MAGNETIC RESONANCE IN MEDICINE 13, 90–102 (1990)

In Vivo ^{13}C Nuclear Magnetic Resonance Investigations of Choline Metabolism in Rabbit Brain

BUDI TUNGGAL, KAY HOFMANN, AND WILHELM STOFFEL*

Institut für Biochemie Medizinisohe Fakultät, Universität zu Köln, Joseph-Stelzmann-Strasse 52, 5000 Köln 41, Federal Republic of Germany

Received November 28, 1988; revised February 14, 1989

The metabolism of choline in rabbit brain was studied by the noninvasive approach of *in vivo* ^{13}C NMR spectroscopy. ^{13}C-Enriched precursors were introduced into the brain. Surgery of the head skin was avoided through controlled localization of the surface coil. For long-term accumulation studies in brain, repeated subcutaneous injections proved to be advantageous over other forms of application. The resorption kinetics was calculated to be zero order which suggests slow delivery from the subcutaneous depots. Choline metabolism was studied by two approaches: [*N*-$^{13}CH_3$]choline and S-[$^{13}CH_3$]methionine were administered separately to adult and myelinating rabbits (Days 5 to 32), respectively, over 4 weeks. [*N*-$^{13}CH_3$]Choline and the $^{13}CH_3$ group of methionine were incorporated into lecithin and sphingomyelin of brain myelin. *In vivo* kinetic studies of the turnover of these labeled structures were carried out. Choline and methionine are readily transported through the blood–brain barrier and utilized by the myelinating brain for the biosynthesis of its phospholipids.

INTRODUCTION

The recent developments in magnetic resonance spectroscopy (NMR) *in vivo* of the most common magnetic resonance-sensitive nucleus, proton, phosphorus, and carbon-13 lend increasing insight into metabolic processes and biological structures particularly when spectroscopy is spatially controlled by a joint imaging techniques.

Phosphorus magnetic resonance spectroscopy has been widely used for the detection of metabolic concentrations of phosphorus-containing compounds such as phosphocreatine, ATP, free phosphate, and phosphate esters in different organs and organisms (*1*). Recently Heindel *et al.* (*2*) reported that the diester concentrations of normal brain tissues differ from those of tumor tissues. However, the small differences in chemical shifts of broad phosphorus signals of the inorganic phosphate and phosphate monoester complicate the interpretation of the spectral data. Another nucleus might therefore be more suitable for this purpose.

A rather limited number of studies have been carried out in the field of carbon-13 spectroscopy. The detection sensitivity of the carbon nucleus is only 1% of the proton sensitivity. This and the low natural abundance of the isotopes result in reduction of sensitivity by a factor of 10.000 which must be overcome to perform metabolic studies by *in vivo* carbon spectroscopy. We used ^{13}C-enriched synthetic simple and com-

* To whom correspondence should be addressed.

0740-3194/90 $3.00

plex lipids to investigate model and biological membranes (*3–6*). In continuation, we applied ^{13}C-enriched intermediates for the study of metabolic pathways in animals or humans by *in vivo* spectroscopy.

So far most studies have been concerned with the short, transient incorporation of high concentrations of labeled compounds and with the monitoring of their metabolic fate immediately after application of the compounds to the organisms (*7*) or the measurement of compounds in high natural abundance (*8*). Accumulation of metabolic precursors or intermediates and their decay in long-term experiments have not been reported.

A precursor molecule very suitable for incorporation into essential structures of cell and myelin membranes of the CNS is choline. It is the precursor of phosphatidylcholine and sphingomyelin, two abundant common phospholipids in neuronal and glial cell membranes, and of the neurotransmitter acetylcholine. The metabolism of choline is well understood. The *N*-methyl groups of choline are metabolically stable. The contribution resulting from *de novo* synthesis of choline by methyl transfer from *S*-adenosyl methionine to ethanolamine, on one hand, and by uptake from the blood circulation into the brain, on the other hand, is not known (*9, 10*). Choline is readily transported through the blood–brain barrier as reviewed by Massarelli *et al.* (*11*). However, the results of studies *in vitro* using radioactive precursors in experiments may differ considerably from the physiologically intact *in vivo* situation.

We report on the accumulation of ^{13}C-labeled [*N*-$^{13}CH_3$]choline into phospholipids in rabbit brain. Furthermore, the *de novo* synthesis of choline by methylation of ethanolamine with *S*-[$^{13}CH_3$]methionine as methyl donor in the brain was investigated. These studies contribute to our understanding of the uptake of choline into the brain via the blood–brain barrier and of the catabolism of choline-containing phospholipids in the brain.

