Toxicities of Methionine and Other Amino Acids

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The means whereby consumption of excessive levels of methionine leads to a reduction in growth and tissue damage was discussed. It was concluded that the effects of methionine on the transport of other amino acids could not account for the toxicity of methionine. The adverse effects due to consumption of excessive levels of methionine appear to be due to an aberrant metabolism of the methyl group. Studies, both *in vitro* and *in vivo,* indicate that at least three pathways are involved in the conversion of the methyl group to CO_2. One involves the synthesis of phospholipid choline and is apparently saturated at low concentrations of methionine. Another involves the formation of sarcosine (*N*-methylglycine) and may account for a portion of the metabolism of methionine at higher concentrations. A pathway competitively inhibited by *S*-methyl-L-cysteine appears to account for a majority of the metabolism of methionine at high concentrations of methionine. It is currently postulated that the toxic product is an intermediate in the degradation of methionine *via* this pathway.

It is not my intention to consider the toxicities of all of the amino acids in this paper since several lengthy reviews on this subject have recently appeared (Harper, 1964; Harper *et al.,* 1970; Sassen, 1955). Rather, my objective is to review only briefly studies which have dealt with the toxicities of amino acids so that their toxicities can be compared and then to deal in depth with one of the most toxic amino acids, methionine.

In studies designed to investigate the relative toxicities of amino acids, comparisons are often made when the amino acids are incorporated into the diet at the same percentage (Daniel and Waisman, 1968; Salmon, 1958; Sauberlich, 1961) or molality (Benevenga and Harper, 1967). Both approaches revealed that methionine was the amino acid which resulted in the greatest growth depression. Another approach is to assess the toxicities in relation to the dietary requirement for the amino acid. In one experiment, unfortunately, involving the use of some amino acids in both the D and L form, the relative toxicities were estimated from the consumption of a twofold excess of the requirement of each of the indispensible amino acids (Russell *et al.,* 1952). Here again methionine resulted in the greatest growth depression. In a summary of these experiments, Harper *et al.* (1970) concluded that consumption of methionine at four times its requirement results in growth depression and tissue damage when incorporated into a diet low in protein, whereas tryptophan, which was considered to be the second most toxic amino acid, could be added at levels in excess of tenfold of its requirement before adverse effects were noted. Although the amino acids have not been classified as to the level required in excess of the requirement before adverse effects are seen, methionine clearly appears to be one of the most toxic.

The remaining portion of this paper will be concerned with the mechanism(s) of how consumption of high levels of methionine (2% or more) might depress growth and cause tissue damage. It will be necessary to evaluate the toxicity of the D- and L-enantiomorphs of methionine to establish if methionine at high levels is toxic because it adversely affects the transport of other amino acids, to determine if methionine must be metabolized before it is toxic and if the metabolism of methionine varies with cellular concentration and, if so, if some aberrant metabolism of methionine at high concentrations may be related to the toxicity.

The relative toxicities of D- and L-methionine have been studied extensively with the conclusion that D-methionine is less toxic than L-methionine (Harper *et al.,* 1970; Muramatsu *et al.,* 1971). This could mean that, on the one hand, D-methionine does not produce the same cellular changes as does L-methionine or, on the other hand, that it causes tissue damage only after being converted to the L-enantiomorph. Thus the damage is less because the cellular concentration of L-methionine may be limited by the rate of conversion of the D isomer. That there may be a limited conversion of D-amino acids to the L-amino acids has been shown by Marrett *et al.* (1964); chicks grew well when D-methionine was used to replace L-methionine only when the diet contained no other D-amino acids.

Additional evidence that D-methionine is not toxic is suggested by experiments on the concentration of individual amino acids required to maximize the multiplication of L cells in culture. Eagle (1955) showed that D-methionine did not support growth when included at concentrations that were tenfold (1 m*M*) the amount of L-methionine required to maximize growth. Nor did the addition of these high levels of D-methionine to a medium adequate in L-methionine (0.1 m*M*) limit growth, suggesting that D-methionine itself was not toxic. However, increasing the concentration of L-methionine tenfold above its optimum resulted in approximately a 50% reduction in the rate of multiplication of L cells. Similar results have been reported by Strecker *et al.* (1970) with Chang's liver cells and HeLa cells in suspension culture. If these results can be extrapolated to the intact animal, the apparent toxicity of D-methionine may be simply due to its conversion to L-methionine.

It is conceivable that the toxicity of the amino acids and, in particular, methionine may be due to a competitive effect on amino acid transport. Christensen (1972) has estimated the relative importance of independent transport systems for the uptake of a number of amino acids (Table I). It must be emphasized that these transport systems were determined for the Ehrlich cell and for red blood cells and, hence, may not be strictly applicable to all tissues. A zero in the table is used to indicate that no significant transport activity was observed. It is evident that the majority of the amino acids shown here are transported by the A and L systems and that, in general, individual amino acids are transported more by one system than by another. The exception to this general rule is methionine which is transported effectively by both A and L systems. This may make it such an effective competitor that it seriously interferes with the uptake of those amino acids that have no alternative routes of absorption.

Evidence that at least part of the adverse effects of excessive levels of dietary methionine may be due in part to their effect on transport is shown by the experiments of Webber (1962). Dogs were infused intravenously with in-

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Table I. Reactivities of α-Amino Acids with Several Transport Systems[a]

Amino acid	gly	A	ASC	L
Gly, sar	+++	++	0	±
Ala, ser	0	+++	+++	+
Met	0	++++	0	++++
Val	0	+	0	++
Leu, ile, phe	0	+	0	++++
His, tyr, trp	0	++	0	++

[a] Adapted from Christensen (1972).

creasing concentrations of individual amino acids at 4 ml/min, and the effects on blood and urinary concentrations of all of the amino acids were noted. Creatinine clearance rate was used as an indication of the glomerular filtration rate. Urine was collected continuously *via* an indwelling catheter. From the concentration of the amino acids in blood and urine and the glomerular filtration rate, the reabsorption of each amino acid was calculated. The effect of the infusion of high levels of methionine on the reabsorption of some of the amino acids measured is shown in Table II.

