Metabolism of Excess Methionine in the Liver of Intact Rat: An in Vivo ^2H NMR Study

Robert E. London,* Scott A. Gabel, and Alex Funk

Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

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ABSTRACT: L-Methionine is the most toxic amino acid if supplied in excess, and the metabolic basis for this toxicity has been extensively studied, with varying conclusions. It is demonstrated here that in vivo ^2H NMR spectroscopy provides a useful approach to the study of the hepatic metabolism of methionine in the anesthetized rat. Resonances corresponding to administered L-[*methyl*-$^2H_3$]methionine, and to the transmethylation product sarcosine, are observed during the first 10-min period after an intravenous injection of the labeled methionine, and the time dependence has been followed for a period of 5 h. A third resonance, assigned to the *N*-trimethyl groups of carnitine, phosphorylcholine, and other metabolites, becomes observable several hours after administration of the deuteriated methionine. In addition, there is a small increase in the intensity of the HDO resonance over the period of the study, which is interpreted to reflect the ultimate oxidation of the labeled sarcosine methyl group via mitochondrial sarcosine dehydrogenase. Additional small ^2H resonances assigned to N^1-methylhistidine and creatine could be observed in perchloric acid extracts of the livers of rats treated with the deuteriated methionine. Inhibition of the flux through the transmethylation pathway is observed in the rat pretreated with the *S*-ethyl analogue of methionine, ethionine. These data provide strong support for the importance of glycine transmethylation in the catabolism of excess methionine.

The metabolism of L-methionine has been extensively studied by a variety of techniques as a consequence of the central role of transmethylation reactions in a broad range of anabolic processes. The role of methylation of DNA in the regulation of gene expression has been of intense recent interest (Doerfler, 1983), and a specific methionine dependence has been noted in many tumor cell lines (Mecham et al., 1983; Hoffman, 1984). Methionine metabolism is also of considerable interest in human pathology. Hypermethioninemia can reflect a specific enzymatic defect (Gaull et al., 1981) and may also be associated with different hepatocellular injuries (Liau et al., 1979).

When present in excess, L-methionine has been reported to be the most toxic of the naturally occurring amino acids (Benevenga, 1974; Mitchell & Benevenga, 1978). Metabolism of excess methionine can occur either via a transmethylation–transsulfuration pathway involving the formation of *S*-adenosylmethionine (Shapiro & Schlenk, 1965) or via a transamination pathway (Benevenga, 1974; Steele & Benevenga, 1978). On the basis of a number of observations including the negligible oxidation of the methyl group of *S*-adenosylmethionine to CO_2 by liver homogenates (Case & Benevenga, 1976), the inhibition of methionine metabolism by *S*-methylcysteine (Case & Benevenga, 1976), the direct observation of intermediates in the transamination pathway (Steele & Benevenga, 1978), and the conclusion that the transmethylation pathway cannot explain the observed toxicity of L-methionine (Mitchell & Benevenga, 1978), it has been proposed that the transamination pathway becomes of primary importance in the metabolism of excess methionine. Alternatively, data of Hardwick et al. (1970) indicate that much of the toxicity associated with the metabolism of excess methionine can be explained in terms of the *S*-adenosylmethionine-mediated pathway, particularly as a consequence of the drop in hepatocellular ATP levels which occurs, analogous to the effects seen with the *S*-ethyl analogue ethionine (Shull, 1962; Farber et al., 1964). Finkelstein and Martin (1986) have carried out a series of systematic measurements of metabolite levels and related enzyme activities in the livers of Sprague-Dawley rats chronically overdosed with various levels of methionine and find that the results can be interpreted within the framework of increased flux through the enzymes of the transsulfuration pathway. Further, Finkelstein (1975) notes that human patients with a deficiency in hepatic methionine adenosyltransferase accumulate methionine, often without apparent toxicity, and patients with a deficiency of cystathionine synthase, an enzyme in the transsulfuration pathway, do not show urinary excretion of the keto compounds characteristic of the transamination pathway. The critical need for in vivo metabolic studies to complement the data of in vitro studies has been emphasized by Livesey (1984).

In view of the considerable interest in determining the metabolic fate of excess methionine, we have carriedout in vivo ^2H NMR studies of the liver of anesthetized rats dosed with L-[*methyl*-$^2H_3$]methionine. The application of in vivo NMR spectroscopy to problems related to metabolic regulation is increasingly appreciated (Iles et al., 1982; Avison et al., 1986). Previous ^{13}C NMR studies utilizing [*methyl*-^{13}C]methionine have included analyses of porphyrin biosynthesis (Scott et al., 1972), as well as studies of intact *Neurospora crassa* cells (Eakin et al., 1975). The use of ^2H NMR as an in vivo probe has provided some data in studies of erythrocytes (York et al., 1982), microorganisms (Hunter et al., 1984; Barrow et al., 1986), and rabbit lens (Aguayo et al., 1987); however, lower field ^2H NMR studies of the liver of intact rats have yielded such limited resolution that it has been unclear whether this technique can provide any insight into metabolic transformations (Brereton et al., 1986; Irving et al., 1986). The advantages and disadvantages of the technique have thus been evaluated in connection with the present high-field NMR

study.