METHODS

Animals

Wistar rats were used for the resorption kinetic studies. The compounds were administered intravenously, intraperitoneally, and subcutaneously (abdominal), respectively. Litters of Chinchilla and New Zealand White rabbits were used for the experiments on accumulation of ^{13}C-enriched precursors in the brain. In a typical experiment, 50 to 70 μmol of labeled compound in 0.2 to 0.4 ml phosphate-buffered saline (PBS) was administered by subcutaneous injections daily for 3 weeks to two 15-day-old animals.

The accumulation experiment started at Day 5 after birth. During their myelogenesis from the 11th to the 20th day (*12*), twice the dose of choline was administered daily. Two rabbits from the same litter were used as controls. NMR spectroscopy was performed on 32- to 56-day-old animals, anesthesized by 0.5 ml/kg body wt Nembutal. The superficial anesthesia lasts about 45 to 60 min. The animals were placed on the surface coil of the spectrometer by means of a homemade adjustable table which allowed correct positioning of the liver and the brain. For spectroscopy of the brain, the fixed double-tuned coil was turned by 180° for the safe and agreeable position of the rabbit during spectroscopy.

Chemicals

^{13}C-labeled methyl iodide and methionine were purchased from Aldrich and used without further purification. Mono- and tri-^{13}C-methyl-labeled choline was synthesized by quarternization of *N,N*-dimethylamino ethanolamine and ethanolamine with [^{13}C]methyl iodide in ethanol solution. Hydrogen iodide released during the reaction was buffered with NaOH. The yellow precipitation of labeled choline iodide was filtered and dried *in vacuo*. [*N*-$^{13}CH_3$]choline iodide was turned into the chloride form and purified by cation-exchange chromatography (Dowex 50 × 2).

Spectrometer and rf Coils

All measurements were performed in a Bruker Biospec 47/40, a superconducting magnet with a field strength of 4.7 Tesla and a horizontal bore of 40-cm diameter, and imaging was achieved with an imaging coil of 34-cm inner bore or with a double-tuned phosphorus-proton or carbon-proton surface coil with a diameter of 5 cm for the X nucleus. In the latter case the resonator coil was used as transmitter and the surface coil as receiver. Spectroscopy was also carried out in this combination, thus utilizing the superior energy of the resonator for decoupling purposes. In most cases, imaging or decoupling was performed with the double-tuned carbon-proton surface coil.

Imaging and in Vivo Spectroscopy

Imaging was supported by the routine package software delivered by Bruker. For spectroscopy purposes we have developed our own measuring package utilizing the normal functions of the spectrometer controlling computer systems. Our spectroscopy package allows simple menu-driven measurements of proton, carbon, and phosphorus nuclei using different methods. Predetermined 90° flip angles localized 1.5 cm above the plane of the surface coil were used for the measurements: repetition time 1 s, total observation time 30 min (1000 accumulations) for the spectra of brain and 6 min (200 accumulations) for each spectrum following the kinetics of the subcutaneous resorption, respectively. For improved spatial localization nonstandard imaging experiments were performed using the surface coil with reduced rf energy. Chemical shifts are reported as ppm relative to the terminal methyl group of fatty acids at 14 ppm. The resonance peak of the labeled choline methyl group and the other carbons have already been identified (*13*). The positioning of the surface coil over the head of the rabbit turned out to be critical for the quantitative aspects of the spectroscopy. The standard Bruker surface coil was supplemented by a homemade device which allows correct positioning of the area of choice outside the magnetic bore before the measurement. By phantom measurements the spatial area was assumed to be 3 cm in diameter with the 90° pulse at a distance of 1.5 cm from the surface of the coil.

Regression Analysis and Stacked Plots

PASCAL programs were developed with the Bruker Aspect 3000 computer. The stack plot program was realized using hidden line algorithm. These programs are available upon request.

TABLE 1

Concentrations of Choline-Containing Compounds in Rat Brain and Turnover of Phosphatidylcholine (*11, 14*)

Compound	Concentration (nmol/g fresh tissue)
Acetylcholine	25
Choline	22
Phosphorylcholine	380
Glycero-3-phosphocholine	400
Choline plasmalogen	620
CDP-choline	50
Phosphatidylcholine	18,000[a]
Sphingomyelin	5,000

[a] 14–27 days.