During the control period, reabsorption of the amino acids shown in Table II was nearly complete. An infusion of 0.88 mmol of methionine/min markedly affected the reabsorption of alanine, serine, glycine, and histidine and had a lesser effect on the reabsorption of valine and phenylalanine. The minus sign for the reabsorption of some of the amino acids suggests a net release of amino acids by the kidney. It is clear that, at the lowest level of infusion of methionine, a dramatic effect on the reabsorption of other amino acids was apparent. Whether this effect was at the level of the transport systems alone or also involved an effect on the kidney was not determined in this experiment. That an alteration in kidney function may be involved is suggested by the observation that as the rate of infusion of methionine increased, the filtration rate fell and the urinary flow relative to the filtration rate increased. This might have been expected since consumption of excessive levels of methionine has caused kidney enlargement and tubular dilation (Klavins *et al.*, 1963; Stekol and Szaran, 1962).

Webber's experiment with alanine (Table III) may, however, shed some light on the effect of methionine on kidney function. It is again apparent that nearly 100% of reabsorption of the amino acids was achieved in the control period. Even at the lowest level of alanine infusion, a marked effect on the reabsorption of serine, threonine, and histidine was apparent. At higher levels of alanine infusion, reabsorption of these amino acids was further reduced without an apparent effect on kidney function. L-Alanine, even at high levels (5 to 10%) in the diet, is not toxic and does not result in an altered rate of growth (Daniel and Waisman, 1968; Harper, 1964). Thus, the toxic effect of methionine may not necessarily be related to its effect on the reabsorption of amino acids by the kidney.

It is interesting that α-aminoisobutyric acid, a nonphysiological model amino acid, when added at a level of 3% to a diet containing 8 or 12% of casein, results in a marked reduction in growth rate, but a rate, however, that is not significantly different from that of pair-fed control animals (Christensen and Cullen, 1968). α-Aminoisobutyric acid does significantly affect the distribution ratio of some of the amino acids in the liver and increases the excretion of some of the amino acids. The amounts of the amino acids lost *via* urine, however, would seem insignificant since, in many cases, the loss was less than one-half of 1% of the requirement of the particular amino acid, and in the most extreme case the loss was less than 3% of the requirement of the amino acid. No tissue

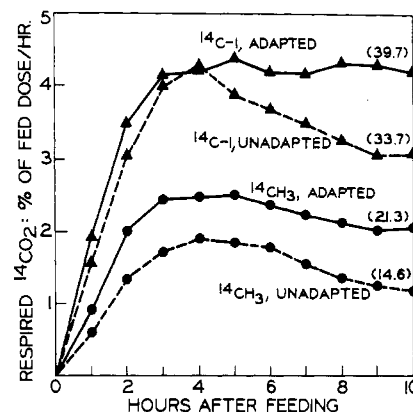

Figure 1. Recovery of $^{14}CO_2$ from labeled L-methionine over a 10-hr period. Rats were fed 10 g of a 10% casein diet in gel form (5 g of dry matter) supplemented with 3% of L-methionine labeled with carbon-14 in either the methyl or carboxyl carbon. The figures in parentheses are the cumulative percentage of the fed dose recovered in CO_2 over the 10-hr period. The techniques used in preparation of the diets and animals in these experiments are identical to those reported earlier (Benevenga and Harper, 1970).

damage was observed in the animals in these experiments (Christensen, 1973); hence, the decreased rate of growth of animals fed high levels of this model amino acid probably resulted from a suppressive effect of high plasma amino acid levels on the appetite control centers. A similar suggestion can be made for the effects of high plasma levels of methionine: supplemental glycine and serine markedly reduce circulating methionine levels and then food intake and growth increase (Harper and Benevenga, 1969).

The studies involving the feeding of high levels of α-aminoisobutyric acid (and other natural amino acids) suggest that depressed growth and food intake resulting from consumption of excessive levels of an amino acid may not necessarily involve tissue damage. Tissue atrophy has always been observed when high levels of methionine have been fed (Harper *et al.*, 1970). The toxicity of methionine must then be due to some feature of its metabolism when cellular concentrations are elevated.

Growth depression from consumption of excessive levels of homocysteine was much less than that observed when methionine was fed. These experiments, however, were somewhat compromised in that homocysteine was given in the DL form. Nevertheless, they suggest that the toxicity of methionine may involve its methyl group rather than the homocysteine moiety (Benevenga and Harper, 1967). This hypothesis is supported by the growth-depressing effects of consumption of high levels of other methyl-containing compounds, *e.g.*, dimethylthetin (Harper *et al.*, 1970) and *S*-methyl-L-cysteine (Case and Benevenga, 1972). Gross tissue damage, similar to that seen when excessive levels of methionine were consumed, was also observed with these other methylated compounds.

Rats fed a diet containing 10% casein supplemented with 3% L-methionine undergo some type of adaptation after a few days which results in an increased oxidation of methionine to CO_2 (Benevenga and Harper, 1970). The effect of the adaptation on the conversion of the methyl and carboxyl carbons to CO_2 is seen in Figure 1. Rats were fed 10 g of the high methionine diet containing either [^{14}C]methyl- or [^{14}C]carboxyl-labeled L-methionine and CO_2 was collected at 1-hr intervals over a 10-hr period. The increased recovery of the fed dose in CO_2 due to the adaptation was 46% for the methyl group, while that of the carboxyl group increased 18%. However, because the recovery of the methyl group in CO_2 is only 40% of that of the carboxyl group, these changes reflect a proportionate increase in the amount of methionine oxidized.

