A Deuterium Surface Coil NMR Study of the Metabolism of D-Methionine in the Liver of the Anesthetized Rat

Robert E. London* and Scott A. Gabel
Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709

Received February 10, 1988; Revised Manuscript Received May 24, 1988

ABSTRACT: The hepatic metabolism of deuteriated D-methionine has been studied in the intact, anesthetized rat using ^2H NMR spectroscopy. The rate of formation of the principal labeled metabolite, [*methyl*-$^2H_3$]sarcosine, from the D-[*methyl*-$^2H_3$]methionine precursor was found to be as rapid as the rate observed previously in NMR studies of the hepatic metabolism of L-methionine. Similarly, rates of clearance of labeled methionine from the liver, formation of *N*-trimethyl-labeled metabolites, and labeling of the HDO pool were all found to be similar to the rates observed in the L-methionine studies. In contrast, all of these metabolic transformations are strongly inhibited by pretreatment of the rats with sodium benzoate, an inhibitor of D-amino acid oxidase. In vivo ^2H NMR studies of sodium benzoate treated rats given L-[*methyl*-$^2H_3$]-methionine exhibit a much more rapid formation of [*methyl*-$^2H_3$]sarcosine than rats given the D enantiomer, consistent with the expectation that the sodium benzoate does not interefere with either the formation of *S*-adenosylmethionine or the subsequent transmethylation of glycine. However, the rates of methionine clearance and formation of deuteriated water are markedly reduced in this study relative to rats receiving the labeled D- or L-methionine without sodium benzoate pretreatment. These results indicate that subsequent to the initial oxidative deamination of the labeled D-methionine, the reamination to give L-methionine is rapid compared with the further degradation of the α-keto acid. Thus, the results are consistent with a dominant contribution of the glycine/sarcosine shuttle to the metabolism of excess D- or L-methionine.

The metabolism of the amino acid methionine has been of intense interest, both as a consequence of the central role of transmethylation reactions in a wide range of biochemical transformations (Borchardt et al., 1986) and as a consequence of the toxicity associated with excess dietary methionine (Kaufman et al., 1960; Stekol & Szaran, 1962; Benevenga & Harper, 1967; Benevenga, 1974; Hardwick et al., 1970). Thus, the consumption of methionine at a level 4 times its requirement results in growth depression and tissue damage when incorporated into a low-protein diet, whereas tryptophan, which in excess is the second most toxic amino acid, must be present in excess of 10-fold above its requirement before adverse effects are observed (Harper et al., 1970). Recent studies of the metabolism of methionine have provided support for both a transmethylation–transsulfuration catabolic pathway (Mudd et al., 1980; Stipanuk, 1986; Finkelstein & Martin, 1986; London et al., 1987) and a transamination pathway with the initial production of the α-keto acid (Case & Benevenga, 1976; Mitchell & Benevenga, 1978; Steele & Benevenga, 1978; Livesey, 1984; Scislowski et al., 1987), and the relative flux through each, as well as the possible significance of each in determining the observed toxicity, remains unclear. A study of the metabolism of D-methionine is of interest in this regard, since many early nutritional studies involved D,L mixtures (Russell et al., 1952), and since the initial metabolism of the D-methionine via D-amino acid oxidase yields the α-keto-γ-methylthiobutyrate transamination product as the first metabolic step (Rechcigl et al., 1960; Brada & Bulba, 1980; Konno & Yasumura, 1984) (Figure 1). More specifically, Livesey (1984) has argued that the relative catabolism of L-methionine via the two pathways may reflect the K_m values for transamination relative to formation of *S*-adenosylmethionine by the action of methionine adenosyltransferase. In this case, the obligate initial transformation of D-methionine to the α-keto acid via the action of D-amino acid oxidase would be expected to virtually shut off flux via the transmethylation pathway. D-Methionine metabolism has also been of interest with regard to the interpretation of studies of the convulsant methionine analogue methionine sulfoximine (Ghittoni & Sellinger, 1974). Recent positron emission tomography studies have employed a combination of D-[^{11}C]- and L-[^{11}C]methionine to separate transport mechanisms from metabolism (Schober et al., 1985; Bergstrom et al., 1987).

We have recently demonstrated that in vivo ^2H NMR provides a useful methodology for following the metabolism of deuteriated L-methionine by the liver of the anesthetized rat (London et al., 1987). The extension of this approach to the study of D-[*methyl*-$^2H_3$]methionine is described in the present studies.

Materials and Methods

Chemicals. D-[*methyl*-$^2H_3$]Methionine was obtained from MSD Isotopes, and the stereochemical purity was verified on a Perkin-Elmer Model 241 polarimeter. Sodium benzoate was obtained from Sigma Chemical Co. The long-acting anesthetic Inactin was obtained from Lockwood Associates Imports, East Lansing, MI.