RESULTS AND DISCUSSION

The overall metabolic turnover particularly of brain and myelin membrane constituents is believed to be low, once oligodendrocytes have developed the myelin sheaths of the axons. The half-life of brain lecithin is summarized in Table 1 together with the lipid composition of rat and human brain (*11, 14–16, 17*). The only two choline-containing species of brain, listed in Table 1, for which the N-CH_3 signal can be detected by present NMR techniques are lecithin and sphingomyelin.

Choline is the precursor of the polar head group of phosphatidylcholine and sphingomyelin, the two most abundant phospholipids, and of the neurotransmitter acetylcholine of the brain. Their biosynthesis is well understood. The contribution by the two sources, transport of choline through the blood–brain barrier and *de novo* synthesis from ethanolamine *in situ*, is however disputed (*11*). Choline is a suitable marker for *in vivo* NMR studies of the biosynthesis and the catabolism of brain phosphatidylcholine and sphingomyelin. In addition the nutritional supply and uptake of choline by the central nervous system can be analyzed. For an effective NMR spectroscopy ^{13}C-enriched precursors were synthesized for the kinetic studies reported below.

Chemical Synthesis of ^{13}C-Labeled Precursors

In previous studies (*3–6*) on biophysical parameters of reconstituted model and biosynthetically labeled biomembranes we applied [N-$^{13}CH_3$]choline and specifically labeled fatty acids as nondisturbing probes in the polar head groups and the hydrophobic region of the membrane bilayer. However, for kinetic studies by *in vivo* NMR spectroscopy the signal intensity of monolabeled choline proved to be unsatisfactory. We therefore synthesized [N,N,N-$^{13}CH_3$]choline to increase the sensitivity.

Application of Labeled Precursor for the Accumulation

Among the modes of application of the compound to be accumulated, direct intracranial or intracerebral injection is and has been the preferred method (*14, 15*). This

procedure bypasses the blood–brain barrier but has several drawbacks: it is invasive, and high concentrations of choline in the brain can cause severe impairment of brain function. Therefore, we compared three modes of administration: intravenous, intraperitoneal, and subcutaneous injections. Initially, accumulation experiments were performed with adult rats. Their smaller weight seemed to make rats preferable to rabbits for accumulation of high concentrations of labeled choline: all three groups received ^{13}C-labeled choline daily$_1$ one by intravenous, the second by intraperitoneal, and the third by subcutaneous injection. Intravenously injected water-soluble compounds such as choline are very rapidly distributed but are secreted mainly by the kidney, whereas intraperitoneally and subcutaneously injected labeled choline is only gradually resorbed, and subcutaneously injected choline is deposited more slowly than intraperitoneally applied choline due to the resorbing surface areas. Therefore, subcutaneous administration became the method of choice and led to the accumulation of reasonably high concentrations of choline in the brain. Intravenously injected choline could not be detected in the whole rat even after 3 weeks of administration, whereas subcutaneously injected choline showed a signal about four times that of the control animal.

Kinetics of Choline Resorption

Figures 1A and B represent the *in vivo* NMR spectra of the area around the injection site immediately after injection and the time-dependent decay of the subcutaneously injected choline (100 μmol/0.5 ml PBS per injection), respectively. The physiological concentration of choline in the blood is about 12 nmol/ml. Figure 2 shows the regression analysis of the integrals of the choline resonance signals. Under the assumption of zero-order kinetics, all choline is cleared from the injection area within 2 hours.

Choline Accumulation in Brain of Myelinating and Adult Rabbits

Figures 3A and B show the standard *in vivo* spectra of rabbit brain and abdomen, respectively, with parts of the adjacent tissues. The spatially localized spectra were obtained using a depth technique, placing a 90° pulse in the center of the brain and a 180° pulse on the surface of the surface coil. Recently a critical evaluation of this technique was published by Pope and Eberl (*18*). Work now in progress is aimed at improving the localization methods by use of the magnetic gradient-based localization technique. Table 2 shows the assignment of NMR signals. Their chemical shifts are given using the terminal methyl group of the fatty acid chain as reference resonance at 14 ppm (*13*). The spectrum of the abdomen clearly shows the dominating signals of fatty acids of triglycerides of the subcutaneous fat tissue. The narrow lineshape indicates the relatively fluid character of the fatty acid chains. The N-CH_3 resonance line of choline is at 54.2 ppm. The spectrum of the brain, on the other hand, with the broadened resonance lines indicates the more rigid character of the hydrocarbon chains of the fatty acyl groups and displays a small signal of choline at the appropriate localization in Fig. 3A. The carbonyl part of the spectrum reflects largely those of peptide bonds of the protein of the myelin membranes.