Table II. Effect of Infusion of High Levels of Methionine on Reabsorption of Other Amino Acids by the Kidney of the Dog[a]

Level infused[b]	Creatinine clearance, ml/min	Urine flow, % of clearance	% reabsorption					
			Ala	Ser	Gly	His	Val	Phe
Control	51.3	4.9	99.6	99.1	99.3	97.1	100.0	100.0
0.88 mmol/min	50.8	15.3	18.1	9.5	−4.9	31.0	61.0	64.0
1.77 mmol/min	36.1	17.7	10.2	10.5	−11.0	−26.0	29.4	7.1
2.65 mmol/min	34.7	37.7	19.5	3.3	−0.3	6.2	32.0	9.1

[a] Adapted from Webber (1962). [b] Solution of amino acids 3.3, 6.6, and 9.9% infused at 4 ml/min.

Table III. Effect of Infusion of High Levels of Alanine on Reabsorption of Other Amino Acids by the Kidney of the Dog[a]

Level infused[b]	Creatinine clearance, ml/min	Urine flow, % of clearance	% reabsorption					
			Ser	Thr	His	Val	Phe	Met
Control	69.1	3.8	99.4	99.8	95.0	100.0	99.0	97.7
1.48 mmol/min	77.1	5.6	23.2	62.0	47.4	100.0	93.8	91.4
2.96 mmol/min	74.7	9.9	33.7	26.0	−19.6	65.7	75.9	76.1
4.44 mmol/min	68.8	13.0	13.0	52.8	3.5	66.7	80.0	73.8

[a] Adapted from Webber (1962). [b] Solution of amino acids 3.3, 6.6, and 9.9% infused at 4 ml/min.

Part of the adaptation to the high methionine diet somehow involves the ability of the animal to utilize glycine and serine in the alleviation of the toxicity (Benevenga and Harper, 1967; Harper *et al.*, 1970). Animals fed the diet high in methionine with 3% supplemental glycine or 4.2% supplemental L-serine require 3 to 4 days before a steady rate of growth (20 g/week) is resumed. The supplementations appear to be immediately beneficial because the animals receiving the supplements do not lose as much weight as the animals consuming the high methionine diet alone. Supplementation of the high methionine diet with glycine prevents the tissue damage normally associated with consumption of excessive levels of methionine (Klavins and Peacock, 1964).

An earlier report (Benevenga and Harper, 1970) showed that glycine or serine supplementation of the high methionine diet significantly enhanced the conversion of both the methyl and carboxyl carbons of methionine to CO_2 only in the adapted rat. However, in recent studies using the unadapted rat, simply increasing the amount of diet from the 4 g previously fed to the 10 g shown here (Figure 2) resulted in a significant stimulation in the conversion of the methionine methyl group to CO_2 when the diet also contained the glycine or serine.

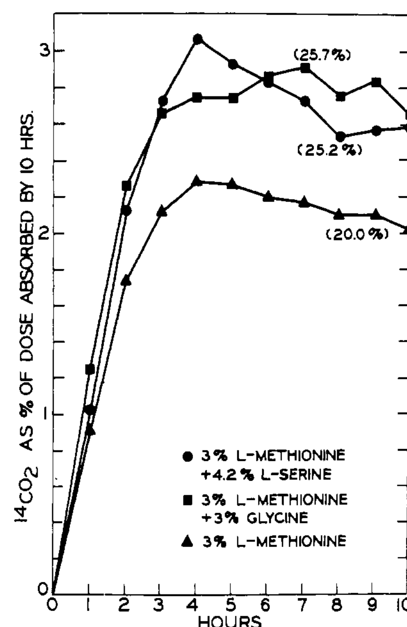

Figure 2. Effect of glycine and serine supplementation on the production of CO_2 from the methyl group of L-methionine. Rats were fed 10 g of a 10% casein diet in gel form (5 g of dry matter) supplemented with 3% L-methionine or this diet with supplemental glycine or serine. CO_2 was collected over a 10-hr period. Figures in parentheses are the percentage of the absorbed dose recovered in CO_2. The percentage of the dose absorbed at 10 hr was 75.0, 83.7, and 75.0 for rats receiving the methionine, methionine + glycine, and methionine + serine diets, respectively.

Figure 3 presents our current understanding of the metabolism of methionine. The metabolism of the methionine methyl group is emphasized because it appears to be involved in the toxicity. The roles that glycine and serine may play in the increase in the conversion of the methyl and carboxyl carbons of methionine to CO_2 are shown. Serine is required for the formation of cystathionine; glycine is required as the methyl acceptor from *S*-adenosylmethionine in the formation of sarcosine by the glycine methyltransferase (Blumenstein and Williams, 1963; Heady and Kerr, 1973). The observation that either glycine or serine alone is effective in alleviation of the toxicity of excessive dietary methionine (Benevenga and Harper, 1967) probably results from the ease of their interconversion (Arnstein and Neuberger, 1953). The central role of the folate system in the metabolism of the methionine methyl group is clear in this figure since it provides methyl groups for the resynthesis of methionine from homocysteine, receives methyl groups from the metabolism of the methionine methyl group *via* dimethylglycine, sarcosine, and possibly formate, and is involved in the conversion of formate to CO_2 (Kutzbach and Stokstad, 1968; Stokstad *et al.*, 1966) and in the interconversion of glycine and serine (Schirch and Mason, 1963). Another possible important pathway involved in the metabolism of the methionine methyl group and its ultimate conversion to CO_2 is its incorporation into phospholipid choline *via* methylation of phosphatidyl ethanolamine, shown at the top portion of this figure. Mackenzie (1955) has suggested that the synthesis of choline is of prime importance in the conversion of normal levels of the methionine methyl group to CO_2. The methyl groups of choline, derived from phospholipid choline, could possibly be converted to CO_2 *via* metabolism in the folate system.

