 1978; Steele and Benevenga 1978; Benevenga and Haas 1979). Steele and Benevenga (1979) have suggested that toxicity of methionine is due to intermediates or products of the transamination pathway (e.g. methylthiopropionate, methanethiol or hydrogen sulfide—see Fig. 1). If degradation of methionine proceeds through two pathways in sheep, one yielding cyst(e)ine, the other toxic intermediates of methionine catabolism, this could have important implications in any approach to manipulate methionine absorption to optimize the supply of *S*-amino acid for wool growth.

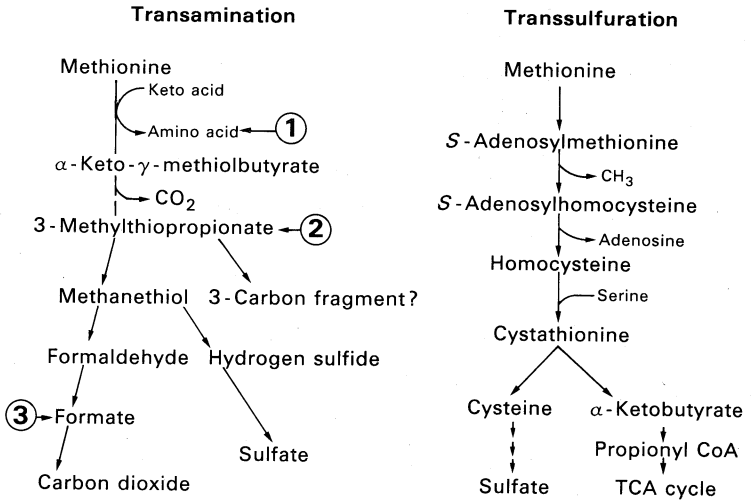

Fig. 1. Comparison of the transamination pathway of methionine degradation with the (well recognized) transsulfuration pathway of methionine degradation. Arrows show points where *S*-methyl-L-cysteine (1), 3-methylthiopropionate (2) or formate (3) are thought to inhibit methionine metabolism *in vitro*.

In the studies reported here, the abilities of sheep liver, kidney cortex, pancreas, muscle, and intestinal mucosa to convert the methyl carbon and carboxyl carbon of methionine to CO_2 were examined using tissue homogenates. This first step, in which tissue distribution of oxidative capacity and the response to inclusion of inhibitors or trapping-pools in the incubation mixture were examined, provides evidence for the existence of alternative pathway(s) of methionine catabolism in the liver and kidneys of sheep. The effects of age or breed on the catabolism of methionine in various tissues were also examined.

Materials and Methods

Animals

In preliminary studies to develop the *in vitro* incubation system, liver was obtained from mature Poll Dorset Horn × Merino wethers and ewes from the Waite Agricultural Research Institute flock.

Experiment Series A

Pure-bred Merino and Poll Dorset Horn sheep were held on good quality grass–legume pasture in the spring growth period. Tissues taken at slaughter from male or female animals in three age classes were used to study *in vitro* the oxidation of the methyl and the carboxyl carbons of methionine in liver, kidney, intestinal mucosa, pancreas and skeletal muscle. Two-week-old suckled lambs were taken from the ewes immediately before slaughter. Three-month-old animals had been weaned for at least 6 weeks and each had well-developed rumens. Mature animals were all approximately 4 years of age. No animal was slaughtered because of obvious deformity or health abnormality. The Poll Dorset Horn males were entire, whereas the Merinos at 3 months and older were castrated males. The age, number and sex of the animals used is shown in Table 1.

Table 1. Age, breed, sex and number of the sheep used in the study

Age	Merino[A]			Poll Dorset[B]	
	Ewe	Wether	Ram	Ewe	Ram
2 weeks	3	—	3	3	3
3 months	3	3	—	3	3
4 years	3	3	—	3	1

[A] Two-week-old animals were obtained from Mr John Burton, Jamestown, S.A. The others were from the University flock.
[B] Animals kindly provided by Mr Malcolm Piggott, 'Illoura', S.A.

