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A Survey of Methionine Adenosyltransferase and Cystathionine γ-Lyase Activities in Ruminant Tissues

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Abstract

The activities of two key enzymes, methionine adenosyltransferase (EC 2.5.1.6) and cystathionine γ-lyase (EC 4.4.1.1), involved in the metabolism of methionine to cyst(e)ine have been studied in the liver, heart, kidney medulla, kidney cortex, pancreas, duodenal wall, spleen and skeletal muscle in the neonatal lamb, unweaned lamb, adult sheep, pre-ruminant calf, ruminant steer and adult goat, and for comparative purposes in the adult rat.

Methionine adenosyltransferase was widely distributed in the tissues of the ruminant species examined, with specific activities in the order of 10^2 nmol *S*-adenosylmethionine formed per milligram protein per 30 min. Greatest activities were found in liver, kidney, spleen and duodenal wall in adult ruminants, and also in skeletal muscle in pre-ruminant lambs and calves. Hepatic methionine adenosyltransferase activity declined with age in both sheep and cattle.

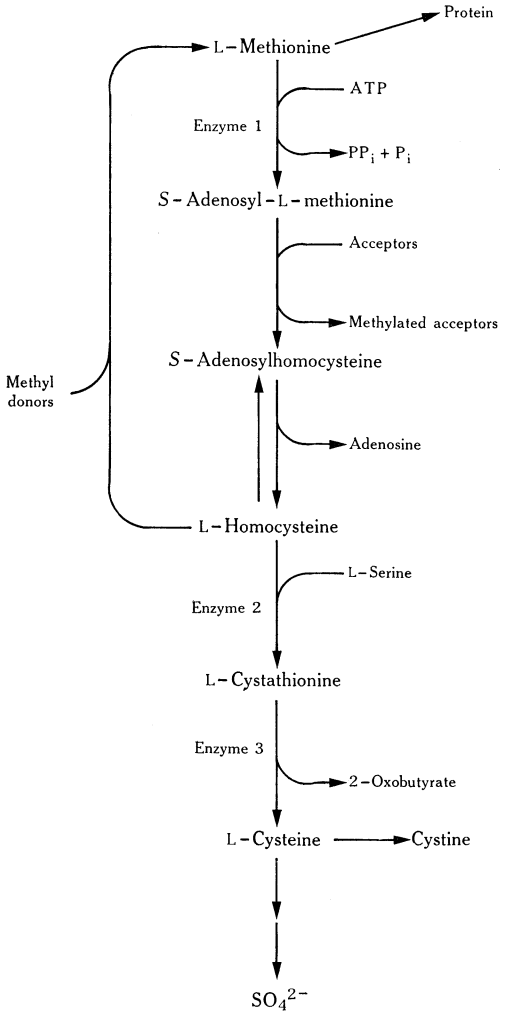
Cystathionine γ-lyase activity was not demonstrable in skeletal muscle or heart in any of the species examined. Greatest activities (in the order of 10 nmol cystathionine degraded per milligram protein per 30 min) were found in liver, kidney and pancreas for all species. Neonatal lamb tissues had the highest activities. In sheep specific activities in liver, pancreas, kidney cortex and spleen declined with age.

Introduction

Both direct and indirect evidence indicate that sulphur-containing amino acids are of critical importance in the nutrition of ruminants, and more specifically that methionine is frequently the first limiting amino acid for sheep (Barry *et al.* 1973; Egan and Walker 1973; Schelling *et al.* 1973).

Methionine is an essential amino acid necessary for protein synthesis, but is also converted to *S*-adenosylmethionine, the primary methyl group donor of mammalian metabolism (Mudd and Cantoni 1964), and is a source of cyst(e)ine for incorporation into proteins, including keratin. In sheep this last pathway may constitute a major drain on available methionine, since cystine is a major constituent of wool. Wool has a nitrogen : sulphur ratio of 4–6 (Spector 1956) compared with body protein, which has a nitrogen : sulphur ratio of about 15, and rumen bacterial protein, which is variously reported to have a nitrogen : sulphur ratio of from 13 (D. J. Walker and C. M. Nader, personal communication) to 20–24 (Bird 1973). It has been shown that wool growth does respond to post-ruminal supplements of methionine or cystine (Reis and Schinckel 1963, 1964; Reis *et al.* 1973*a*).