Physiological Methods. Experimental conditions were generally identical with those described previously in studies of the hepatic metabolism of deuteriated L-methionine. Briefly, the studies utilized male Sprague-Dawley rats weighing 275–300 g, which were surgically prepared by the removal of a circle of muscle directly over the liver, followed by suturing the skin. This procedure allowed subsequent monitoring of hepatic metabolites with an externally placed surface coil (London et al., 1985). Four rats were then anesthetized with Inactin, a catheter was inserted into the jugular vein, and the rat was placed in the NMR probe. Subsequent to obtaining control ^2H NMR spectra, rats were dosed via the catheter with 100 mg of D-[*methyl*-$^2H_3$]methionine dissolved in 1–1.5 mL of normal saline. A second series of four rats received an additional intraperitoneal injection of sodium benzoate (0.9

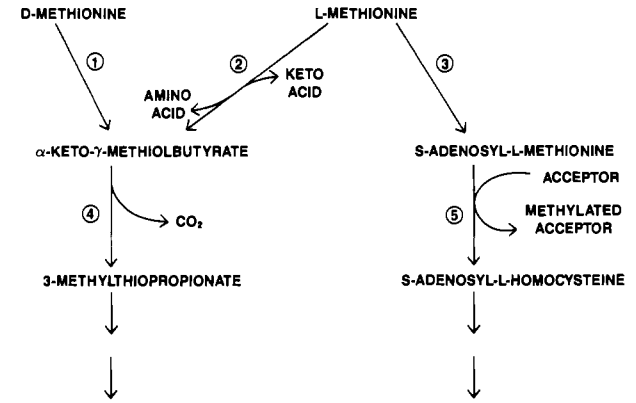

FIGURE 1: Proposed metabolic pathways for the catabolism of D- and L-methionine. Enzymatic steps indicated correspond to (1) D-amino acid oxidase, (2) transamination, (3) methionine adenosyltransferase, (4) oxidation of α-keto-γ-methylthiobutyrate, and (5) glycine methyltransferase.

g/kg weight) 1 h prior to the intravenous dose of D-methionine. This dose is slightly below the 1 g/kg dose of sodium benzoate used by Brada and Bulba (1980) in studies of the inhibition of *S*-adenosylethionine formation from D-ethionine. However, under the conditions of the studies reported here, 50% of the rats treated with the higher sodium benzoate dose did not survive the 5-h experiment, while all of the rats dosed with 0.9 g/kg of sodium benzoate survived. This increased mortality may reflect the combined effects of the sodium benzoate and the anesthetic. We note that in the rats that survived the higher dose, there was no significant difference in the response to D-methionine as monitored by ^2H NMR.

As an additional control study, two rats were treated with 0.9 g/kg of sodium benzoate and subsequently given an intravenous dose of 100 mg of L-[*methyl*-$^2H_3$]methionine. The in vivo hepatic metabolism of the L-methionine was then monitored as in the D-methionine studies described below.

In Vivo NMR Measurements. In vivo NMR measurements were carried out on a Nicolet NT-360 NMR spectrometer using a home built-probe with concentric surface coils tuned to ^2H (1.3-cm diameter) and ^1H (2-cm diameter), as described previously (London et al., 1987). All studies were carried out without the use of a lock; magnet drift has been measured at less than 3×10^{-3} ppm/h. Optimal resolution of in vivo ^2H resonances was obtained by using a double-exponential apodization function, which is effectively a combination of a Gaussian multiplication with LB > 0 and an exponential multiplication with LB < 0 (Lindon & Ferrige, 1979):

$$F'(t) = F(t) \exp(at - bt^2)$$

Resonance assignments were made on the basis of the observed ^1H chemical shifts of model compounds as described previously (London et al., 1987).

All in vivo ^2H NMR studies utilized a block size of 1K data points and a total sweep width of ±1 kHz, corresponding to an 0.256-s acquisition time. This is sufficient to permit nearly complete relaxation of the hepatic HDO resonance, which has been reported to exhibit a T_1 value of 129 ms (Block & Parekh, 1987). Consistent with this expectation, inclusion of an additional interpulse delay of 0.5 s led to an increase of 16% in the intensity of the HDO resonance. The spin–lattice relaxation times of the other metabolites observed in the studies are not readily measured since the concentrations are time dependent, but these higher molecular weight molecules are likely to have shorter T_1 values. Thus, it is probable that the resonances are almost fully relaxed under the conditions of the studies.

With the exception of the estimation of tissue concentrations based on comparisons between the intensities of ^2H resonances of HDO and the other metabolites, all of the conclusions of the studies are based on changes in metabolite levels.

Results

As discussed previously (London et al., 1987), the background ^2H NMR spectrum obtained with the surface coil positioned over the abdominal region of the surgically modified rat exhibits resonances corresponding to HDO and to CHD groups of fatty acids present in lipoproteins, in membranes, or as triglycerides. Based on the natural abundance of ^2H and assuming that the tissue is 70% water lead to an estimation of 12 mM for the HDO concentration. Addition of D-[*methyl*-$^2H_3$]methionine yields the expected resonance at 2.1 ppm (Figure 2). Surprisingly, the formation of [*methyl*-$^2H_3$]sarcosine from the labeled methionine proceeded as rapidly as was observed previously with the deuteriated L-methionine precursor (London et al., 1987). Additionally, as in the L-methionine studies, a resonance attributed to *N*-trimethyl-labeled species became apparent approximately 2 h into the study and continued to increase progressively after that time (Figure 2). The *N*-trimethyl resonance continues to increase beyond the time period involved in the study, as noted previously (London et al., 1987). It is emphasized that the observed *S*-methyl resonance reflects the sum of D- and L-methionine present in the liver. As discussed more fully under Discussion, the rapid formation of labeled sarcosine implies an even more rapid racemization of the D-methionine, so that the [L-methionine]/[D-methionine] ratio increases progressively during the study.