Experiment Series B

Mature Dorset × Merino wethers and ewes drawn from a flock grazing good quality grass–legume spring pasture were slaughtered and liver and kidney tissue used *in vitro* in studies on metabolite inhibition of methionine oxidation.

Tissue Collection and Preparation

Sheep were killed by exsanguination and the liver, left kidney (experiment series A and B), duodenum, pancreas, and semitendinosis muscle (experiment series A only) were rapidly excised. The liver was weighed and a portion (*c.* 100 g) taken from the tip of the ventral lobe. The left kidney was weighed after removal of the fat and capsule and a sample of the cortex (*c.* 20 g) was retained. Preliminary studies had shown that the conversion of the carboxyl carbon of methionine to CO_2 in homogenates of kidney medulla was about one-tenth the rate of the cortex; mixtures of cortical and medullary tissue were thus avoided. The pancreas was freed of adherent tissue and weighed. Liver, kidney cortex and pancreas samples were coarsely chopped with scissors. In experiment series A the proximal duodenum (a 30-cm section) was washed with 0·9% (w/v) NaCl solution, slit longitudinally, re-washed with saline, gently blotted dry, and the mucosa scraped off with a microscope slide. The complete left semitendinosis muscle was removed and weighed. A central cross-sectional portion was chopped several times with a Mickle tissue chopper (The Mickle Laboratory Engineering Co., Gomshall, Surrey, England), set at 0·4 mm.

Tissues were kept on ice and were homogenized within 15 min of slaughter in 4 volumes of ice-cold 0·25 M sucrose in a Potter–Elvehjem glass–Teflon homogenizer kept in ice.

Incubation System

All reactions for experiment series A and B were carried out in 50-ml Erlenmeyer flasks (Corning 4980) closed with a No. 33 Subaseal stopper to which was attached a stainless-steel spring wire coil which projected into the gas space in the flask. Carbon dioxide was collected in a polypropylene test tube (10 by 25 mm) containing 0·5 ml of a 1 : 1 mixture of ethanolamine–methylcellosolve, the tube being suspended in the wire coil over the reaction mixture.

In homogenates of liver and kidney, 3-methylthiopropionate, at concentrations which varied from 0·25 to 10 mM (Fig. 3), suppressed the rate of production of $^{14}CO_2$ from both methyl and carboxyl carbons of methionine, the maximum depression being to 20% of control values.

Incorporation of 2–40 mM *S*-methyl-L-cysteine into the reaction mixture also resulted in a similar reduction of the rate of $^{14}CO_2$ production from both methyl and carboxyl carbons of methionine for both liver and kidney homogenates (Fig. 3). The inhibition observed at 10–20 mM *S*-methyl-L-cysteine suggests that the maximum inhibition would be 20% of control values.