Materials and Methods

Physiological Methods. L-[*methyl*-$^2H_3$]Methionine was obtained from MSD Isotopes and used without further purification. Male Sprague-Dawley rats weighing 275–300 g were surgically prepared as described previously (London et al., 1985). One to two days prior to NMR study, an incision was made directly over the liver, and a circle of muscle approximately 3 cm in diameter was removed. The skin was then sutured. In this way, the layer of intervening muscle was removed to optimize NMR observation of heaptic metabolism without the need for coil implantation, direct exposure of the liver, or the use of imperfect pulse localization methods. All rats were anesthetized with inactin (Lockwood Assoc. Imports, East Lansing, MI; Smith et al., 1987). One hundred milligrams of the deuteriated methionine was dissolved in 1–1.5 mL of normal saline and administered via intravenous injection through a polyethylene tube cannulated into the jugular vein. Perchloric acid extracts of liver were prepared as described previously (Smith et al., 1987).

In Vivo NMR Measurements. In vivo NMR measurements were made on a Nicolet NT-360 NMR spectrometer using a probe design similar to that previously described (London et al., 1985) with two modifications. First, an aluminum probe body has been substituted for the acrylic body in order to more effectively screen out noise introduced via the room temperature shims. Second, the probe design included two concentric coils, each of which was part of an independent radio-frequency circuit. The deuterium observe coil consisted of eight turns, 1.3 cm in diameter, and was tuned to 55.42 MHz. A second single-turn coil tuned to the proton resonance frequency of 361.0 MHz was used for shimming. The homogeneity of the magnetic field over the volume under study, always an important consideration in NMR studies, is particularly critical for in vivo ^2H NMR studies as a consequence of the small range of chemical shifts characterizing the metabolites. The use of an independent coil tuned to the proton resonance frequency for shimming has been found to give more consistent results than reliance on the proton signals detected through observe coils tuned to other frequencies. Additionally, the probe design, in which the orientation of the coil is fixed, allows for high reproducibility in the placement of the coil relative to the static magnetic field, affording substantial reproducibility of the shimming parameters. All studies were carried out in the unlocked mode; magnet drift has been measured at less than 3×10^{-3} ppm/h.

Although reasonable qualitative data can be obtained by using standard exponential filtering of the data [line broadening (LB) = 10 Hz], improved and more readily quantitated spectra were obtained by using resolution enhancement techniques (Ferrige & Lindon, 1978). After evaluation of several related approaches, optimal spectra were obtained by using the "DM" apodization function described in the Nicolet operating manual. This operation, developed by Lindon and Ferrige (1979), is effectively a combination of a Gaussian multiplication with LB > 0 and an exponential multiplication with LB < 0:

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

However, the Nicolet program includes only a single parameter, LB, from which a and b are calculated.

In Vitro NMR Measurements. For assignment purposes, in vitro ^1H NMR studies of model compounds (Sigma Chemical Co.) and in vitro ^2H NMR studies of hepatic extracts were also carried out. The former were performed on either a Nicolet NT-360 or a GE Model GN 500 NMR spectrometer, and the latter on the NT-360. All in vitro spectra were obtained at an ambient probe temperature of 21 °C, and the chemical shifts were referenced to the HDO resonance set at 4.8 ppm. The use of ^1H NMR measurements to establish standards for the interpretation of ^2H NMR studies requires in principle the incorporation of the appropriate isotope shift corrections. For methyl groups, isotope shifts are expected to enter the comparison as a consequence of two factors. First, the deuterium resonance of the CD_3 group will be shifted upfield as a consequence of the presence of the two geminal deuterium nuclei. Second, the ^1H resonance of the HDO standard will similarly be shifted upfield relative to the ^2H resonance of HDO as a consequence of the geminal deuterium nucleus. The magnitude of these effects was estimated by comparing the ^1H chemical shift observed for the methyl resonance of unlabeled choline with the ^1H chemical shift observed for the residual protons in commercial (98 atom %) [$^2H_9$]choline (MSD Isotopes), which arises from the CHD_2 groups. The latter resonance was found to be shifted upfield by 0.03 ppm so that, assuming a similar isotope shift for the ^1H HDO resonance, the methyl proton shifts must be reduced by 0.045 ppm for comparison with the deuteron shifts.

Although the above procedure was found to give reasonable agreement with the ^2H NMR shifts determined for perchloric acid extracts, the multiple corrections involved in the above procedure can introduce errors into such comparisons. Hence, a second approach was developed, based on the recognition that although isotope shift effects must enter a comparison of deuterium and proton NMR data, the relative shifts of the methylated metabolites determined from ^1H NMR will be preserved. In order to utilize this fact, ^2H NMR spectra of liver extracts were recorded both before and after being doped with 1 mg of [*methyl*-$^2H_9$]choline. By setting the ^2H shift of the choline in ppm equal to the ^1H shift determined for unlabeled choline, all other methyl group shifts should be directly comparable. Hence, this approach does not require any explicit corrections for isotope shifts.