A plot of the mean intensities of the resonances corresponding to methionine, sarcosine, *N*-trimethyl-containing metabolites, and HDO, which have been normalized relative to the initial HDO resonance, is shown in Figure 3A. The zero time values in the figure actually correspond to the first 10-min period during which the labeled methionine is injected. Standard errors obtained in the series of four studies are also indicated. These data are essentially identical with the results obtained previously with the L-methionine-treated rats. As in the previous case, the HDO resonance is observed to increase significantly relatively late in the study, reflecting the excess rate of production of HDO over its loss due to diffusion into other tissues.

As discussed previously (London et al., 1987), the intensity data can be approximately converted to concentration by comparison with the initial HDO resonance, by noting that a factor of 1/3 must be introduced in the analysis of the methionine and sarcosine data and a factor of 1/9 in the *N*-trimethyl metabolites, due to the presence of 3 and 9 deuterons, respectively. It is also necessary to emphasize that since the coil may be sensing some HDO from other tissues, the calculation is probably a lower limit for the concentration of these metabolites. On the basis of this analysis and with a concentration of 12 mM for the HDO, the mean initial concentration of D-methionine in the liver is determined to be 2.2 mM. This value is reduced to 1.9 mM if a correction for the overpulsing of the HDO resonance is included.

As shown in Figure 1, the initial step in the metabolism of D-methionine is presumably the formation of the corresponding α-keto acid via the action of peroxisomal D-amino acid oxidase (Leighton et al., 1975; Brada & Bulba, 1980). Sodium benzoate is known to inhibit the activity of D-amino acid oxidase in vitro (Klein & Kamin, 1941; Barlett, 1948) and has been shown to inhibit the deamination of ethionine in vivo based on the reduced levels of *S*-adenosylethionine formed from

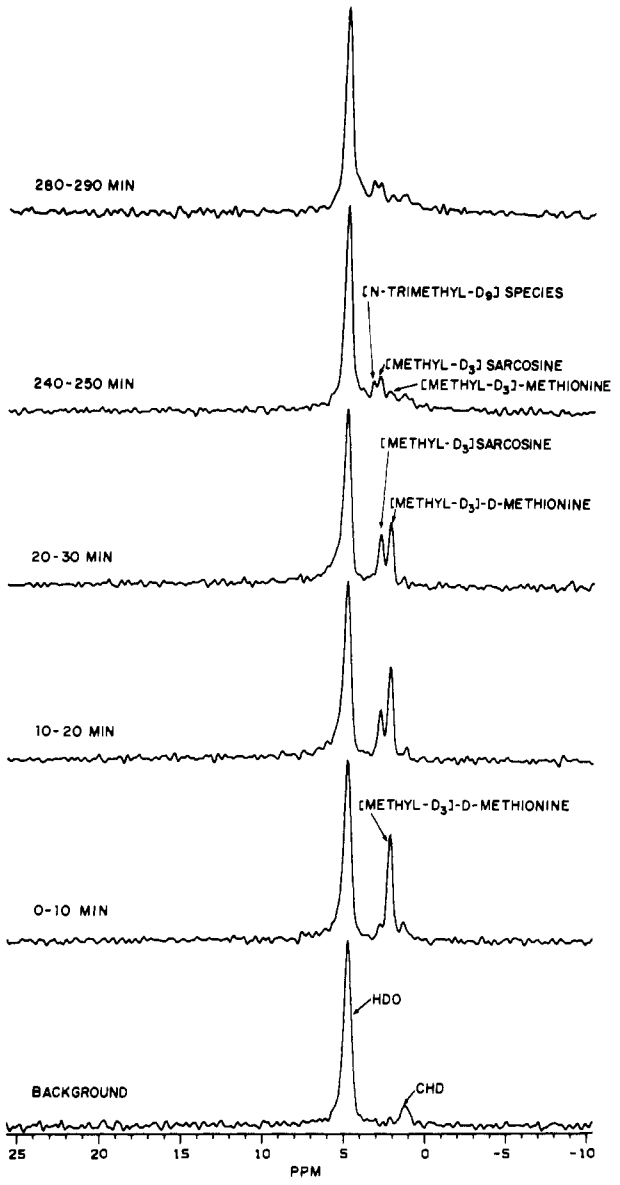

FIGURE 2: Series of ^2H NMR spectra obtained by using the surface coil probe described under Materials and Methods positioned over the abdomen of the surgically modified rat. The lower trace corresponds to the background resonances assigned as indicated to HDO and CHD groups of lipids. The D-[*methyl*-$^2H_3$]methionine was introduced via the intravenous cannula at time zero, and spectra shown are referenced to that time. As discussed in the text, the resonance labeled D-[*methyl*-$^2H_3$]methionine actually corresponds to both D and L isomers, with the L/D ratio presumably increasing over the course of the study. Each spectrum corresponds to a 10-min accumulation time (2340 scans), a total sweep width of ±1 kHz, and a block size of 1K data points. All spectra have been resolution enhanced using double-exponential multiplication and a parameter of 10 Hz.