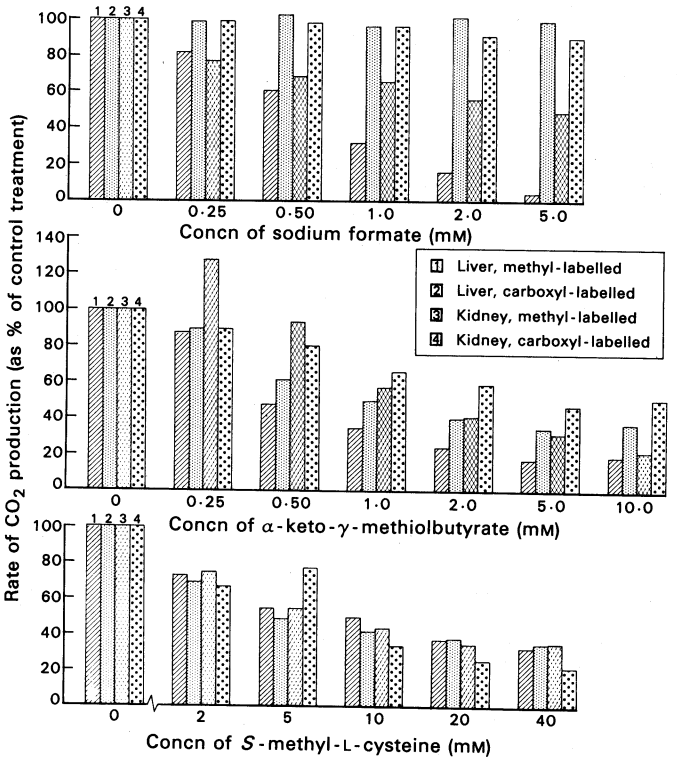

Fig. 3. Effects of 0–5 mM sodium formate, 0–10 mM 3-methylthiopropionate (MTP), or 0–40 mM *S*-methyl-L-cysteine (SMC) on recovery of $^{14}CO_2$ from carboxyl- or methyl-labelled methionine with homogenates of adult sheep liver and kidney cortex. Zero concentrations are controls.

Effect of Age on Methionine Metabolism in Tissues from Merino and Poll Dorset Sheep (Experiment Series A)

The hourly rates (nmole/g) of conversion of the labelled methyl or carboxyl carbon of methionine to $^{14}CO_2$ by homogenates of five tissues from Merino sheep are presented in Fig. 4. There were no significant differences associated with breed or sex. Rates of $^{14}CO_2$ production from the carboxyl or methyl carbon of methionine by liver homogenates from 2-week-old lambs were significantly lower than those with 3-month-old or 4-year-old sheep ($P<0·01$). There was no evidence of increased activity per gram of liver in sheep older than 3 months of age.

The capacity for the conversion of the carboxyl carbon of methionine to $^{14}CO_2$ in liver and kidney homogenates was relatively high in sheep in all age groups. Conversely the rate of oxidation of the methyl carbon of methionine was very low in homogenates of liver and kidney from 2-week-old lambs. In ruminant lambs three months of age, the rate