Metabolites which result from methylation of their precursors by *S*-adenosylmethionine (*e.g.*, creatine, RNA, DNA, carnitine, 3-methylhistidine, or choline, etc.) are, however, either not converted to CO_2 or are converted at very low rates. Thus, it is unlikely that these methyl acceptors contribute significantly to the conversion of the

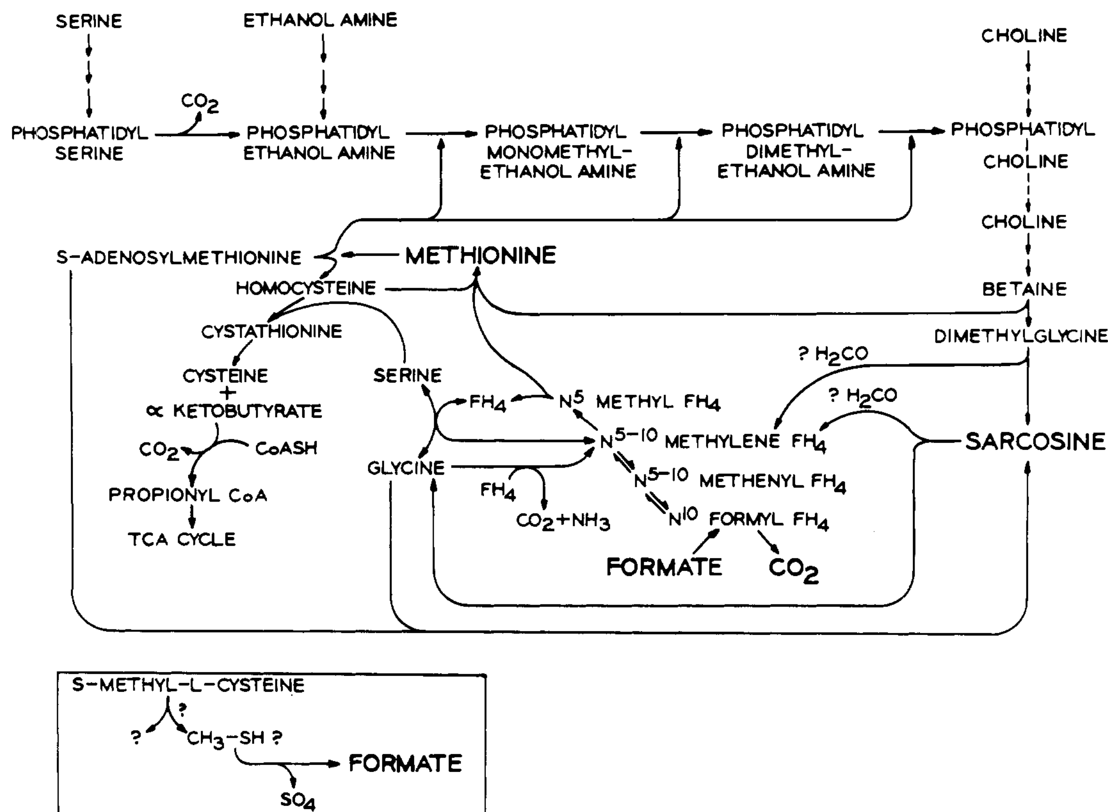

Figure 3. Diagram of the metabolism of methionine emphasizing the metabolism of the methyl group.

methionine methyl group to CO_2 either in the supplemented or unsupplemented animal consuming high levels of methionine. On the other hand, we have shown that the recovery of the methyl group of methionine as CO_2 was 35% of the dose in animals consuming the high methionine diet, and in those consuming the diet supplemented with glycine or serine, the recovery was approximately 40% (Benevenga and Harper, 1970). Therefore, other pathways for the metabolism of the methionine methyl group must be available to account for the high rate of its conversion to CO_2. The conversion of the methionine methyl group to CO_2 *via* formation of sarcosine or *via* a pathway similar to that shown in the inset shown for *S*-methyl-L-cysteine will be discussed later in this paper.

Initially, however, we investigated the incorporation of the methionine methyl group into phospholipid choline and its subsequent oxidation to CO_2. Therefore, large amounts of choline chloride (2.79%) or betaine hydrochloride (3.1%) or sarcosine (1.78%) were added to a diet containing 1.5% of [^{14}C]methyl-L-methionine in an attempt to decrease the rate of conversion of the methionine methyl group to CO_2 by expanding the pools of these intermediates (Case and Benevenga, 1970). The results of these experiments are shown in Table IV. It is clear that high levels of these theoretical intermediates did not diminish the conversion of the methyl group to CO_2. Indeed, the conversion was significantly enhanced by two of these compounds, indicating that we knew little about the conversion of the methionine methyl group to CO_2.

Although the results in Table IV suggest that the methyl group of methionine need not be incorporated into phospholipid choline prior to its oxidation, more direct proof was necessary. If the incorporation of the methyl group into choline could be inhibited without affecting the conversion of the methionine methyl group to CO_2, then the importance of this pathway could be evaluated. Early work by Wells and Remy (1961) had indicated that 2-amino-2-methyl-1-propanol inhibited methylation of phosphatidyl ethanolamine, a precursor of phospholipid choline. This was tested in a liver slice system by including various levels of 2-amino-2-methyl-1-propanol with [^{14}C]methyl-L-methionine. Incorporation of the methionine methyl group into phospholipid choline, which was recovered as the reinecke salt, was markedly reduced by this compound as was CO_2 production, even at high concentrations of methionine. However, another inhibitor, methoxyethanol, at concentrations of 20 m*M* in some experiments or 40 m*M* (Figure 4), reduced markedly the incorporation of the methionine methyl group into phospholipid choline without having an effect on CO_2 production when the level of methionine was high. It is important to note the differences in the scale used for the recovery of the methionine methyl group in phospholipid choline and CO_2 in Figure 4. At all levels of methionine, the addition of 40 m*M* methoxyethanol significantly depressed the recovery of the methyl group in phospholipid choline after the 2-hr incubation period. We have interpreted the recovery of carbon-14 in phospholipid choline as representative of total entry of the methyl group into the phospholipid choline pool rather than as a fraction of the total

Table IV. Effect of Choline, Betaine, or Sarcosine on the Recovery of the Methionine Methyl Group in CO_2

Diet[a]	% of fed dose recovered in CO_2 over 12 hr
10% casein + 1.5% L-methionine	31.0a[c]
10% casein + 1.5% L-methionine + 2.79% choline Cl[b]	36.4b
10% casein + 1.5% L-methionine + 3.15% betaine HCl[b]	39.9b
10% casein + 1.5% L-methionine + 1.78% sarcosine[b]	34.2ab

[a] Labeled with [^{14}C]methyl-L-methionine. [b] 0.2 mol/kg diet. [c] Means followed by different letters are significantly different, $p > 0.01$.