D-ethionine subsequent to the administration of sodium benzoate (Brada & Bulba, 1980). Deuterium NMR studies were carried out on rats pretreated with sodium benzoate (0.9 g/kg) 1 h prior to the administration of D-[*methyl*-$^2H_3$]methionine. A series of ^2H NMR spectra obtained in vivo is shown in Figure 4. As is apparent from these data, the rate of formation of the labeled sarcosine is markedly reduced relative to the rate observed in rats not pretreated with benzoate. Thus, even during the 20–30-min accumulation period, only a low level of labeled sarcosine is visible in the spectra. This level gradually rises, but the level of methionine (presumably in this case, the resonances arise principally from D-methionine) remains elevated throughout the period of observation. A plot of the mean resonance intensity as a function of time, nor-

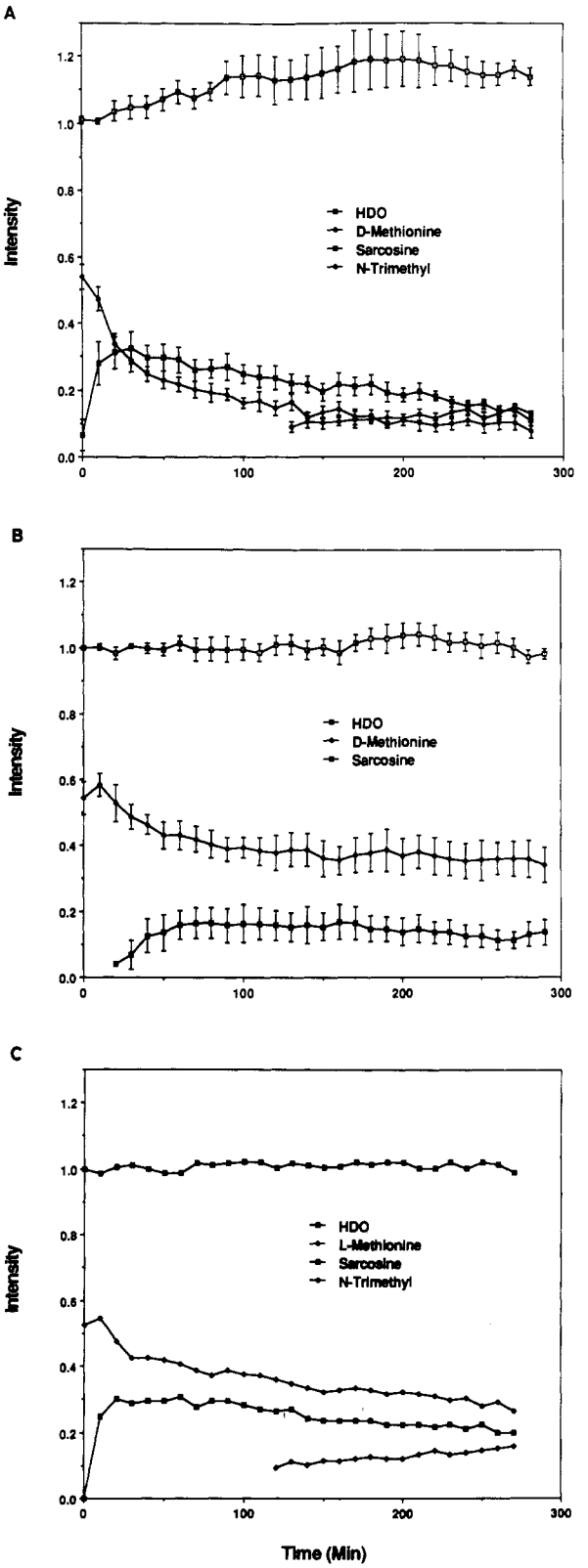

FIGURE 3: Relative intensities, normalized with respect to the intensity of the initial HDO resonance in each study, of the ^2H resonances of the observed metabolites plotted as a function of time after the injection of deuteriated methionine via intravenous catheter, as indicated. (A) Rats dosed with D-[*methyl*-$^2H_3$]methionine; (B) rats dosed with D-[*methyl*-$^2H_3$]methionine 1 h subsequent to receiving an intraperitoneal dose of 0.9 g/kg sodium benzoate; (C) rats dosed with L-[*methyl*-$^2H_3$]methionine 1 h subsequent to receiving an intraperitoneal injection of sodium benzoate. Calculated standard errors for the studies shown in (A) and (B) (n = 4) are indicated; average values for two rats are plotted in (C). Observed metabolites are methionine (●), sarcosine (■), *N*-trimethyl-deuteriated compounds (○), and HDO (□).