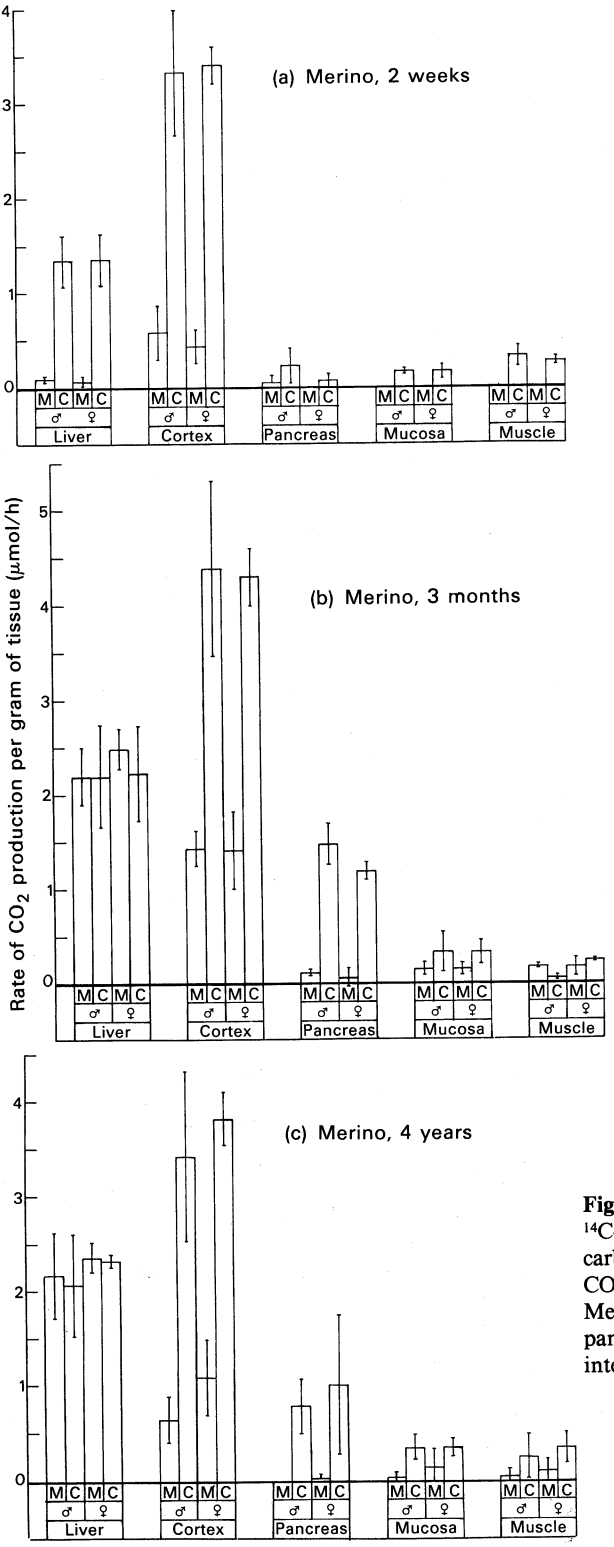

Fig. 4. Rate of conversion of ^{14}C-labelled methyl (M) and carboxyl (C) of methionine to CO_2 by tissue homogenates from Merino sheep liver, kidney, pancreas, mucosa of the small intestine, and muscle.

of $^{14}CO_2$ production from the methyl carbon of methionine in liver homogenate was equal to that from the carboxyl carbon. The rate of oxidation of the methyl carbon of methionine by kidney homogenates from 3-month-old lambs was threefold that observed with kidney homogenate from 2-week-old lambs. Despite this, the rate of $^{14}CO_2$ production from methyl carbon was still only 30% of that for the carboxyl carbon of methionine. Of the other tissues examined, only homogenates of pancreas showed relatively rapid rates of $^{14}CO_2$ production from carboxyl carbon of methionine, approximately one-half to one-fifth the rate exhibited by homogenates of liver and kidney cortex.

Discussion

Experiments on inhibition of methionine catabolism were undertaken with liver and kidney cortex taken from mature crossbred (Merino × Dorset) sheep because other tissues displayed much lower rates of methionine oxidation.

In Fig. 1, two metabolic pathways of methionine degradation are shown, and the steps at which *S*-methyl-L-cysteine (SMC), 3-methylthiopropionate (MTP) and sodium formate are believed to inhibit metabolism are indicated. SMC is a competitive inhibitor of methionine catabolism (Case and Benevenga 1976), possibly effective at the transamination step. Formate and MTP have been identified as intermediates in methionine degradation by the transamination pathway in rats (Case and Benevenga 1976; Mitchell and Benevenga 1978; Steele and Benevenga 1978, 1979). Inhibition by these two compounds of $^{14}CO_2$ production from ^{14}C-labelled methionine may thus be either by the dilution of the pool of the respective intermediates, or by inhibition of activity of enzyme(s) leading to their production.

The effect of formate on the conversion of the methyl carbon of methionine to CO_2 in homogenates of liver and kidney cortex of sheep (Fig. 3) is similar to that reported in the rat (Case and Benevenga 1976) and the pig (Benevenga and Haas 1979). No attempt was made in this experiment to recover ^{14}C-labelled formate from the incubation mixture when either [^{14}C-methyl]- or [^{14}C-carboxyl]methionine was the substrate. However, the extent of inhibition of CO_2 production from the methyl carbon of methionine by formate in the liver is consistent with the conversion of the methyl carbon to CO_2 via formate in the sheep. Although formate substantially depressed the conversion of the methyl carbon of methionine to CO_2 by homogenates of kidney cortex, the extent of inhibition was much less than that observed with liver homogenates. This is the first report of marked difference between homogenates of liver and kidney in suppression by formate of CO_2 production from the methyl carbon of methionine. This difference may indicate that another pathway for conversion of the methyl carbon of methionine to CO_2 is present in the kidney cortex, or that a volatile intermediate such as methanethiol is formed more rapidly in kidney. Methanethiol is thought to be a precursor of formate in the transamination pathway, and could also be recovered in the ethanolamine trap (Steele and Benevenga 1979).