Comparatively little is known about the metabolism of methionine in sheep and other ruminants at the cellular level. The transsulphuration pathway (Fig. 1) whereby methionine is converted to cyst(e)ine has been well established in rat and human



tissues (Finkelstein 1970). Although Downes *et al.* (1964) observed that [^{35}S]methionine injected intradermally was incorporated into wool at the injection site as [^{35}S]cystine, the relative activities of the enzymes in the transsulphuration pathway in ruminant tissues have not been systematically studied.

The present study was undertaken to survey the levels of two of the enzymes in the transsulphuration pathway in various tissues of a number of ruminant species. The two enzymes are the first one in the pathway, ATP : L-methionine *S*-adenosyltransferase (methionine adenosyltransferase, EC 2.5.1.6), and L-cystathionine cysteine-lyase (deaminating) [cystathionine γ-lyase, EC 4.4.1.1; also formerly known as L-homoserine hydro-lyase (deaminating) (EC 4.2.1.15)].

Fig. 1. Transsulphuration pathway in mammalian tissue (after Finkelstein 1970). Enzymes 1, 2 and 3 are methionine adenosyltransferase, cystathionine β-synthase (not studied) and cystathionine γ-lyase respectively.

Materials and Methods

Animals

Sheep. The three adult sheep (2–3 years old) used were a pasture-fed Suffolk wether and two penned wethers fed lucerne, one a Merino and the other a Dorset × (Merino × Dorset) crossbred. Three pasture-fed crossbred lambs [Dorset × (Merino × Dorset)], one male and two female, were slaughtered at 3–4 months of age (weight 30–35 kg). Three female neonatal lambs, each the smaller of a set of twins and half-sibs of the lambs described above, were less than 48 h old and weighed 3·0–3·4 kg.

Goats. Both goats were male, of mixed breed, 5 years old, and pasture-fed. Goat 2 had been fasted for 1 week before slaughter.

Cattle. Both calves were milk-fed Friesian males, 3 weeks old, and weighed 53–56 kg. Four Hereford × Shorthorn cross steers were 15–18 months old and weighed about 250 kg.

Rats. Three female Hooded Wistar rats, each weighing about 150 g, were fed on a pelleted rat diet (Charlick's Mouse Cubes, Adelaide) from weaning.

Sample Preparation

Tissues from all animals were taken and treated similarly; tissue samples were removed immediately after slaughter and placed in ice-cold 0·9% KCl containing 0·1 mM disodium EDTA. Each

sample was minced finely with a razor blade, and then homogenized in two volumes of cold 0·9% KCl–0·1 mM disodium EDTA in a Potter–Elvejhem glass–Teflon homogenizer. The homogenate was centrifuged at 8000 *g* for 2 min to remove cell debris. Sample preparation was carried out as rapidly as possible and the methionine adenosyltransferase assays were begun within 2 h of sample removal.