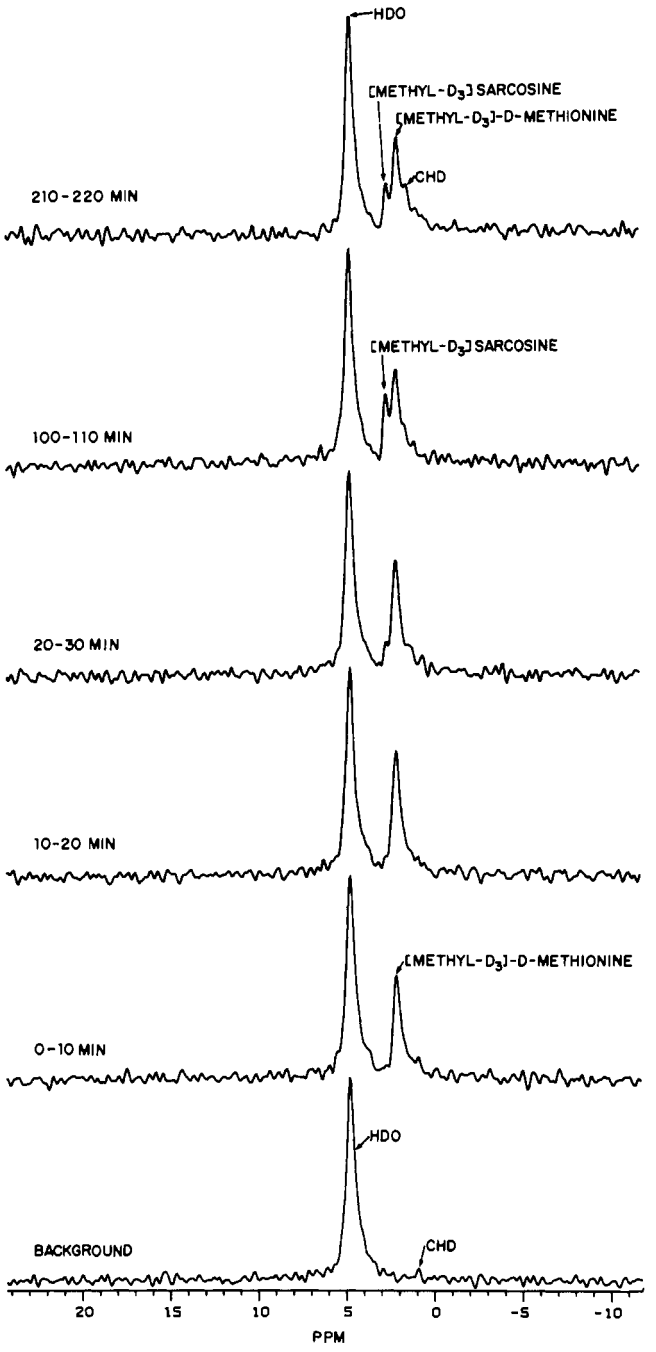

FIGURE 4: Series of ^2H NMR spectra obtained as described in Figure 2 on rats which received an intraperitoneal injection of sodium benzoate, 0.9 g/kg, 1 h prior to receiving the D-[*methyl*-$^2H_3$]methionine. The indicated times are referenced to the initiation of the methionine injection at $t = 0$.

malized to the initial HDO level, is shown in Figure 3B. As can be seen from this figure, the D-methionine resonance actually increases in intensity during the second 10-min period of observation, presumably reflecting the gradual clearance of the labeled material from the blood and the low rate of hepatic metabolism in the rats pretreated with benzoate. The loss of intensity of this resonance is markedly slower than observed for the untreated animals. The level of sarcosine increases more slowly, and to a lower value than in the rats not treated with benzoate. No significant resonance arising from *N*-trimethyl-containing metabolites became visible during the period of the studies. Finally, the HDO resonance intensity remains essentially unchanged over the period of the study. This observation presumably reflects the low rate of oxidation of the deuteriated methyl group and supports the conclusion that the increase in the intensity of this resonance observed in the rats receiving L- or D-methionine is significant and reflects oxidation of the deuteriated methyl group.

As a further control, two rats were treated with 0.9 g/kg sodium benzoate prior to receiving 100 mg of L-[*methyl*-$^2H_3$]methionine via the intravenous catheter. The time dependence of the ^2H resonances, normalized relative to the initial HDO resonance and averaged for the two studies, is shown in Figure 3C. From these data, it is apparent that the formation of the labeled sarcosine from L-methionine proceeds rapidly, reaching an intensity ratio of 0.3 relative to the initial HDO peak, which is very similar to observations in the L-methionine study (London et al., 1987) and to the present D-methionine study in the rats not treated with sodium benzoate (Figure 3A). However, the subsequent clearance of the deuteriated L-methionine and sarcosine from the liver is much slower in the sodium benzoate treated rats than in the untreated L- or D-methionine-dosed rats. Consistent with the observation that much of the deuterium remains in the observed metabolites, the HDO resonance is essentially constant for the duration of the NMR studies. It is therefore concluded that (1) sodium benzoate administration does not interefere with the formation of sarcosine and, by inference, *S*-adenosylmethionine from L-methionine and (2) the sodium benzoate does exert an additional effect on the hepatic metabolism of L-methionine.