Addition of MTP depressed $^{14}CO_2$ production from both [^{14}C-methyl]- and [^{14}C-carboxyl]methionine by sheep liver and kidney homogenates. The percentage depression in recovery of the methyl carbon in CO_2 was greater than that for the carboxyl carbon of methionine at each graded level of MTP in both liver and kidney homogenates. It has been shown that MTP inhibits metabolism of KMBA in rat liver (Steele and Benevenga 1978) and that labelled carbon from methyl-labelled methionine can be recovered in MTP isolated from the incubation mixture with rat liver homogenates (Steele and Benevenga 1978). Hence our results with sheep liver and kidney homogenates are consistent with inhibitory effects of MTP at both the level of the conversion of the carboxyl carbon of KMBA to CO_2, and at the level of MTP oxidation by expanding the MTP pool and diluting the labelled methyl carbon in that pool.

The effect of SMC on the conversion of the methyl and carboxyl carbons of methionine to CO_2 was broadly similar to the effect of MTP, although the concentration of SMC required was much greater and there was no evidence of a greater effect on the methyl carbon than on the carboxyl carbon.

Overall, the differential inhibitory effects of formate, MTP and SMC on the metabolism of the methyl and carboxyl carbons of methionine in liver and kidney homogenates from the sheep do not support the hypothesis that the transsulfuration pathway is the sole major pathway for methionine catabolism. Observations are consistent with the operation of the transamination pathway for methionine catabolism (Fig. 1) described in the rat (Case and Benevenga 1976; Mitchell and Benevenga 1978; Steele and Benevenga 1978, 1979). The means whereby CO_2 is produced from both carboxyl and methyl carbons of methionine after maximum inhibition by these three inhibitors, particularly in kidney cortex homogenates, remains to be elucidated.

The rates of methionine oxidation in different tissues of sheep (Fig. 4) form a pattern similar to that described in the rat (Mitchell and Benevenga 1978). This reflects poorly the distribution of methionine adenosyltransferase (EC 2.5.1.6) and cystathionine-γ-lyase (EC 4.4.1.1) activities between tissues of adult sheep (Radcliffe and Egan 1974), but is similar to that reported for these two enzymes in the adult rat (Finkelstein 1967). More work is required to determine whether the tissue distribution of enzymes associated with transsulfuration and transamination pathways of methionine metabolism can be correlated with observed metabolism of specifically labelled methionine by tissue homogenates.

Table 2. Estimated capacity of sheep organ homogenates for the oxidation of the carboxyl or methyl carbons of methionine to CO_2

Total organ activity was calculated from specific activity of the homogenate and organ weights. The value for kidney is for both kidneys assuming that two-thirds of the kidney weight is cortex. The value for muscle was obtained by using the activity per gram of semitendinosis muscle and the regression equations of Tulloh (1964) where muscle mass is related to empty body weight (i.e. log muscle weight = 0·544 + 1·018 log empty body weight). Values given are means ± s.d.

Sheep breed	Age	Organ capacity (μmol/h) Liver	Kidney	Pancreas	Muscle
		Carboxyl carbon oxidation			
Merino[A]	2 weeks	240± 60	160±30	1± 1	750± 125
	3 months	1050±120	450±90	40±10	850± 580
	4 years	1750±370	510±60	60±20	4300±2650
Poll Dorset Horn	2 weeks	250± 75	170± 60	10± 10	630± 60
	3 months	1200±340	520±130	40± 10	1400± 840
	4 years	1770±370	600±200	160±140	2020±1560
		Methyl carbon oxidation			
Merino	2 weeks	13± 8	25±11	0·2±0·4	3± 7
	3 months	1100± 90	150±35	2±2	1300±550
	4 years	1880±370	120±55	1±1	580±770
Poll Dorset Horn	2 weeks	46± 56	29±18	11±23	210± 440
	3 months	1280± 60	150±90	0·6± 1·0	1080±1385
	4 years	1600±540	100±78	3± 6	85± 168

[A] $N = 6$ for all experiments except for Poll Dorset Horn 4 years where $N = 4$.