In addition to the effects noted above, the temperature dependence of the water resonance must be considered in comparing in vivo and in vitro data. It was found that setting the ^2H resonance of HDO at 4.8 ppm for the in vitro studies and at 4.7 ppm for the in vivo studies gave close agreement for the chemical shifts of the deuterium resonances of the methylated metabolites.

Results

The background ^2H NMR spectrum obtained prior to the addition of labeled metabolites is shown as the lower trace in Figure 1. The spectrum was obtained with a 1.3-cm diameter surface coil (Ackerman et al., 1980) positioned over the liver of an anesthetized Sprague-Dawley rat prepared as described under Materials and Methods. As discussed by Brereton et al. (1986), the only observable background resonances arise from naturally abundant deuterons in HDO and in the CHD groups of fatty acids present in membranes, in lipoproteins, or as triglycerides. Although the spectrum shown in Figure 1 was acquired over a period of 10 min, an adequate signal to noise ratio to permit observation of the background water resonance can be obtained in several pulses. The HDO resonance serves as a convenient internal shift standard (δ = 4.7 ppm). Additionally, this resonance provides an approximate intensity standard to allow quantitation of deuterium-labeled metabolites, assuming that the metabolism of such molecules does not significantly perturb the size of the HDO pool. With

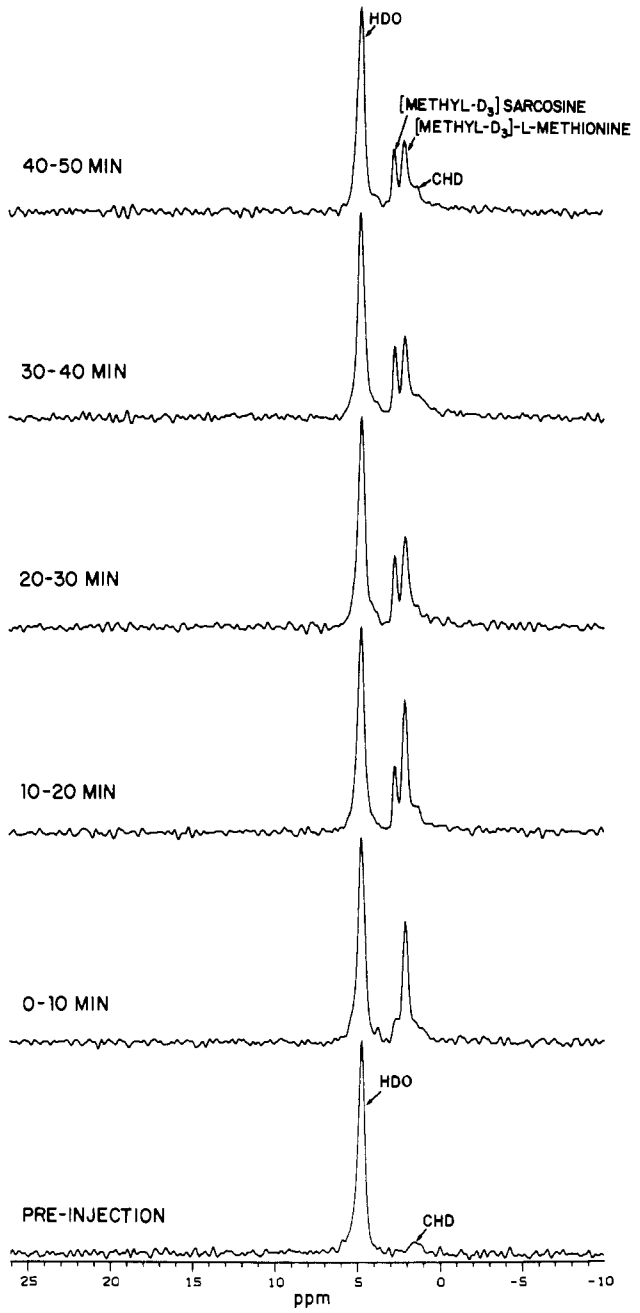

FIGURE 1: ^2H NMR spectra obtained by using a 1.3-cm diameter surface coil tuned to 55.4 MHz and placed above the liver of an anesthetized rat prepared as described under Materials and Methods. The background deuterium resonances arising from HDO and CHD species are apparent in the initial spectrum (lower trace). A series of spectra obtained subsequent to an intravenous injection of 100 mg of L-[*methyl*-$^2H_3$]methionine is illustrated. Each spectrum corresponds to 2340 accumulations obtained over 10 min, with a total sweep width of ±1000 Hz and a block size of 1K data points. All spectra have been resolution enhanced by using a double-exponential multiplication function set at 10 Hz.

a proton concentration in pure water of 110 M, a natural deuterium abundance of 0.0156%, and a figure of 70% water for biological tissue, the HDO resonance is determined to correspond to 12 mM deuterons. It is noted, however, that using this value to estimate levels of intracellular metabolites will lead to an underestimation of cellular concentrations due to contributions from extracellular HDO and from HDO resonances from any other tissues which are picked up by the surface coil.