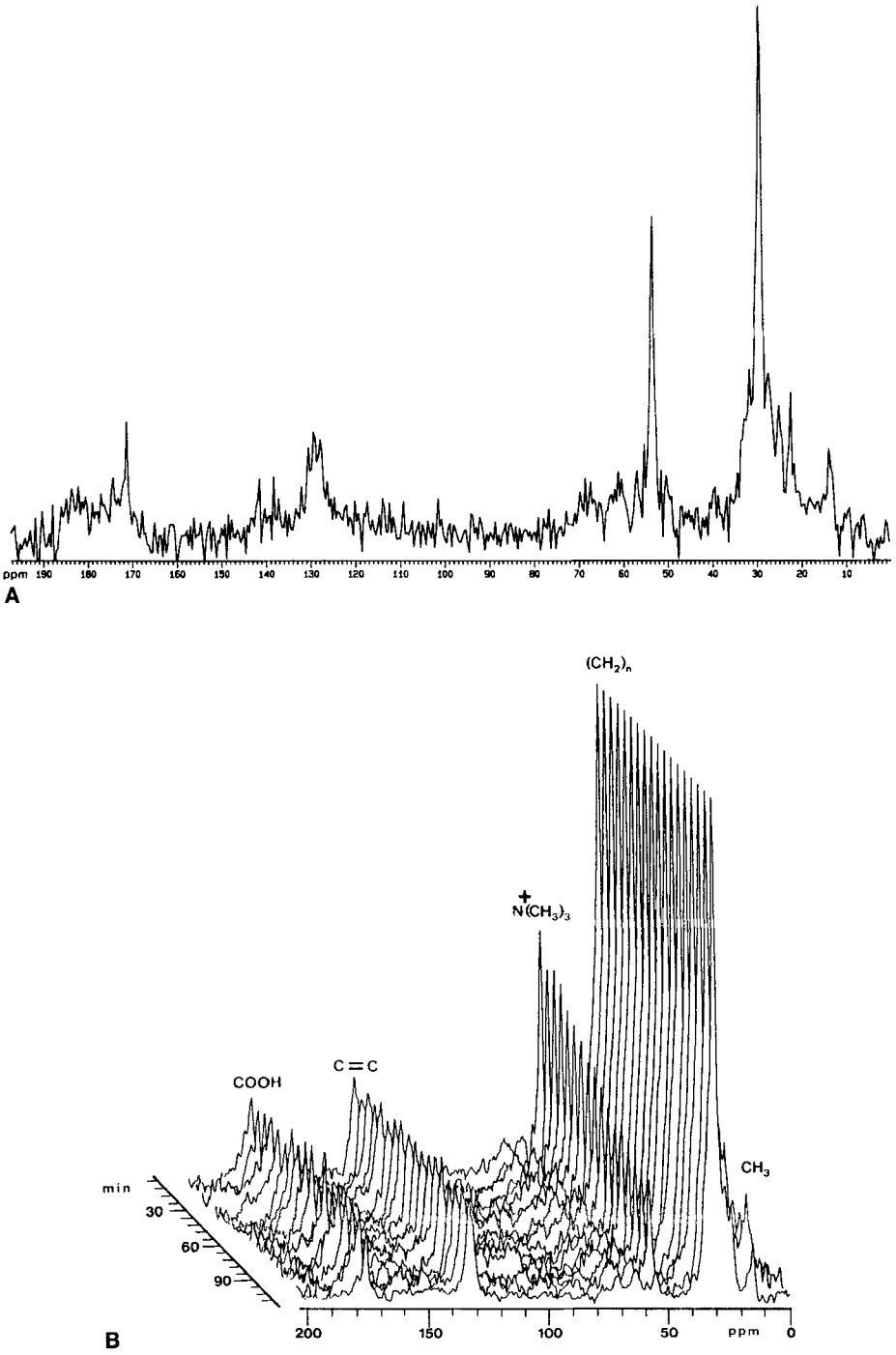

FIG. 1. *In vivo* ^{13}C NMR spectra of rabbit subcutis. Spectra were obtained at 50.3 MHz with a double-tuned surface coil and a ^{13}C-90° pulse adjusted to the localization of rabbit brain and abdomen, respectively. A Waltz-16 decoupling was used during acquisition. Each spectrum represents 1000 acquisitions. The surface coil was adjusted to the localization of the subcutaneous injection site. (A) Spectrum of rabbit subcutis. (B) Kinetics of choline resorption from the injection site. Time scale in minutes after subcutaneous injection.