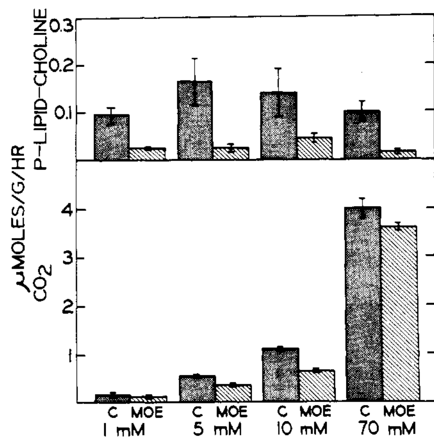

Figure 4. Effect of methoxyethanol on the recovery of the methyl group of methionine in phospholipid choline and carbon dioxide with increasing concentrations of methionine *in vitro* using rat liver slices. Control (C)40 m*M* methoxyethanol (MOE), 1 m*M*, 5 m*M*, 10 m*M*, and 70 m*M* are the concentrations of methionine in the incubation medium. The data are presented as the mean ± the standard error of the mean. Each incubation contained 500 mg of liver slices (0.4 mm thick) in 5 ml of Kreb's ringer phosphate (pH 7.4). Boiled tissue was used as blank. Incubations were carried out over the 2-hr period in a shaker bath at 37° while being continuously gassed with oxygen. CO_2 was trapped in ethanolamine-methylcellosolve. One-hundred percent of added $Na^{14}CO_2$ was recovered in test experiments. Rates were linear with time over a 2-hr incubation period. Total phospholipid was extracted with ethanol and ethanol ether and, finally, ether. The total extracts were pooled. Choline was freed by refluxing with saturated barium hydroxide and then isolated as the reinecke. Free choline was then generated from the reinecke with silver sulfate and the radioactivity in the isolated choline determined by standard liquid scintillation technique.

flux because the half-life of phospholipid choline relative to the length of these experiments is quite long. Estimation of the half-life of liver phospholipid choline with labeled choline appears to be difficult because of the complex kinetics of the decay of its specific activity; estimates vary from 4 to 24 hr (Krause and Beamer, 1972) to 2 to 4 days, depending on whether the first or second component of the decay curve is used to estimate half-life (Pasternak and Friedrichs, 1970). The incorporation of the methyl group of methionine into phospholipid choline did not increase with methionine concentration (Figure 4); however, that recovered in CO_2 materially increased as the concentration of methionine was raised from 1 to 70 m*M*. Thus, while at a methionine concentration of 1 m*M*, the recoveries of the methionine methyl group in phospholipid choline and CO_2 were similar, the proportion of the methionine methyl carbon recovered in phospholipid choline diminished to approximately 2% of that recovered in CO_2 when the concentration of methionine was 70 m*M*. The decline in CO_2 production after the addition of methoxyethanol was significant at the 5 and 10 m*M* levels of methionine and was much greater than the decrease in the incorporation of the methyl group into phospholipid choline. These results suggest that while the incorporation of the methionine methyl group into phospholipid choline may be important in the conversion of the methyl group to CO_2 at low concentrations of methionine, this would not appear to hold at the higher concentrations of methionine. Obviously, other pathways come into play in the conversion of the methionine methyl group to CO_2 at higher cellular concentrations of methionine.

It was decided to reinvestigate the role of glycine methyltransferase in the conversion of the methionine methyl group to CO_2 *via* transfer of the methyl group from *S*-adenosylmethionine to glycine to form sarcosine. Rats readily convert the methyl group of sarcosine to CO_2 (Case and Benevenga, 1970; Horner and Mackenzie, 1950; Weinhouse and Friedmann, 1952). The effects of increasing sar-

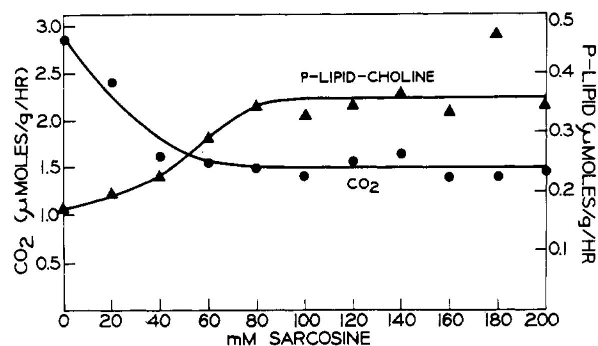

Figure 5. Effect of the concentration of sarcosine in the incubation medium on the recovery of the methyl group of methionine in CO_2 and phospholipid choline using the liver slice system. The concentration of methionine in the medium was 70 m*M* and the concentration of sarcosine varied from 0 to 120 m*M*. Each pair of points represents a single incubation.