Injection of 100 mg of L-[*methyl*-$^2H_3$]methionine, as described under Materials and Methods, results in the appearance of an additional ^2H resonance at 2.1 ppm (Figure 1). The effective hepatic concentration of the deuteriated methionine is determined, taking into account the presence of three deuterons per molecule, to be 2.2–2.6 mM in a series of five studies immediately postinjection. By the 30–40-min accumulation period, hepatic methionine levels had been reduced to an average of 61% ± 3% of the initial values. A second resonance

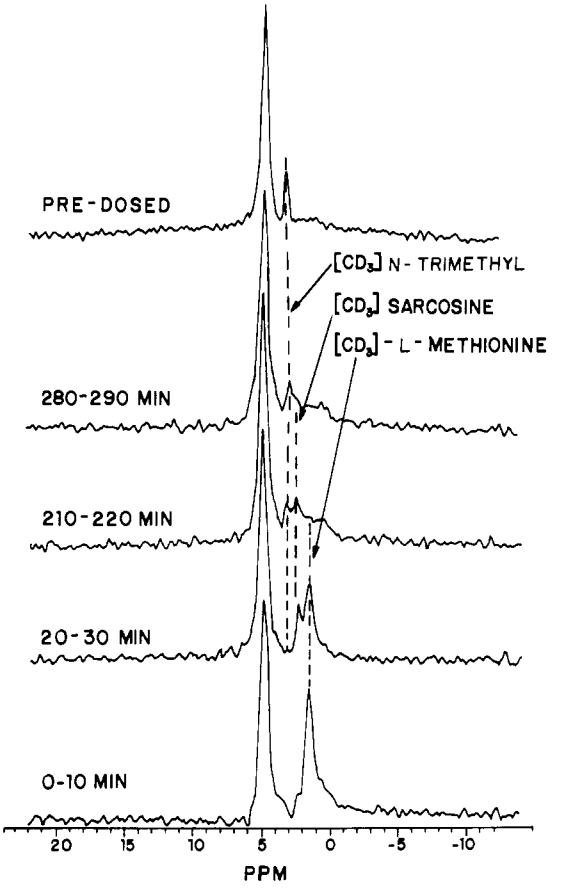

FIGURE 2: In vivo ^2H NMR spectra corresponding to the times indicated with the resonance assignments given as discussed in the text. Resolution enhancement of these spectra was achieved by the use of a double-exponential weighting function and a 10-Hz line-broadening parameter. Other spectral parameters were as described in Figure 1. The times indicated are referenced to the intravenous injection of deuteriated methionine at $t = 0$. The upper trace was obtained from a rat dosed the previous day with 0.2 g of [*methyl*-$^2H_3$]methionine.

Table I: Methyl Proton Chemical Shifts[a] of Some Common Hepatic Metabolites and Model Compounds

compound	^1H shift (ppm)
4-(methylthio)-2-oxobutyric acid	2.08
methionine	2.14[b]
N-methylphenylalanine	2.64
sarcosine	2.70
N-methylaspartic acid	2.71
S-adenosylmethionine	2.98[b]
creatine	3.00
phosphocreatine	3.01
phosphorylcholine	3.15
choline	3.17
carnitine	3.18
betaine	3.22
N^3-methylhistidine	3.64
N^1-methylhistidine	3.66
N^1-methylanserine	3.81[c]
N-methylnicotinamide	4.43

[a] Chemical shifts are referenced to the HDO resonance at $\delta = 4.80$ ppm. [b] Shifts taken from Stolowitz and Minch (1981). [c] Anserine methyl shift taken from Arus et al. (1984).

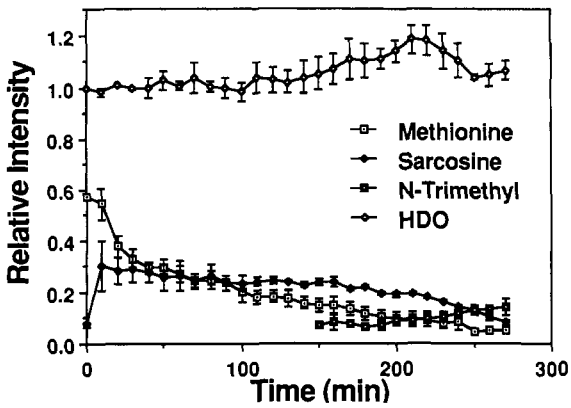

FIGURE 3: Relative intensities, normalized with respect to the intensity of the initial HDO resonance in each study, of the deuterium resonances of the observed metabolites, plotted as a function of the time after the initiation of an intravenous injection of [*methyl*-$^2H_3$]-methionine. The data points represent mean values (peak heights) corresponding to 10-min accumulation periods, and calculated standard errors are included. Data correspond to methionine (□), sarcosine (♦), *N*-trimethyl-containing metabolites (■), and HDO (♦).