Enzyme Assays

Methionine adenosyltransferase activity was assayed by the method of Lombardini *et al.* (1970) as modified by A. M. Snoswell and G. Henderson (personal communication). The assay mixture consisted of a premixed incubation medium containing, in 500 μl: tris acetate (pH 8·2), 100 μmol; magnesium acetate, 100 μmol; L-methionine, 10 μmol; ATP, 5 μmol; [8-^{14}C]ATP, 0·2 μmol containing 150 × 10^3 disintegrations/min; dithiothreitol, 2 μmol. To 500 μl of incubation medium were added yeast inorganic pyrophosphatase, 2 units; tissue homogenate, 100–200 μl; and water to a final volume of 1 ml. The reaction was begun by adding the tissue homogenate and the mixture was incubated at 37°C for 30 min. At the end of the incubation period a 100-μl aliquot was removed, placed in a microcentrifuge tube containing 1 ml of water plus 200 μl of a suspension of ion-exchange resin [5 g of Dowex 1 × 10 (200–400 mesh) in 10 ml of water] and shaken thoroughly to adsorb any unreacted [^{14}C]ATP. The suspension was then centrifuged for 2 min at 8000 *g*. A 1-ml portion of the supernatant containing the [^{14}C]-*S*-adenosylmethionine formed in the reaction was removed and placed in a scintillation vial containing 10 ml of toluene–Triton (2 : 1) scintillation mixture (Patterson and Green 1965). A control with no tissue homogenate was included in each assay series. A standard sample of each batch of incubation medium was prepared and counted in a Packard scintillation counter with each lot of samples as an external standard.

Each batch of incubation medium was assayed enzymically for ATP concentration using hexokinase and glucose-6-phosphate dehydrogenase (Lamprecht and Trautschold 1963).

Enzyme activity was calculated from the equation: activity units (μmol) = c × (observed radioactivity − control radioactivity) ÷ radioactivity of ^{14}C standard, where c is the concentration (mM) of ATP in the reaction mixture and radioactivity is expressed as disintegrations per minute.

Cystathionine γ-lyase activity was assayed by a modification of the technique of Greenberg (1962). The reaction mixture contained, in a final volume of 1 ml: L-cystathionine, 1 μmol; pyridoxal phosphate, 5 μmol; mercaptoethanol, 7·5 μmol; disodium EDTA, 7 μmol; potassium phosphate (pH 7·5), 100 μmol; and tissue homogenate, 100 μl. The reaction was begun by adding the tissue homogenate and allowed to continue for 30 min at 37°C. It was terminated by adding 1 ml of 6% (w/v) perchloric acid and centrifuging down the precipitated protein. The cyst(e)ine formed was estimated by the acid ninhydrin method of Gaitonde (1967). A zero time control was carried out for each sample to correct for endogenous cyst(e)ine.

Protein was measured by the Biuret method (Dawson *et al.* 1969) with bovine serum albumin as standard. Results were analysed for statistical significance of differences using Student's *t*-test and analysis of variance.

Results and Discussion

Table 1 presents the specific activity of methionine adenosyltransferase in various tissues of a number of ruminant species and in rat tissue. The specific activities of the enzyme in the tissues of the rat were higher than those reported by Mudd *et al.* (1965), a difference possibly associated with differences in the respective assay systems used. Mudd *et al.* (1965) employed 0·118 mM methionine in their assay system compared with 10 mM methionine used here. Published values for the K_m for L-methionine with rat liver preparations range from 0·56 mM (Cox and Smith 1969) to 0·91 mM (Pan and Tarver 1967), and Mudd and Cantoni (1958) reported a K_m of 2·2 mM for L-methionine with a rabbit liver preparation. It is therefore likely that the activities reported by Mudd *et al.* (1965) were limited by substrate concentrations and hence were less than maximal.

Present results also differ from those of Mudd *et al.* (1965) in that readily measurable methionine adenosyltransferase activity was widely distributed in the tissues of the

rats and also of the ruminant species examined, and not mainly confined to the liver. While activities in liver were consistently high, those of spleen and kidney cortex were as great or greater in sheep at all ages, in adult goats, and in adult rats (whole kidney). The relative activity of methionine adenosyltransferase in skeletal muscle, cardiac muscle and kidney medulla varied widely. In the species examined, these tissues ranked from lowest activity relative to other tissues from the same animal to relatively high activities comparable to that of liver. This variability cannot be attributed, from these studies, to species, age or diet.

Table 1. Methionine adenosyltransferase activity in various tissues
Values are specific activity (nmol per milligram protein per 30 min) ± S.E.M.