DISCUSSION

A comparison of the data summarized in Figure 3A for the metabolism of D-[*methyl*-$^2H_3$]methionine with the analogous data presented previously for the metabolism of an equivalent dose of L-[*methyl*-$^2H_3$]methionine (London et al., 1987) indicates that with regard to all parameters which can be determined with ^2H NMR—clearance of the labeled methionine from the liver, formation of labeled sarcosine, formation of *N*-trimethyl-labeled species, and enrichment of the water with deuterium—the results are identical within experimental error. The very rapid formation of labeled sarcosine can in principle be interpreted to reflect either the rapid racemization of the labeled D-methionine to L-methionine with subsequent formation of *S*-adenosyl-L-methionine, as shown in Figure 1, or the formation of *S*-adenosyl-D-methionine and its subsequent utilization as a donor of the labeled methyl group in the reaction catalyzed by glycine methyltransferase (Ogawa & Fujioka, 1982; Wagner et al., 1985). The latter possibility can, in fact, be eliminated since it has been demonstrated that ATP:L-methionine adenosyltransferase will not accept D-methionine as a substrate (Lombardini & Talalay, 1970). Hence, it must be concluded that the inversion of D-methionine, which proceeds via formation of the α-keto acid and subsequent transamination to yield L-methionine, proceeds sufficiently rapidly under the dosage conditions utilized in the study to saturate methionine adenosyltransferase, leading to a transmethylation rate which is equivalent to that observed upon direct administration of L-methionine. The participation of the peroxisomal D-amino acid oxidase in the conversion of D- to L-methionine is further supported by the strong inhibition of the methionine metabolism in rats pretreated with sodium benzoate, a known inhibitor of this enzyme. Brada and Bulba (1980) have observed an analogous reduction in the rate of formation of *S*-adenosylethionine from D-ethionine in rats treated with sodium benzoate. Analogous to the studies of *S*-adenosylethionine formation from D-ethionine, the sodium benzoate dose that the rat will tolerate is insufficient to completely inhibit sarcosine formation and, by inference, the formation of *S*-adenosylmethionine.

The control studies on sodium benzoate treated, L-[*methyl*-$^2H_3$]methionine-dosed rats exhibit a much more rapid initial rate of formation of [*methyl*-$^2H_3$]sarcosine than the group receiving sodium benzoate plus D-[*methyl*-$^2H_3$]methionine. This observation is consistent with the expectation that the sodium benzoate inhibits D-amino acid oxidase but will not inhibit methionine adenosyltransferase or glycine *N*-methyltransferase. However, the control studies utilizing benzoate plus labeled L-methionine also exhibit a marked retardation in the rates of clearance of the L-[*methyl*-$^2H_3$]methionine and the [*methyl*-$^2H_3$]sarcosine as well as the rate of labeling of the water with ^2H, relative to the rats receiving L- or D-methionine only. These observations indicate that sodium benzoate has an additional effect on hepatic methyl group metabolism. There are several possible bases for such effects. One mechanism could involve the reduction of hepatic glycine levels as a result of the conjugation of glycine with benzoate to form hippuric acid. Such an effect is consistent with the reduced rate of clearance of hepatic methionine but would not explain the elevation in hepatic sarcosine and the reduced rate of sarcosine clearance. Additional effects on sarcosine metabolism could arise as a consequence of a reduced flux through sarcosine dehydrogenase (Figure 5) due either to the reduced availability of the tetrahydrofolate cofactor or to a direct inhibition of the enzyme by the benzoate. Depletion of the tetrahydrofolate pool might arise if the oxidation of the methylenetetrahydrofolate is significantly impaired, while the direct inhibition of the enzyme could reflect the formation of a charge transfer complex of benzoate with the flavin cofactor of the enzyme (Cook et al., 1984). There are precedents for the formation of such complexes [e.g., see Miura and Miyake (1987)]. Inhibition of sarcosine dehydrogenase flux via either mechanism could also reduce the clearance of hepatic methionine by limiting the availability of glycine. Inhibition of this flux is also consistent with the observed reduction in the rate of appearance of deuterium in the water resonance.

An increase in the level of the *N*-trimethyl-labeled metabolites was observed in the sodium benzoate treated rats dosed with L-methionine, but not D-methionine (Figure 3C). This increase is similar to that observed in the studies using D- or L-methionine without sodium benzoate pretreatment. The difference is proposed to reflect the greater production of *S*-adenosylmethionine in the sodium benzoate/L-methionine controls, relative to the sodium benzoate/D-methionine group. As discussed previously (London et al., (1987), several metabolites, including choline, phosphorylcholine, glycerophosphorylcholine, carnitine, and betaine, exhibit very similar methyl proton or deuteron shifts, precluding an analysis on the basis of the ^2H NMR data alone. On the basis of the established pathways of methylation of phosphatidylethanolamine to yield phosphatidylcholine (Stekol & Szaran, 1962; Pelech & Vance, 1984), as well as the relatively high levels of phosphorylcholine found in the liver (Dawson, 1956), it is probable that this metabolite makes a dominant contribution to the observed resonance. Finkelstein and Martin (1986) have shown that the concentration of hepatic betaine declines significantly in rats receiving a high level of dietary methionine. However, it is possible that under the conditions of this study, an initial depletion of the betaine pool might be followed by a resynthesis of labeled betaine from labeled choline.

As in the previous study of the metabolism of deuteriated L-methionine, the principle metabolic fate of the label appears to flow through the methyl group of sarcosine and ultimately into water, as reflected by the gain in intensity of the HDO resonance. These data, coupled with the cytosolic localization of glycine methyltransferase (Ogawa & Fujioka, 1982; Wagner et al., 1985) and the mitochondrial localization of sarcosine dehydrogenase (Cook et al., 1984), are therefore consistent with the conclusion of a dominant contribution by a glycine/sarcosine shuttle, in which sarcosine functions to transport the labeled methyl group into the mitochondrion where it is further oxidized by sarcosine dehydrogenase, yielding 5,10-methylenetetrahydrofolate (Figure 5). The methylene group can be subsequently utilized for a variety of anabolic reactions or further catabolized to formaldehyde, formate, or CO_2. The recent demonstration of allosteric regulation of glycine methyltransferase by the pentaglutamyl derivative of 5-methyltetrahydrofolate (Wagner et al., 1985) suggests that the initial formation of sarcosine via this enzyme may be a critical regulatory point in this catabolic pathway. According to this model, the glycine need only be present in catalytic amounts, although the NMR data indicate the presence of millimolar levels of labeled sarcosine. As noted previously (London et al., 1987), no significant deuterium kinetic isotope effect is anticipated in the methyl transfer reactions; however, a significant effect would be predicted for the sarcosine dehydrogenase reaction (reaction 2, Figure 5), so that the levels of [*methyl*-$^2H_3$]sarcosine observed in the present study are presumably above those which would be produced in a study utilizing unlabeled D-methionine.