cosine concentrations (0 to 200 m*M*) were tested on the recovery of the methionine methyl group in CO_2 and in phospholipid choline using the liver slice system and a high concentration of methionine (70 m*M*). The results from one series of these experiments are shown in Figure 5. The maximal reduction in CO_2 production from the methionine methyl group occurred at a concentration of sarcosine of 40 m*M*. Interestingly, as the sarcosine concentration increased from 40 to 80 m*M*, an increase in the recovery of the methionine methyl group in phospholipid choline was observed. This may explain why sarcosine, at a dietary concentration of 1.78%, did not affect the production of CO_2 from methionine *in vivo* (Table IV). Calculations from that experiment based on animal size, amount of sarcosine consumed, and an estimation of the emptying rate of the stomach indicated that the concentration of sarcosine in body water may have risen as high as 5 m*M*, an amount that would not drastically lower the recovery of methionine methyl group in CO_2, if the results of the *in vitro* experiment (Figure 5) can be extrapolated to the intact animal. The mechanism whereby high levels of sarcosine depress the conversion of the methionine methyl group to CO_2 is not known. It would be logical to suggest that sarcosine acts as a trapping pool, which would be consistent with the observations of Horner and Mackenzie (1950), or some breakdown product of sarcosine, such as formate, or some combination of the two may act as trapping pools. Mackenzie (1955) has shown that methoxyacetate at five times the concentration of sarcosine depresses by 85% the production of formaldehyde from sarcosine. In a preliminary experiment, the effect of methoxyacetate on the production of CO_2 from the methionine methyl group was investigated with the liver slice system (Mitchell and Benevenga, 1973b). At 70 m*M* methionine, CO_2 production from the methyl group was 2.7 μmol/g of liver/hr, whereas, when methoxyacetate was included at 40 m*M*, the production of CO_2 decreased to 1 μmol/g of liver/hr (Mitchell and Benevenga, 1973a). While the inhibition of CO_2 production from the methionine methyl group by methoxyacetate suggests that sarcosine is an intermediate in the conversion of the methionine methyl group to CO_2, the relative importance of this pathway has not been assessed. Horner and Mackenzie (1950) found that 3% of the methionine methyl group was converted into sarcosine in rats consuming control diets containing 5% of sarcosine as a trapping pool. The rate of conversion of the methionine methyl group to CO_2 *via* pathways generating sarcosine, as shown in Figure 3, may well change with the level of methionine, but even at low levels of methionine (5 m*M*), relatively high concentrations of sarcosine (120 m*M*) did not prevent production of CO_2 from the methionine methyl group (Table V). This suggests that yet another pathway may be avail-

Table V. Effect of Sarcosine on the Metabolism of the Methionine Methyl Group in Liver Slices[a]

	µmol/g/hr	
	CO_2	Phospholipid choline
Control	0.488 (±0.02)[b]	0.271 (±0.03)
120 mM sar	0.146 (±0.01)	0.391 (±0.015)

[a] The concentration of methionine in the incubation medium was 5 mM. [b] ± S.E. of mean.

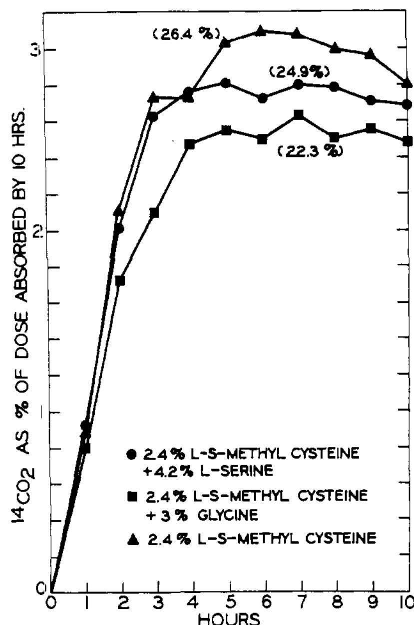

Figure 6. Effect of glycine and serine supplementation on the recovery of the methyl group of *S*-methyl-L-cysteine in CO_2 over 10 hr. Rats were fed 10 g of a 10% casein diet in gel form (5 g of dry matter) supplemented with 0.3% of L-methionine + 2.4% of *S*-methyl-L-cysteine or this diet supplemented with glycine or serine. Figures in parentheses are the percentage of the absorbed dose recovered in CO_2 over the 10-hr collection period. The percent of the dose absorbed by 10 hr for the animals consuming *S*-methyl-L-cysteine alone or supplemented with glycine or serine were 61.2, 59.4, and 56.4%, respectively.

able for the production of CO_2 from the methionine methyl group.

It has been known for some time that the methyl group of *S*-methyl-L-cysteine is rapidly converted to CO_2 in the intact rat (Horner and Kuchinskas, 1959) and in a cell-free system (Kuchinskas, 1965) and that *S*-methyl-L-cysteine will not alleviate a cysteine deficiency (Armstrong and Lewis, 1951; Grau and Almquist, 1943). Because incubation of *S*-methyl-L-cysteine with a preparation containing the methionine adenosyltransferase does not increase the release of inorganic phosphate from ATP (Cantoni, 1951) and because *S*-methylcysteine is a poor inhibitor of this enzyme (Cox and Smith, 1969), it has been suggested that this amino acid is not activated to an *S*-adenosylmethionine-like compound. These observations plus those of Canellakis and Tarver (1953) that a mitochondrial preparation produces methyl mercaptan from *S*-methylcysteine and methionine and that methyl mercaptan is rapidly oxidized to CO_2 and sulfate in the intact rat lead to the speculation that *S*-methyl-L-cysteine may be used as an analog in the study of the metabolism of methionine by pathways which do not involve the activation of methionine to *S*-adenosylmethionine (Figure 3).

Initial studies (Case and Benevenga, 1972) revealed that incorporation of 2.4% of *S*-methyl-L-cysteine into a 10% casein diet resulted in growth depressions comparable to those observed with 3% of L-methionine. Furthermore, gross examination of these animals revealed the darkened

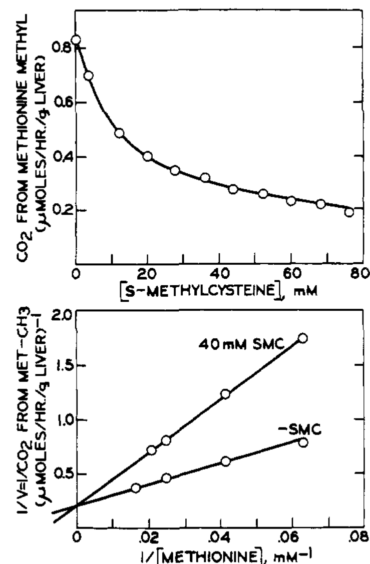

Figure 7. Top panel: Effect of *S*-methyl-L-cysteine on the conversion of the methionine methyl group to CO_2 by a rat liver homogenate system. The methionine concentration in the medium was maintained at 10 m*M*. Bottom panel: Lineweaver–Burk plot of the conversion of the methionine methyl group to CO_2 without and with *S*-methyl-L-cysteine added to a final concentration of 40 m*M*. In this system 2 ml of a 20% liver homogenate in 0.25 *M* sucrose was incubated in 10 m*M* potassium phosphate buffer at pH 6.5, with 5 m*M* $MgCl_2$ and 1 m*M* ATP. The concentration of the substrate and inhibitors was varied. Tissues were incubated for 1 hr at 37° in a shaker bath under flowing oxygen conditions. CO_2 was collected as described in Figure 4.