Tissue	Goat: 1	Goat: 2[A]	Neonatal lambs ($n = 3$)	Lambs ($n = 3$)	Adult sheep ($n = 3$)	Calves ($n = 2$)	Steers ($n = 4$)	Rats ($n = 3$)	Rats[B]
Duodenum	130	390	69±20	150±17	227±223	128±0	306±67	458±248	1·1
Skeletal muscle	0	110	106±5	230±15	116±92	624±45	64±2[C]	50±4	n.d.[D]
Liver	80	50	247±23	103±24	98±52	927±2	230±37	146±21	17
Pancreas	140	190	72±4	40±6	76±25	110±9	52±2	242±122	4
Kidney cortex	130	200	115±14	187±13	196±72	116±2	102±23	507±113[E]	5[E]
Kidney medulla	190	50	17±8	70±15	68±38	74±26	51±13		
Heart	150	180	44±12	110±10	55±40	309±35	63±6	357±46	0·5
Spleen	180	500	194±6	437±29	525±287	89±9	87±2	437±75	1·2

[A] Goat 2 was fasted for 1 week before slaughter. [B] From Mudd *et al.* (1965).
[C] $n = 2$. [D] Not determined. [E] Whole kidney.

Table 2 presents the specific activities of cystathionine γ-lyase found in the various tissues. These values are of an order of magnitude similar to those reported by Mudd *et al.* (1965) in the rat. The highest activity was found in the liver of neonatal lambs. Unlike methionine adenosyltransferase, which was found to a greater or lesser extent in all the tissues assayed, cystathionine γ-lyase was undetectable in any sample of skeletal muscle and heart. The metabolism of methionine to cyst(e)ine is dependent upon the presence of a complete operative transsulphuration pathway, and it seems likely that this pathway is present in ruminant liver. Though not assayed in this study, cystathionine β-synthase activity has been reported by Mudd *et al.* (1965) at a level of activity about one-tenth that found in rat liver. Pancreas and kidney cortex are also rich in both methionine adenosyltransferase and cystathionine γ-lyase and may, like these tissues in the rat (Mudd *et al.* 1965), contain the entire mechanism of the transsulphuration pathway.

Finkelstein (1962) studied the effect of age on the activities of methionine adenosyltransferase, cystathionine γ-lyase and cystathionine β-synthase in rat tissue. Hepatic methionine adenosyltransferase activity declined with increasing age, while cystathionine β-synthase and cystathionine γ-lyase levels increased. Our data also show a decline in specific activity of methionine adenosyltransferase in liver when neonatal lambs are compared with 3–4-month-old lambs that were their half-sibs ($P < 0·05$).

This difference was also apparent in calves and older steers ($P < 0 \cdot 05$), although these were of different breeds. In contrast to observations in the rat (Finkelstein 1962), cystathionine γ-lyase activity in liver was higher in neonatal lambs than in older lambs ($P < 0 \cdot 01$).

Difference in diet rather than age alone may have contributed to observed differences in enzyme activities in the two classes of lambs. For example, a reduced cystathionine γ-lyase activity was found in rat liver when the dietary methionine supply was low and the methionine : cystine ratio was also low (Finkelstein and Mudd 1967). In contrast Shannon *et al.* (1972) reported a marked increase in hepatic cystathionine γ-lyase activity in the rat as the dietary methionine : cystine ratio decreased.

Table 2. Cystathionine γ-lyase activity in various tissues

Values are specific activity (nmol per milligram protein per 30 min) ± S.E.M.