The present ^2H NMR studies of the metabolism of D-methionine indicate a very significant flux through the transamination pathway (subsequent to the initial oxidation step) which is apparently sufficient to provide a level of L-methionine able to saturate methionine adenosyltransferase. However, the flow is in the opposite sense, i.e., amination rather than deamination, to that which would characterize the catabolic pathway for L-methionine. This observation is consistent with conclusions reached by Cooper and Meister (1972) on the transamination of methionine via glutaminase II. In this enzyme system, glutamine serves as the amino group donor in the conversion of α-keto-γ-methylthiobutyrate to methionine. The α-ketoglutaramate product is rapidly eliminated as a consequence of either the rapid nonenzymatic cyclization to 5-hydroxypyrrolidone carboxylate or, enzymatically, the action of ω-amidase to yield α-ketoglutarate plus ammonia. Thus, transamination via glutaminase is essentially irreversible under physiological conditions. The glutaminase-catalyzed reactions provide a viable interpretation for the observations of a high transamination flux in the direction of synthesis of L-methionine from α-keto-γ-methylthiobutyrate, but minimal catabolism of L-methionine via the transamination pathway.

As noted above, the question of the relative flux of excess methionine through the transmethylation and transamination pathways (Figure 1) has been the subject of a considerable degree of investigation. Most recently, evidence has been obtained indicating that the transamination pathway may be significant in skeletal muscle since the enzymes of the transmethylation pathway are weak or absent in this tissue (Davis & Lee, 1985; Lee & Davis, 1986; Scislowski et al., 1987). However, most evidence suggests that the transsulfuration pathway represents the major catabolic route for excess methionine in liver (Mudd et al., 1980; Stipanuk, 1986). The formation of the transamination intermediate α-keto-γ-methylthiobutyrate in liver is frequently not observed [e.g., see Ozturk et al. (1986)]. Recent studies on a human adult with hepatic methionine adenosyltransferase deficiency (Gahl et al., 1988) indicate that although in this extreme case catabolic flux through the transamination pathway can be observed, this flux is insufficient to maintain methionine levels

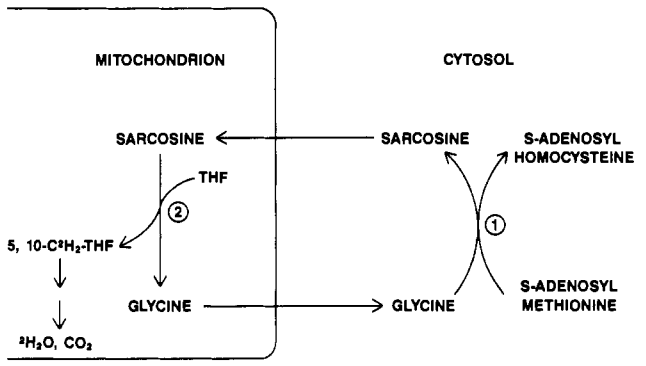

FIGURE 5: Glycine/sarcosine shuttle transports methyl groups into the mitochondrion where they are oxidized. Enzyme activities indicated correspond to (1) glycine methyltransferase and (2) sarcosine dehydrogenase.

in the normal range. The present results are also consistent with recent biochemical studies on the hepatic metabolism of D-methionine in rats (Sugiyama & Muramatsu, 1987), leading to the conclusion that D-methionine is converted to L-methionine and that the latter preferentially enters the transsulfuration pathway.

Ghittoni and Sellinger (1971) have studied the uptake and metabolism of D-methionine by rat brain, and the effects of the convulsant methionine sulfoximine on this metabolism. Clearly, the rapid inversion of D- to L-methionine by hepatic enzymes can make a major contribution to the observed metabolite levels in the brain, even at the earliest (0.5 h) time points utilized in the study. The potent inhibition of this inversion by sodium benzoate suggests that this agent might prove useful for separating out the effects of hepatic racemization, depending on its uptake and activity in other organs such as brain. Finally, it is noted that in contrast to the data presented here which clearly indicate that D-methionine can function effectively as a precursor of L-methionine for the rat, studies on plasma and urinary amino acid levels in adult humans indicate a rather poor utilization of D-methionine (Stegink et al., 1986).

ACKNOWLEDGMENTS

We thank Alex Funk for work on the ^2H surface coil probe used in these studies.

Registry No. D-Methionine, 348-67-4; sarcosine, 107-97-1.