spleen and anemia normally seen in rats consuming high levels of methionine (Harper *et al.*, 1970). However, addition of 3% of glycine or 4.2% of L-serine to a diet containing 2.4% of *S*-methyl-L-cysteine labeled in the methyl group did not increase the percentage of the absorbed dose recovered in CO_2 over a 10-hr period (Figure 6). This is in contrast to the results obtained when glycine and serine were added to a diet containing 3% of L-methionine (Figure 2). The failure of glycine or serine to enhance the production of CO_2 from the methyl group of *S*-methyl-L-cysteine is consistent with the hypothesis that this compound is not activated to an *S*-adenosylmethionine-like compound with transfer of the methyl group to glycine. Additional evidence for this notion is available from the observations of Horner and Kuchinskas (1959) that only traces of the methyl carbon from *S*-methyl-L-cysteine appear in choline or creatinine, while twice as much of the radioactivity from the methyl group of *S*-methylcysteine, relative to that of methionine, is recovered in CO_2.

Addition of 1.2% of *S*-methyl-L-cysteine to a diet containing 3% of L-methionine depressed by 40% the production of CO_2 from the methyl group of methionine; when the level of *S*-methylcysteine was increased to 2.4%, the production of CO_2 from the methionine methyl group was depressed further to 50% (Case and Benevenga, 1972). Similar depressions in CO_2 production from L-methionine-*1*-^{14}C were observed when 1.2 or 2.4% of *S*-methyl-L-cysteine were incorporated into the diet. High levels of methionine in the converse experiment also reduced the production of CO_2 from the methyl group of *S*-methylcysteine (Case and Benevenga, 1972). These observations suggest that *S*-methyl-L-cysteine and methionine may compete at a transport site or at some early site of catabolism. *In vitro* studies were carried out using a liver homogenate system to test whether *S*-methyl-L-cysteine might inhibit the metabolism of the methionine methyl group *via* an effect on transport.

The top portion of Figure 7 shows the effect of increasing concentrations of *S*-methyl-L-cysteine on the conver-

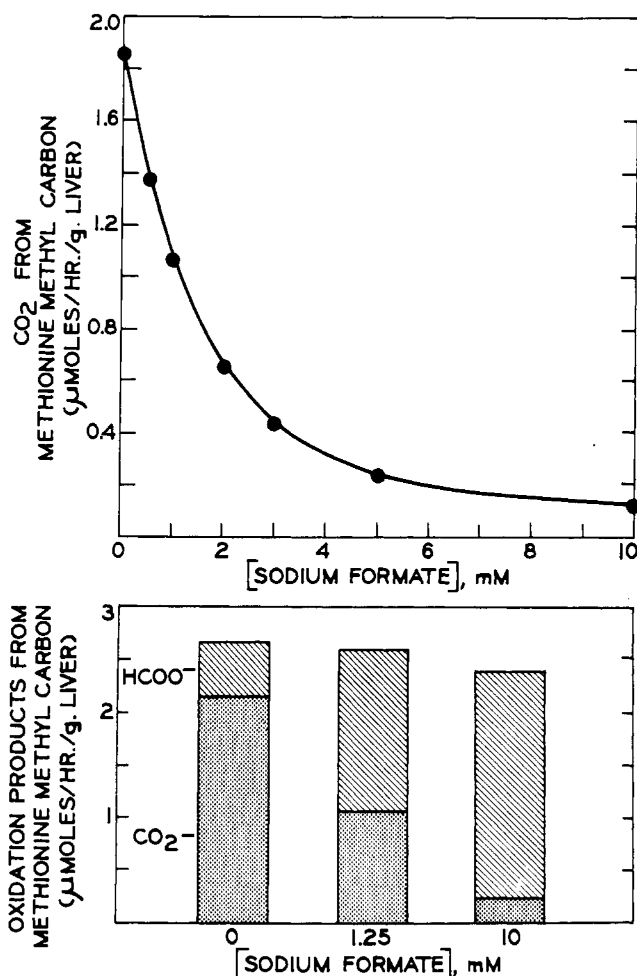

Figure 8. Top panel: Effect of increasing formate (final concentrations of 0 to 10 m*M*) on the recovery of the methionine methyl group in CO_2 in the rat liver homogenate system. Bottom panel: Recovery of the methionine methyl group in CO_2 and formate with increasing concentrations of formate added to the incubation system. The liver homogenate system used here is described in the legend of Figure 7.

sion of the methionine methyl group to CO_2 in a liver homogenate system when the concentration of methionine was held constant at 10 m*M*. The maximum depression of CO_2 probably occurs at concentrations of *S*-methyl-L-cysteine in excess of 80 m*M*. If, however, the maximum depression is assumed to occur at this concentration, 12 to 15 m*M* *S*-methylcysteine causes a 50% reduction in the rate of conversion of the methionine methyl carbon to CO_2. This concentration of *S*-methyl-L-cysteine is equivalent to our best estimate of the k_m for the conversion of the *S*-methyl-L-cysteine methyl group to CO_2 in this same system. Experiments in which methionine was used to inhibit CO_2 production from the methyl group of *S*-methylcysteine revealed that a 50% reduction in CO_2 production occurred at a concentration of methionine of 50 to 60 m*M*, which is the approximate k_m of methionine for CO_2 production. It is recognized that this type of kinetic analysis may not be valid for a multicomponent system; however, a more detailed analysis of the competition awaits purification of the components of the system. The pattern of the double reciprocal plot for the conversion of the methionine methyl carbon to CO_2 without and with 40 m*M* *S*-methylcysteine, shown in the bottom portion of Figure 7, is suggestive of competitive inhibition. The competition shown *in vivo* and that *in vitro* in the homogenate system is consistent and suggests that the competition may occur at some initial point in metabolism rather than at some transport site.