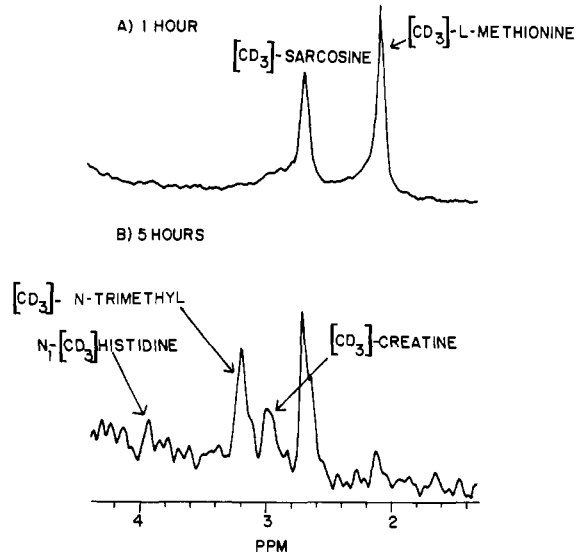

FIGURE 4: ^2H NMR spectra of perchloric acid extracts of the livers of rats obtained at (A) 1 h (upper trace) and (B) 5 h (lower trace) after administration of 100 mg of L-[*methyl*-$^2H_3$]methionine via intraperitoneal injection. Chemical shifts are referenced to HDO set at $\delta = 4.8$ ppm. Spectra were obtained by using 8K data points and a sweep width of ±1 kHz for a 1.02-s acquisition time, and multiplied by a Gaussian function with LB = 1.5 Hz. Spectra correspond to 28 332 transients (A) and 23 532 transients (B). Chemical shifts are in good agreement with the standards listed in Table I, and this agreement is improved slightly by taking into account the isotope shifts as discussed under Materials and Methods.

at 2.7 ppm becomes visible almost immediately, and an additional resonance at 3.15 ppm becomes observable after several hours (Figure 2). In order to determine the identity of these metabolites, ^1H NMR spectra of common hepatic metabolites containing *N*-methyl groups were obtained, and the methyl shifts are summarized in Table I. A comparison with the results of the in vivo study indicates that the metabolite corresponding to the 2.7 ppm chemical shift corresponds to an *N*-methyl amino acid. Since the activity of the glycine methyltransferase is known to be the highest of methyltransferases in adult liver (Ogawa & Fujioka, 1982; Heady & Kerr, 1973), the assignment of this resonance to *N*-methylglycine (sarcosine) can be made with confidence. As discussed below, this assignment is consistent with previous suggestions regarding the role of glycine as the primary methyl group acceptor under conditions of excess methionine administration.

As shown in Figure 2, the 3.15 ppm resonance observed after several hours is also obtained in rats dosed the previous day with 0.2 g of [*methyl*-$^2H_3$]methionine. Hence, this resonance appears to correspond to a metabolite which does not turn over rapidly. On the basis of the shifts in Table I and on the fact that phosphorylcholine levels in the liver are known to be in the millimolar range (Dawson, 1956), this resonance is tentatively assigned primarily to phosphorylcholine. Other compounds containing *N*-trimethyl groups, particularly glycerophosphorylcholine, betaine, and carnitine, may also make small contributions to the observed resonance. The corresponding methyl resonances cannot be distinguished in vivo or in extracts due to the very small chemical shift differences.

A plot of the mean intensities of the ^2H resonances obtained in three separate studies as a function of time is shown in Figure 3. As is typically the case in surface coil studies, the system is not closed so that metabolites can enter and exit the region of observation. Hence, total resonance intensity is not necessarily conserved. Nevertheless, the total intensity under the methionine curve integrated over the first 270 min of the study is similar to that under the sarcosine curve. It is also noted that the labeled sarcosine forms very rapidly from the labeled methionine; a significant sarcosine resonance can be observed during the first 10-min accumulation period in all studies. The further extrapolation of the data of Figure 3 to estimate metabolite levels requires a correction for the different number of deuterons in the observed metabolites. Both methionine and the sarcosine derived from it will contain three deuterons per molecule. Since the initial pool of *N*-dimethyl species in the cells, e.g., proteins containing dimethyllysine residues, is expected to be relatively small in comparison with the methionine dose, the deuterium resonance assigned to the *N*-trimethyl species arises primarily from methyl-$^2H_9$-labeled molecules. Hence, the extrapolation of the data in Figure 3 to an estimate of the relative concentrations of the HDO, methionine, sarcosine, and *N*-trimethyl-containing metabolites requires that the intensities be multiplied by 1, $^1/_3$, $^1/_3$, and $^1/_9$, respectively. Finally, it is noted that the HDO resonance exhibits a small increase in intensity late in the study, which would presumably reflect the ultimate oxidation of the CD_3 group of sarcosine via sarcosine dehydrogenase in the mitochondria.