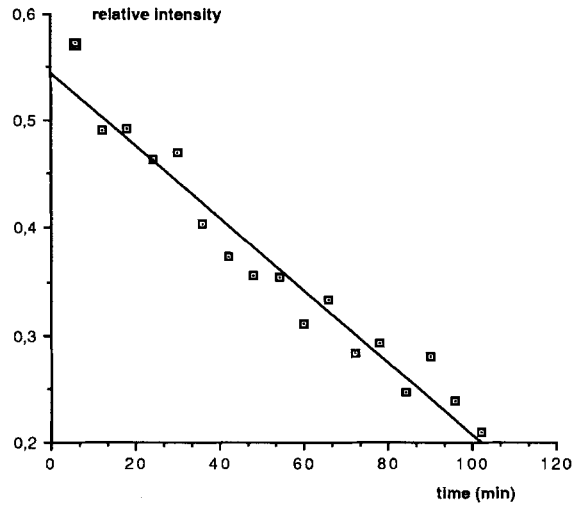

FIG. 2. Regression analysis of the choline signal intensity of the choline resorption from the subcutaneous depot. Relative intensities are given in arbitrary units related to the integrals of the CH_2 groups of Fig. 1B.

The proportion of phospholipids in gray matter cells of the CNS is small compared with that of the lipid-rich myelin of white matter. Subcutaneous fat tissue of the skull contributes negligibly to the [*N*-CH_3]choline resonance line, as demonstrated by control measurements in which a small rf flip angle was adjusted to obtain only spectra of the skin.

Myelogenesis of the CNS is the most intensive period of anabolic metabolism in brain during the life span of the animal. This is made immediately apparent if the accumulation of [*N*-$^{13}CH_3$]choline subcutaneously administered to adult and myelinating rabbits is followed by integration of the 54.2-ppm signal. Only a small signal contributed by the natural abundant choline in phospholipids of the brain is visible in the *in vivo* NMR spectra of control animals: 50 to 75 μmol [*N,N,N*-$^{13}CH_3$]choline or *S*-[$^{13}CH_3$]methionine was subcutaneously administered to newborn rabbits starting at Day 5 after birth for 28 days. This dose was injected twice between Days 10 and 20 of the myelination period (*12*). Control animals received unlabeled choline and methionine, respectively.

NMR spectroscopy requires immobilization of the animal by anesthesia. This bears a high risk for newborn and young animals. We therefore followed the accumulation of our probes without anesthesia in whole-body NMR spectroscopy, whereas spectroscopy of the brain was performed with rabbits anesthesized only for the time intervals indicated in the legend to Fig. 5B during the chase period.

Figure 4 is a magnetic resonance image made of the brain of a 33-day-old rabbit using the surface coil. The spatial localization of the coil for spectroscopy was optimized under the control of magnetic resonance imaging (MRI) as demonstrated in this figure.

Figure 5A is the spectrum of the brain of a 33-day-old animal in the choline accumulation cohort during the myelination period. The accumulation of labeled choline into phospholipids is extremely high, whereas adult animals treated in the same way