Tissue	Goat: 1	Goat: 2[A]	Neonatal lambs ($n = 3$)	Lambs ($n = 3$)	Sheep ($n = 3$)	Calves ($n = 2$)	Steers ($n = 4$)	Rats[B]
Duodenum	4·4	1·3	0	10·7±1	14·9±1·4	0	0	4·3
Skeletal muscle	0	0	0	0	0	0	0	n.d.[C]
Liver	9·5	16·6	101±31	7·8±0·7	3·7±1·2	13·1±3·1	9·7±1·8	54
Pancreas	27·1	12·9	32±7	4·0±0	5·1±1·0	12·5±2·9	0	28
Kidney cortex	17·0	20·2	56±12	15·1±1·9	7·8±1·9	24·9±2·5	18·7±5·4	20[D]
Kidney medulla	4·7	0	0	19·3±5·2	5·3±2·9	4·7±4·7	18·2±5·3	
Heart	0	0	0	0	0	0	0	0
Spleen	3·2	1·5	13·2±2·5	3·3±0·7	0·87±0·60	0	2·0±0·4	0·02

[A] Goat 2 was fasted for 1 week before slaughter. [B] From Mudd *et al.* (1965).
[C] Not determined. [D] Whole kidney.

Reis *et al.* (1973*a*) found methionine to be less effective than cystine as a supplement promoting wool growth. Although the transsulphuration pathway permits the conversion of methionine to cyst(e)ine, the evidence suggests a rate-limiting step in that pathway.

Reis *et al.* (1973*b*) observed that when sheep were given abomasal infusions of large amounts of methionine, free methionine in plasma was elevated nearly 100-fold, but only relatively low concentrations of free taurine and cystine were to be found. They speculated that this apparent bottleneck in the metabolism of methionine may be due to a reduced availability of serine, since plasma serine concentration is reduced, or to a decrease in cystathionine β-synthase activity, or both. Daniel and Waisman (1969) observed such a decrease in cystathionine β-synthase activity in rats receiving large amounts of methionine. Another possible site for the control of the transsulphuration pathway is alteration of the activity of the first enzyme in the pathway, methionine adenosyltransferase. Such a control mechanism would also conserve ATP (Farber 1973). A study of the changes (if any) in the activity of enzymes in the transsulphuration pathway under various dietary conditions or with methionine loading in sheep is necessary to clarify the metabolism of methionine and its role in wool growth.

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References

Barry, T. N., Fennessy, P. F., and Duncan, S. J. (1973). Effect of formaldehyde treatment on the chemical composition and nutritive value of silage. III. Voluntary intake, live-weight gain, and wool growth in sheep fed the silages with and without intraperitoneal supplementation with DL-methionine. *N.Z. J. Agric. Res.* **16**, 64–8.

Bird, P. R. (1973). Sulphur metabolism and excretion studies in ruminants. XII. Nitrogen and sulphur composition of ruminal bacteria. *Aust. J. Biol. Sci.* **26**, 1429–34.

Cox, R., and Smith, R. C. (1969). Inhibition of *S*-adenosylmethionine formation by analogues of methionine. *Arch. Biochem. Biophys.* **129**, 615–19.

Daniel, R. G., and Waisman, H. A. (1969). Adaptation of the weanling rat to diets containing excess methionine. *J. Nutr.* **99**, 299–306.

Dawson, R. M. C., Elliot, D. C., Elliot, W. H., and Jones, K. M. (1969). 'Data for Biochemical Research.' p. 618. (Oxford University Press: London.)

Downes, A. M., Sharry, L. F., and Till, A. R. (1964). The fate of intradermal doses of labelled amino acids in sheep. *Aust. J. Biol. Sci.* **17**, 945–59.

Egan, A. R., and Walker, D. J. (1973). Resource allocation and ruminant protein production. Proc. 3rd World Conf. Anim. Prod., Melbourne. pp. 83–92. (CSIRO: Melbourne.)

Farber, E. (1973). ATP and cell integrity. *Fed. Proc.* **32**, 1534–9.

Finkelstein, J. D. (1962). Methionine metabolism in mammals. Effects of age, diet, and hormones on three enzymes of the pathway in rat tissues. *Arch. Biochem. Biophys.* **122**, 583–90.

Finkelstein, J. D. (1970). Control of sulfur metabolism in mammals. In 'Symposium: Sulfur in Nutrition'. (Eds O. H. Muth and J. E. Oldfield.) pp. 46–60. (Avi Press: Westport, Conn.)