In order to obtain further support for the resonance assignments made above, ^2H NMR spectra were obtained on perchloric acid extracts of the livers of rats given an intraperitoneal injection of 100 mg of L-[*methyl*-$^2H_3$]methionine. Spectra were obtained from rats sacrificed at 1-, 2-, 5-, and 15-h post-methionine administration, and the results obtained at 1 and 5 h are shown in Figure 4. As discussed under Materials and Methods, the ^2H chemical shifts obtained on the extracts were compared with the ^1H methyl shifts of the reference compounds by directly adding 1 mg of [*methyl*-$^2H_9$]choline to the extract and setting the resultant shift in ppm equal to that determined by ^1H NMR of choline. The observed shifts are consistent with the assignments of the sarcosine and *N*-trimethyl resonances. In addition, resonances at 2.97 and 3.91 ppm are observed in the 5-h extract, and these are tentatively assigned to [*methyl*-$^2H_3$]creatine and to N^1-[*methyl*-$^2H_3$]histidine, either in free form or in a peptide. The methylated histidine is the only metabolite expected to exhibit a significant pH-dependent chemical shift, possibly explaining the larger shift difference between the metabolite resonance and that of the model compounds. The time dependence of the resonance intensities derived from the extracts is quali-

tatively consistent with the in vivo observations, with the *N*-trimethyl resonance not visible at 1 h but clearly visible after 5 h. These limited time-dependent data obtained at the cost of four animals again underline the value of in vivo spectroscopy for obtaining real time data on individual experimental animals.

In order to further probe the nature of the observed transmethylation reactions, studies were carried out on rats pretreated with ethionine, the *S*-ethyl analogue of methionine. Ethionine treatment is known to produce a range of metabolic perturbations which include the following: (1) interference with transmethylation reactions (Stekol, 1965); (2) decrease in cellular ATP levels as a consequence of sequestering of the adenine base as *S*-adenosylethionine (Shull, 1962; Farber et al., 1964); (3) ethylation of various cellular components including ethanolamine, acetylguanidine, and nucleic acids (Stekol & Weiss, 1950; Stekol et al., 1960, 1963; Winnick & Winnick, 1959); and (4) fatty degeneration of the liver (Glaser & Mager, 1972). The latter effect has also been monitored by ^{13}C NMR studies of the excised liver of a rat treated with ethionine (Bloch, 1982). In order to determine whether analogous perturbations could be observed in vivo, two rats were predosed with 1 g/kg of DL-ethionine via intraperitoneal injection, and ^{2}H NMR observations were initiated 5-h post-administration. In both studies, data from one of which are shown in Figure 5, ethionine pretreatment was found to significantly inhibit both the disappearance of the methionine resonance and the appearance of the sarcosine resonance. Thus, at 220-min post-methionine administration, the methionine resonance was roughly 70% of the initial value. These observations are consistent with those of Stekol et al. (1963), who noted that in vitro, *S*-adenosyl-L-ethionine depressed the transfer of methyl groups from *S*-adenosyl-L-methionine. In addition to these differences, a resonance close to the position of the endogenous CHD peak (1.5 ppm) was observed to be significantly elevated in the studies of the ethionine-pretreated rats. It is unlikely that this resonance arises from any of the known methionine metabolites. As discussed above, the primary labeled metabolite formed via the transmethylation pathway is sarcosine. Metabolites formed via the transamination pathway presumably exhibit chemical shifts which are very close to that of methionine itself, as supported by the ^{1}H shift determined for 4-(methylthio)-2-oxo-butyrate (Table I). Instead, this resonance most probably arises from the natural-abundance methylene CHD groups of fatty acids which will be enhanced due to the physiological action of ethionine. Thus, since the ethionine-induced fatty liver has been monitored by natural-abundance ^{13}C NMR (Bloch, 1982), it is not surprising that natural-abundance ^{2}H NMR is also sensitive to this effect.