Age, but not breed or sex, appeared to influence the capacity of homogenates of liver and of kidney to oxidize the methyl carbon of methionine to CO_2, the rate being far lower for tissues from 2-week-old lambs than in those from ruminant lambs 3 months of age (Fig. 4). There was also a 1·5–2·0-fold difference in the rate of conversion of the carboxyl carbon of methionine to CO_2 in homogenates of liver and kidney from the 2-week- and 3-month-old lambs. These results can be compared with the general pattern of development of methionine adenosyltransferase in the liver and kidney of the rat (Finkelstein 1967) and of cystathionine-γ-lyase in the liver and kidney of the rat (Finkelstein 1967) and the sheep (Radcliffe and Egan 1974). Finkelstein (1967) found no change with age in the specific

activity of methionine adenosyltransferase in homogenates of kidney from the rat, whereas that in the liver of young rats was 40% lower than that in liver from adult rats. Similar comparisons made for cystathionine-γ-lyase in homogenates from the liver and kidney showed that the activities of tissues from young rats were half those in tissues from adults. Radcliffe and Egan (1974) reported that the specific activity of methionine adenosyltransferase in the kidney of 2-week-old lambs was twice that of the 4-year-old sheep whereas activity of the same enzyme in liver from the lamb was less than half that in the older sheep. The activity of cystathionine-γ-lyase in both the kidney and liver of adult sheep was only about one-tenth the activity in the respective tissues from neonatal lambs.

The divergence between the reported activity of some enzymes of the transsulfuration pathway and the pattern of activity observed in these experiments for the oxidation of the methyl and carboxyl carbons of methionine lends further support to the view that the transsulfuration pathway of methionine metabolism, suggested as the major catabolic pathway of methionine (Finkelstein 1970), cannot alone account for methionine catabolism.

On the basis of organ weights, 70–80% of the sheep's capacity for methionine oxidation is in liver and muscle (calculated as in Table 2). The capacity of muscle tissue for methionine decarboxylation is low on a per unit weight basis (see Fig. 4) but could account for 30–50% of the whole-body capacity for methionine decarboxylation. However, the concentration of substrates and co-factors used in studies on rates of catabolism by tissue homogenates are not those of the tissues *in situ*. Furthermore homogenization of a tissue removes cellular and some subcellular barriers. Thus reactions, or rates of reaction may be observed *in vitro* that are not possible *in vivo*. If the potential rate of catabolism of the methyl or carboxyl carbons of methionine is estimated as in Table 2, adding the activities of liver, kidney, pancreas and muscle, this estimated tissue capacity for methionine catabolism exceeds by 100- to 1000-fold the rate of irreversible loss of methionine measured in intact sheep (Fennessy *et al.* 1978). Therefore studies on methionine catabolism by organs *in situ* are necessary to gain quantitative information on site, rate and pathway of methionine catabolism.

The results reported here provide evidence for existence of *S*-adenosylmethionine-independent metabolism of methionine in sheep. The inhibitor studies are consistent with the view that the transamination pathway for methionine degradation is present in sheep. Though further work is now essential to determine directly the quantitative significance of the transsulfuration pathway and the *S*-adenosylmethionine-independent pathway(s) in the whole animal, our results support the view that intermediates formed in the transamination pathway, which are known to be toxic, may be responsible for adverse effects of high or even moderate levels of methionine supplementation for intestinal absorption in sheep.

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