REFERENCES

Bartlett, G. R. (1948) *J. Am. Chem. Soc. 70*, 1010–1011.
Benevenga, N. J. (1974) *J. Agric. Food Chem. 22*, 2–9.
Benevenga, N. J., & Harper, A. E. (1967) *J. Nutr. 93*, 44–52.
Bergstrom, M., Muhr, C., Lundberg, P. O., Bergstrom, K., Lundqvist, H., Antni, G., Fasth, K.-J., & Langstrom, B. (1987) *J. Comput. Assisted Tomography 11*, 384–389.
Block, R. E., & Parekh, B. C. (1987) *Magn. Res. Med. 5*, 286–289.
Borchardt, R. T., Creveling, C. R., & Ueland, P. M. (1986) *Biological Methylation and Drug Design*, Humana, Clifton, NJ.
Brada, Z., & Bulba, S. (1980) *Res. Commun. Chem. Pathol. Pharmacol. 30*, 341–360.
Case, G. L., & Benevenga, N. J. (1976) *J. Nutr. 106*, 1721–1736.
Cook, R. J., Misono, K. S., & Wagner, C. (1984) *J. Biol. Chem. 259*, 12475–12480.
Cooper, A. J. L., & Meister, A. (1972) *Biochemistry 11*, 661–671.
Davis, E. J., & Lee, S.-H. C. (1985) *Biochem. J. 229*, 19–29.
Dawson, R. M. C. (1956) *Biochem. J. 62*, 693–696.
Finkelstein, J. D., & Martin, J. J. (1986) *J. Biol. Chem. 261*, 1582–1587.
Gahl, W. A., Bernardini, I., Finkelstein, J. D., Tangerman, A., Martin, J. J., Blom, H. J., Mullen, K. D., & Mudd, S. H. (1988) *J. Clin. Invest. 81*, 390–397.
Ghittoni, N. E., & Sellinger, O. Z. (1971) *J. Neurobiol. 2*, 153–168.
Hardwick, D. F., Applegarth, D. A., Cockcroft, D. M., Ross, P. M., & Calder, R. J. (1970) *Metab., Clin. Exp. 19*, 381–391.
Harper, A. E., Benevenga, N. J., & Wohleuter, R. M. (1970) *Physiol. Rev. 50*, 428–558.
Kaufman, N., Klavins, J. V., & Kinney, T. D. (1960) *Arch. Pathol. 70*, 331–337.
Klein, J. R., & Kamin, H. (1941) *J. Biol. Chem. 138*, 507–512.
Konno, R., & Yasumura, Y. (1984) *J. Nutr. 114*, 1617–1621.
Lee, S.-H. C., & Davis, E. J. (1986) *Biochem. J. 233*, 621–630.
Leighton, F., Coloma, L., & Koenig, C. (1975) *J. Cell Biol. 67*, 281–309.
Livesey, G. (1984) *Trends Biochem. Sci. (Pers. Ed.) 9*, 27–29.
Lindon, J. C., & Ferrige, A. G. (1979) *J. Magn. Reson. 36*, 277–280.
Lombardini, J. B., & Talalay, P. (1970) *Adv. Enzyme Regul. 9*, 349–384.
London, R. E., Gabel, S. A., & Funk, A. (1987) *Biochemistry 26*, 7166–7172.
London, R. E., Galvin, M. J., Thompson, M., Jeffreys, L., & Mester, T. (1985) *J. Biochem. Biophys. Methods 11*, 21–29.
Meister, A. (1965) *Biochemistry of the Amino Acids*, 2nd ed., Academic, New York.
Mitchell, A. D., & Benevenga, N. J. (1978) *J. Nutr. 108*, 67–78.
Miura, R., & Miyake, Y. (1987) *J. Biochem. (Tokyo) 101*, 581–589.
Mudd, S. H., Ebert, M. H., & Scriver, C. R. (1980) *Metab., Clin. Exp. 29*, 707–720.
Ogawa, H., & Fujioka, M. (1982) *J. Biol. Chem. 257*, 3447–3452.
Ozturk, M., Lemonnier, F., Cresteil, D., & Lemonnier, A. (1986) *Biochem. Pharmacol. 35*, 4223–4228.
Pelech, S. L., & Vance, D. E. (1984) *Biochim. Biophys. Acta 779*, 217–251.
Rechcigl, M., Jr., Loosli, J. K., & Williams, H. H. (1960) *Proc. Soc. Exp. Biol. Med. 104*, 448–452.
Russell, W. C., Taylor, M. W., & Hogan, J. M. (1952) *Arch. Biochem. Biophys. 39*, 249–252.
Schober, O., Meyer, G.-J., Stolke, D., & Hundeshagen, H. (1985) *J. Nucl. Med. 26*, 98–99.
Scislowski, P. W. D., Hokland, B. M., Davis-van Thienen, W. I. A., Bremer, J., & Davis, E. J. (1987) *Biochem. J. 247*, 35–40.
Steele, R. D., & Benevenga, N. J. (1978) *J. Biol. Chem. 253*, 7844–7850.
Stegink, L. D., Bell, E. R., Filer, L. J., Jr., Ziegler, E. E., Andersen, D. W., & Seligson, F. H. (1986) *J. Nutr. 116*, 1185–1192.
Stekol, J. A., & Szaran, J. (1962) *J. Nutr. 77*, 81–90.
Stipanuk, M. H. (1986) *Annu. Rev. Nutr. 6*, 179–209.
Sugiyama, K., & Muramatsu, K. (1987) *Agric. Biol. Chem. 51*, 3411–3413.
Wagner, C., Briggs, W. T., & Cook, R. J. (1985) *Biochem. Biophys. Res. Commun. 127*, 746–752.