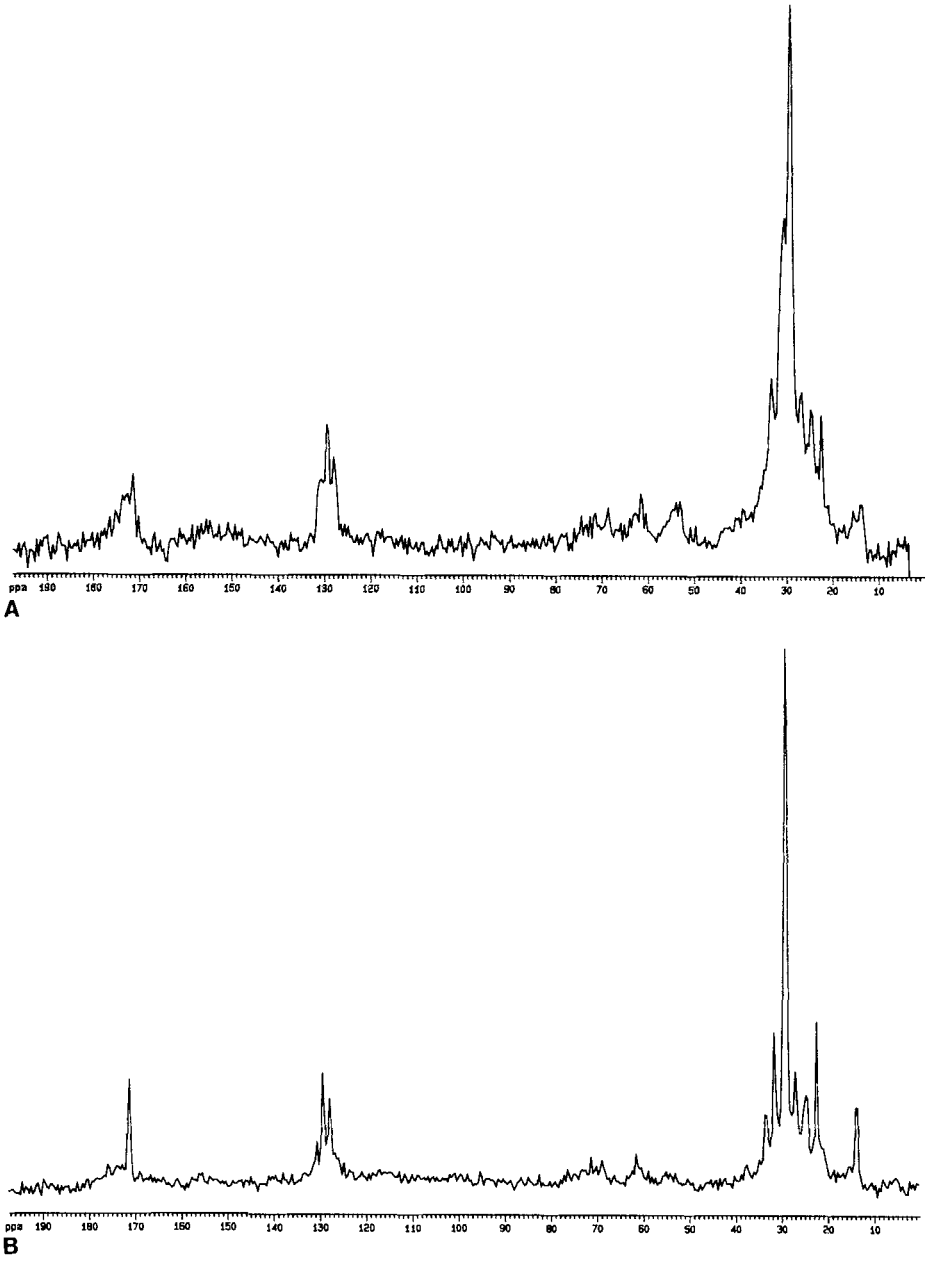

FIG. 3. *In vivo* ^{13}C NMR spectra of rabbit brain and abdomen. Spectra were obtained as described in the legend to Fig. 1. (A) Control spectrum of rabbit brain. (B) Control spectrum of rabbit abdomen.

show only natural-abundance choline in brain phospholipids. Approximately 30 to 40% of the phospholipids in rabbit brain contained labeled choline during this period of [*N,N,N*-$^{13}CH_3$]choline administration.

TABLE 2

Assignments of ^{13}C Resonances Relative to the Terminal Methyl Group of the Fatty Acids (14.0 ppm) (9)

RCOOR	172.0
$-CH=CH-$	128.0–130.0
$-CH_2-CH_2-COOR$	34.0
$-CH_2-CH_2-CH_3$	32.1
$-(CH_2)_n-$	28.0–30.0
$-CH=CH-CH_2-CH_2-$	27.0
$-CH=CH-CH_2-CH=CH-$	25.5
$-CH_2-CH_2-COOR$	24.4
$-CH_2-CH_2-CH_3$	22.4
$-CH_2-CH_3$	14.0

Turnover Studies

The concentration of the incorporated choline allows kinetic studies of its turnover in the main choline-containing constituents of the brain, phosphatidylcholine and sphingomyelin. The interpretation of the signal intensity should take into account a further mass development of the brain. However, control imaging experiments showed that no measurable increase in mass occurred over the period of the experiment. After myelogenesis the increase in brain mass slows down considerably and