Finkelstein, J. D., and Mudd, S. H. (1967). Transsulfuration in mammals: the methionine-sparing effect of cystine. *J. Biol. Chem.* **242**, 873–80.

Gaitonde, M. K. (1967). A spectro-photometric method for the direct determination of cyst(e)ine in the presence of other naturally occurring amino acids. *Biochem. J.* **104**, 627–33.

Greenberg, D. M. (1962). Cystathionine and homoserine cleavage. In 'Methods in Enzymology'. (Eds S. P. Colowick and N. O. Kaplan.) Vol. V, pp. 936–42. (Academic Press: New York and London.)

Lamprecht, W., and Trautschold, I. (1963). Adenosine-5′-triphosphate. Determination with hexokinase and glucose-6-phosphate dehydrogenase. In 'Methods of Enzymatic Analysis'. (Ed. H. U. Bergmeyer.) pp. 543–51. (Academic Press: New York.)

Lombardini, J. B., Coulter, A. W., and Talalay, P. (1970). Analogues of methionine as substrates and inhibitors of the methionine adenosyl transferase reaction. Deductions concerning the conformation of methionine. *Mol. Pharmacol.* **6**, 481–99.

Mudd, S. H., and Cantoni, G. L. (1958). Activation of methionine for transmethylation. III. The methionine activating enzyme of bakers' yeast. *J. Biol. Chem.* **231**, 481–92.

Mudd, S. H., and Cantoni, G. L. (1964). Biological transmethylation, methyl group neogenesis, and other "one-carbon" metabolic reactions dependent upon tetrahydrofolic acid. In 'Comprehensive Biochemistry. Vol. 15. Group Transfer Reactions'. (Eds M. Florkin and E. H. Stotz.) pp. 1–47. (Elsevier: Amsterdam.)

Mudd, S. H., Finkelstein, J. D., Irreverre, F., and Laster, L. (1965). Transsulfuration in mammals. Microassays and tissue distributions of three enzymes of the pathway. *J. Biol. Chem.* **240**, 4382–92.

Pan, F., and Tarver, H. (1967). Comparative studies on methionine, selenomethionine, and their ethyl analogues as substrates for methionine adenosyl transferase from rat liver. *Arch. Biochem. Biophys.* **119**, 429–34.

Patterson, M. S., and Green, R. C. (1965). Measurement of low energy β-emitters in aqueous solution by liquid scintillation counting of emulsions. *Anal. Chem.* **37**, 854–7.

Reis, P. J., and Schinckel, P. G. (1963). Some effects of sulphur-containing amino acids on the growth and composition of wool. *Aust. J. Biol. Sci.* **16**, 218–30.

Reis, P. J., and Schinckel, P. G. (1964). The growth and composition of wool. II. The effect of casein, gelatin and sulphur-containing amino acids given per abomasum. *Aust. J. Biol. Sci.* **17**, 532–47.

Reis, P. J., Tunks, D. A., and Downes, A. M. (1973*a*). The influence of abomasal and intravenous supplements of sulphur-containing amino acids on wool growth rate. *Aust. J. Biol. Sci.* **26**, 249–58.

Reis, P. J., Tunks, D. A., and Sharry, L. F. (1973*b*). Plasma amino acid patterns in sheep receiving abomasal infusions of methionine and cystine. *Aust. J. Biol. Sci.* **26**, 635–44.

Schelling, G. T., Chandler, J. E., and Scott, G. C. (1973). Postruminal supplemental methionine infusion to sheep fed high quality diets. *J. Anim. Sci.* **37**, 1034–9.

Shannon, B. M., Howe, J. M., and Clark, H. E. (1972). Interrelationships between dietary methionine and cystine as reflected by growth, certain hepatic enzymes, and liver composition of weanling rats. *J. Nutr.* **102**, 557–62.

Spector, W. S. (1956). 'Handbook of Biological Data.' pp. 90–1. (Saunders: Philadelphia.)

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