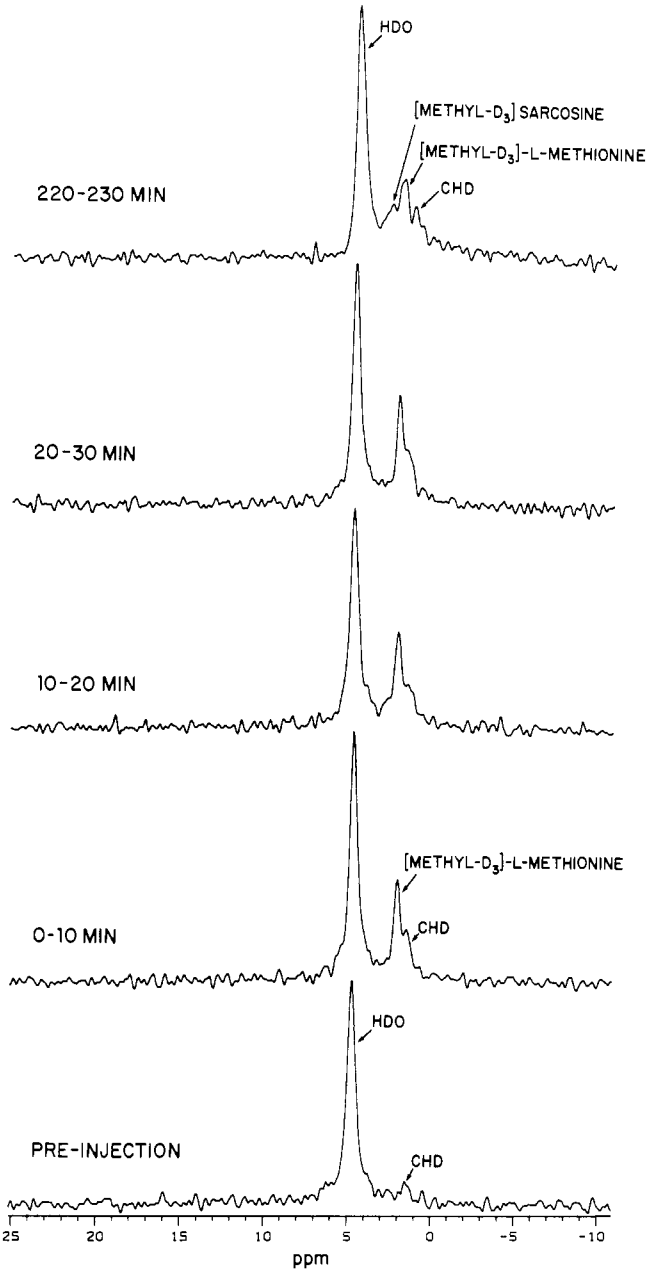

FIGURE 5: Series of ^{2}H NMR spectra obtained as described in Figure 1, using double-exponential multiplication with LB = 10 Hz, on a rat 5 h after the administration of 1 g/kg L-ethionine. The times indicated in the spectra are referenced to the intravenous injection of the deuteriated methionine at $t = 0$.

Discussion

Methodology. These studies demonstrate the utility of ^{2}H NMR for following transmethylation reactions in vivo. High-resolution studies can be carried out as a consequence of the relatively narrow line widths, and resolution is further enhanced by utilizing double-exponential filtering, as in Figure 1. Additionally, a comparison with a previously published in vivo ^{2}H NMR (Brereton et al., 1986) carried out at 4.7 T indicates the importance of utilizing a high magnetic field strength. The use of deuterium as an in vivo metabolic probe has several important advantages: (1) The natural abundance of ^{2}H is extremely low, so that the background resonances are minimal. This situation is in significant contrast with the case of ^{13}C in which natural-abundance resonances from lipid carbons and, in some cases, polysaccharides dominate the spectra. Subtraction of spectra to eliminate such resonances in vivo is more difficult than in analogous in vitro studies, in part as a consequence of movement or other time-dependent changes. In our experience, analogous positioning of a ^{13}C-tuned surface coil yields spectra which are overwhelmed by lipid resonances, and most reported in vivo studies of hepatic metabolism have utilized the exposed liver (Stevens et al., 1982; Reo et al., 1984; Siegfried et al., 1985; Stromski et al., 1986). (2) Deuterium relaxation behavior of small metabolites, and particularly methyl groups, is ideal for such studies. Spin–lattice relaxation times are sufficiently short to minimize problems arising from overpulsing, but transverse relaxation times are sufficiently long so that the line width will generally be dominated by field inhomogeneity or sample heterogeneity, as with spin $^{1}/_{2}$ nuclei. (3) As has been noted for ^{13}C studies (Cross et al., 1984), there will typically be a significant degree of metabolite localization, reducing the need for additional

localization strategies. This situation contrasts with that for ^{31}P studies, in which the predominant metabolites are widely distributed and localization strategies become essential. Preliminary studies suggest that ^{2}H NMR spectra similar to those reported here can be obtained in the absence of any rat surgery, with only slightly reduced resolution. As a consequence of these factors, in vivo ^{2}H NMR studies are simpler to carry out than analogous ^{1}H, ^{13}C, or ^{31}P studies.

Alternatively, the most significant limitations of in vivo ^{2}H NMR are (1) the metabolic perturbations resulting from ^{2}H isotope effects, (2) the limited resolution which can be obtained in such spectra due to the small chemical shift range, and (3) the possibility of proton–deuteron exchange in some labeled metabolites. Deuterium isotope effects will only be significant if the metabolic transformations involve direct oxidation of the C–D bond (Kresge, 1977). In the present study, such effects would be expected to perturb minimally the rate of formation of sarcosine from methionine but could significantly reduce the rate of clearance of sarcosine if direct oxidation is involved. The limited spectral resolution obtainable is a more general problem, but, as determined here, resolution of several tenths of a ppm can be obtained in favorable cases, making the selection of metabolic processes critical to the success of the approach. Not surprisingly, considerably better resolution can be obtained in ^{2}H NMR studies of microorganisms carried out in conventional NMR probes (Hunter et al., 1984), although it is often unclear whether the observed resonances correspond to intra- or extracellular metabolites.