Early work by Horner and Mackenzie (1950) and Weinhouse and Friedmann (1952) showed that the methionine methyl carbon was incorporated into the methyl group of sarcosine and that the methyl groups of both sarcosine and methionine could be trapped as formate in an expanded formate pool. This, of course, would be expected if glycine methyltransferase contributes significantly to the metabolism of methionine (Figure 3). Since the capacity of the rat to convert the methyl carbon of methionine and *S*-methylcysteine to CO_2 *in vivo* is similar (met 17 μmol/hr; smc 15 μmol/hr) when the diet contains high levels of either methionine (3%) or *S*-methyl-L-cysteine (2.4%), it was of interest then to investigate the relative efficiency of trapping of the methyl carbons of methionine and *S*-methylcysteine in an expanded formate pool. These results could shed light on the relative importance of a pathway which does not involve activation of methionine to *S*-adenosylmethionine prior to the conversion of its methyl carbon to CO_2.

Increasing the concentration of formate from 0 to 10 m*M* in the liver homogenate system dramatically lowered the recovery of the methyl carbon of methionine as CO_2 (top panel of Figure 8). The bottom portion of this figure shows that addition of up to 10 m*M* concentrations of formate did not alter the metabolism of methionine since the summation of the production of formate and CO_2 with increasing concentrations of formate essentially equaled that of the production of CO_2 in the control incubations. Preliminary experiments using [^{14}C]methyl-labeled *S*-methylcysteine showed that formate also reduced the recovery of this methyl carbon in CO_2 but also depressed the total metabolism of the methyl group as the formate level increased from 0 to 10 m*M*.

A recent *in vivo* experiment using the formate loading principle described by Weinhouse and Friedmann (1952) showed that after correction for the fraction of formate oxidized to CO_2, approximately 64% of the methyl groups of methionine and sarcosine and 100% of that from *S*-methylcysteine could be trapped in an expanded formate pool. The process which generates formate and the immediate precursors of formate *in vivo* and *in vitro* has not been identified, but undoubtedly these processes will be the subject of future experiments. The *in vivo* and *in vitro* experiments with methionine and *S*-methylcysteine showing their mutual competition, the observation that at high concentrations the methyl carbons of both are converted to CO_2 at essentially equal rates, and the observation that the growth depression and tissue damaging effects due to consumption of excessive levels of both amino acids are similar all lend credence to the hypothesis that a pathway exists for the metabolism of methionine which does not involve its activation to *S*-adenosylmethionine; *S*-methylcysteine may be used as a means of studying factors which affect this pathway.

It is obvious from this review that the metabolism of methionine is complex and that the means by which its excessive consumption results in the tissue damage are still to be determined. However, it seems unlikely that the toxic effects of excessive dietary methionine can be ascribed to its effect on transport of other amino acids. More significantly, it would seem that various pathways involved in the conversion of the methionine methyl group to CO_2 come into play at different cellular concentrations of methionine. Probably the incorporation of the methyl group into phospholipid choline occurs at all methionine levels and is, therefore, more important in total methionine methyl group metabolism at lower methionine concentrations. The glycine methyltransferase pathway may become involved as higher methionine levels are reached, and may explain the beneficial effects of glycine and serine on growth and on the catabolism of methionine. The *S*-methyl-L-cysteine-like pathway may be involved only at very high cellular levels of methionine and may be the

pathway that is involved in the toxicity. We currently hold that at high levels of dietary methionine, the metabolism of excess methionine follows a pathway similar to that shown for *S*-methyl-L-cysteine (inset, Figure 3), possibly resulting in the production of methyl mercaptan which may be toxic. We are not yet sure, however, about production of methyl mercaptan from methionine in the *S*-methylcysteine-like pathway, but in some exciting preliminary studies, carried out under anaerobic conditions, volatile ^{35}S was obtained from sulfur-labeled methionine.

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Chemistry of the Staphylococcal Enterotoxins

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The staphylococcal enterotoxins are simple, low molecular weight proteins that are relatively heat resistant. They contain one disulfide bridge which can be reduced without affecting the toxicity or antigenicity. At least a part of the tyrosyl and methionine residues and the carboxyl and amino groups can be substituted without adversely affecting the toxicity of the molecule. The individual enterotoxins can be separated into several proteins which are identical in every respect except for their isoelectric points.

The staphylococci produce many biologically active substances which have been studied to various degrees. One group of substances which has received much attention in recent years is the enterotoxins, the causative agents of staphylococcal food poisoning. The enterotoxins are produced by the staphylococci in some foods and in culture media in the laboratory. The ingestion of these substances by humans produces a variety of symptoms, the most common being vomiting and diarrhea in 2–6 hr. The illness is relatively mild, normally lasting only a few hours to 1 day. This illness is not a reportable disease, and its true incidence is unknown since most cases are never seen by a physician and hence go unrecognized. Some have the opinion that staphylococcal poisoning is not of great importance; however, according to Morbidity and Mortality Weekly Reports (1972), 45% of all food-borne disease outbreaks in 1971 was due to staphylococcal food poisoning.

The purification of a protein that caused emesis in monkeys led to the discovery that the staphylococci produce more than one enterotoxin (Bergdoll *et al.*, 1959a), the basis for differentiation being their reaction with specific antibodies. This basis was used in establishing a nomenclature, designating them as enterotoxins A, B, C, etc. (Casman *et al.*, 1963). To date, enterotoxins A (Cas-

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