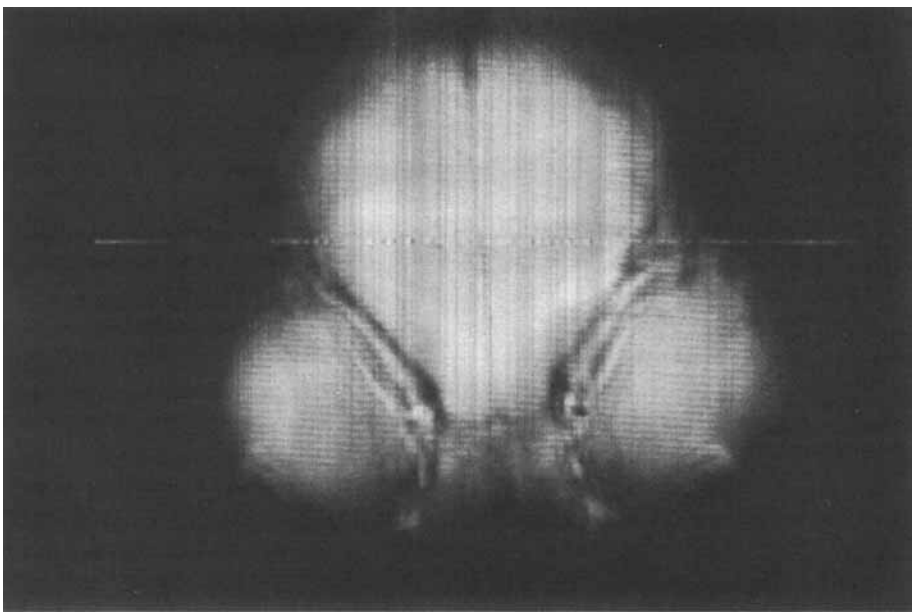

FIG. 4. Magnetic resonance image of rabbit brain, showing the area sensitive to the rf field of the surface coil used for the spectroscopy. The surface coil serves both for spectroscopy and for imaging; therefore, the area apparent in the image is identical to the area probed by spectroscopy.