Methionine Metabolism. Questions related to the metabolism of methionine have been of broad interest in connection with various studies as a consequence of the central role of transmethylation processes. When supplied in excess over other amino acids, L-methionine is the most toxic amino acid (Benevenga, 1974). The toxic effects of methionine can be alleviated by simultaneously feeding glycine or serine, with glycine being the more effective (Benevenga & Harper, 1967). Benevenga and co-workers (Case & Benevenga, 1976; Mitchell & Benevenga, 1978; Steele & Benevenga, 1978) and Livesey (1984) have questioned the importance of the transmethylation–transsulfuration pathway in the degradation of excess methionine. Instead, it is suggested that direct transamination of methionine eliminates the need for methyl group acceptors which are required for the transmethylation pathway, as well as the excessive drop in cellular ATP levels due to sequestering of the adenine base as *S*-adenosylmethionine. Clearly, the relative importance of such degradation pathways is a function of whether the excessive methionine load is chronic or acute, and of other nutritional parameters. The present results demonstrate that under an acute load of approximately 300 mg/kg body weight of methionine, there is sufficient glycine to allow the bulk of labeled methyl groups to be transferred via glycine *N*-methyltransferase in vivo. As discussed by Finkelstein and Martin (1986), the predominance of the transmethylation pathway for the degradation of excess methionine requires a readily available, nonessential methyl acceptor which is physiologically inert and which can be readily regenerated. The glycine methyltransferase reaction fulfills these criteria. A role for this enzyme in processing excess methionine is also consistent with its induction in the liver of rats chronically overdosed with methionine (Ogawa & Fujioka, 1982b). This effect contrasts with other transmethylation enzymes studied, such as guanidoacetate methyltransferase (Ogawa & Fujioka, 1982b).

Although the presence of the deuterium label is not expected to significantly retard the transmethylation process, a significant isotope effect would be predicted for the subsequent sarcosine dehydrogenase reaction, as well as for the further oxidation of 5,10-CD_2-tetrahydrofolate in the mitochondrion. Consequently, the clearance of sarcosine and the recycling of glycine to accept additional methyl groups would be retarded. Thus, the present study tends to represent the worst case for the transmethylation process, which nevertheless appears to play a dominant role in the metabolism of excess methionine. It is also noted that, in addition to the oxidation of methionine, excretion can play a significant role in the clearance of sarcosine from the body, and significant levels of sarcosine are found in the urine of untreated Sprague-Dawley rats (Tulip et al., 1986).

The reasons for differences in the relative flux through transmethylation and transamination pathways obtained in different studies are not completely clear, but the recent identification of an important allosteric effector of glycine *N*-methyltransferase, 5-methyltetrahydrofolate-Glu_5, indicates the potential importance of regulation at this step (Wagner et al., 1985). Differences in the level of this effector can arise, for example, as a consequence of differences in previous dietary history which could influence the level of *S*-adenosylmethionine, which is in turn an allosteric inhibitor of the methylenetetrahydrofolate reductase involved in the formation of 5-methyltetrahydrofolate. Additionally, glycine availability could be a significant regulatory factor in determining the transmethylation flux in situations involving an acute dose of methionine.

The inhibition of the transmethylation flux which is observed in rats pretreated with L-ethionine may reflect a combination of several factors. These include the following: (1) The available glycine pool may be reduced as a consequence of the formation of *N*-ethylglycine, which is known to occur via the transethylation reaction from *S*-adenosylethionine (Winnick & Winnick, 1959). (2) Transethylation reactions from *S*-adenosylethionine can increase the *S*-adenosylhomocysteine pool, and this intermediate is known to function as a powerful inhibitor of most transmethylation reactions (Cantoni et al., 1978). (3) Excessive *S*-adenosylethionine levels, which remain elevated for long periods of time (Stekol, 1965), may compete with *S*-adenosylmethionine for binding to glycine *N*-methyltransferase.

The gradual appearance of the 3.15 ppm *N*-trimethyl resonance presumably does not arise from further methylation of sarcosine, which is largely excreted or oxidized. Studies utilizing methionine containing radioactive methyl groups have shown that much of the label is found in nucleic acids, proteins, and phospholipid choline (Stekol & Weiss, 1950; Stekol & Szaran, 1962). These transmethylations provide a basis for the production of [*methyl*-$^{2}H_9$]carnitine, which is formed from the trimethyllysine residues of proteins, and [*methyl*-$^{2}H_9$]-phosphorylcholine, which can be formed via the methylation of phosphatidylethanolamine to give phosphatidylcholine, followed by phospholipase C activity to yield phosphorylcholine. The ^{2}H NMR resonances of the labeled lipids and proteins will be relatively broad and are not readily observed in vivo. A small but significant broad background is observed in the ^{2}H NMR spectrum of the rat predosed with the deuteriated methionine (Figure 2).

Registry No. Met, 63-68-3; Sar, 107-97-1; creatine, 57-00-1; phosphorylcholine, 107-73-3; carnitine, 541-15-1; N^1-methylhistidine, 332-80-9; ethionine, 13073-35-3.

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