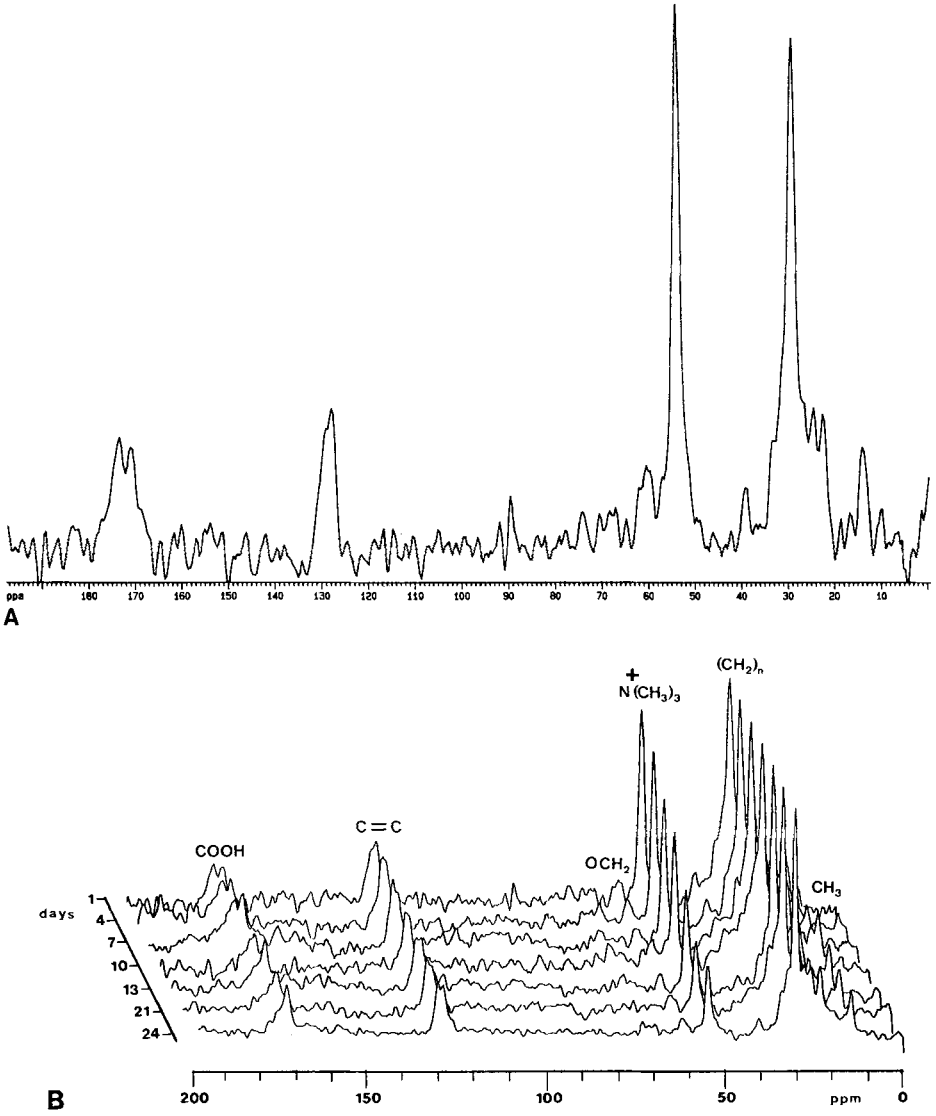

FIG. 5. *In vivo* ^{13}C NMR spectra of rabbit brain following [*N*-$^{13}CH_3$]choline accumulation. Spectra were obtained as described in the legend to Fig. 1. (A) ^{13}C NMR spectrum of the rabbit brain under study, zero-time spectrum of decay period. (B) Kinetics of the choline catabolism in the brain. Time scale is given in days.

therefore is negligible compared to the dramatic decrease in the choline signal which reflects the kinetics of the turnover and not a dilution effect due to mass increase.

Figure 5B shows the spectra obtained from Day 33 to Day 80 after birth. The catabolism is of first order and has a half-life of 20 days as calculated by the regression analysis (Fig. 6). This is in agreement with previous results (*14–16*). From Day 110 after birth, no labeled choline could be detected in the brain of the choline-treated

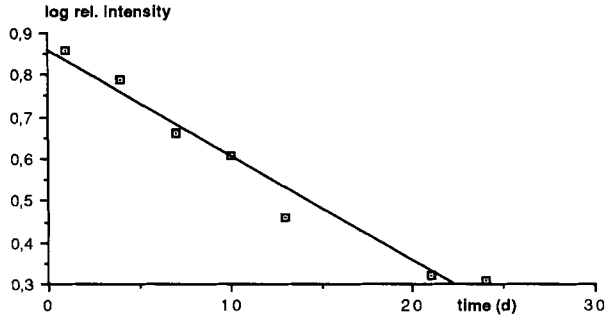

FIG. 6. Regression analysis of the choline signal intensity of choline catabolized in the brain.

rabbits above the normal level. The data suggest that there might be only one pool of choline phospholipids in the myelin of CNS.

Jungalwala and Dawson (*14*) and Miller and Morell (*15*) investigated the turnover of myelin phospholipids in adult and developing rat brain by injection of ^{3}H-labeled compounds intracranially into rats. Concentrations of the labeled phospholipids in the brains of the sacrificed rats were determined. Their results and ours obtained by noninvasive NMR spectroscopy are in good agreement. Noninvasive *in vivo* NMR spectroscopy now provides an alternative method for studying metabolism in a single animal. It avoids the biological heterogeneity that arises from the need for different animals for different points in kinetic isotope experiments from the compound analysis. However, at the moment the NMR technique does not permit the discrimination of the different compounds with the common ^{13}C-enriched precursor incorporated; nor can the regional distribution of the labeled components be resolved. A more advanced magnetic gradient-based targeting method might evaluate the sophistication of the measurement.

These results suggest that choline is intensively transported into and out of the brain by the blood circulation. The fate of the choline from the catabolized choline phospholipid is not known. No accumulation of choline in other parts of the body during the whole experiment could be detected by NMR apparently due to the dilution in the choline pool of the whole-body and urinary secretion.

De Novo Synthesis of Choline in the Brain

Ansell and Spanner (*19*) reported that brain tissue had only 1% methyltransferase activity as compared with liver and that injections of ethanolamine did not produce considerable amounts of choline in experiments *in vivo*. From the specific activities of radioactive choline in arterial and venous blood, Kewitz and Pleul (*20*) concluded that choline must be produced in the brain, but the choline concentrations were not determined. Numerous reports on this subjects with varying conclusions have been reviewed carefully by Masarelli *et al.* (*11*). Recently, London *et al.* (*21*) and Steele and Benevenga (*22*) studied the metabolism of excess methionine in rat liver by deuterium NMR. No evidence was obtained that lecithin is the end product of the transmethylation that occurs in the liver. So far no experiments have targeted the catalytic

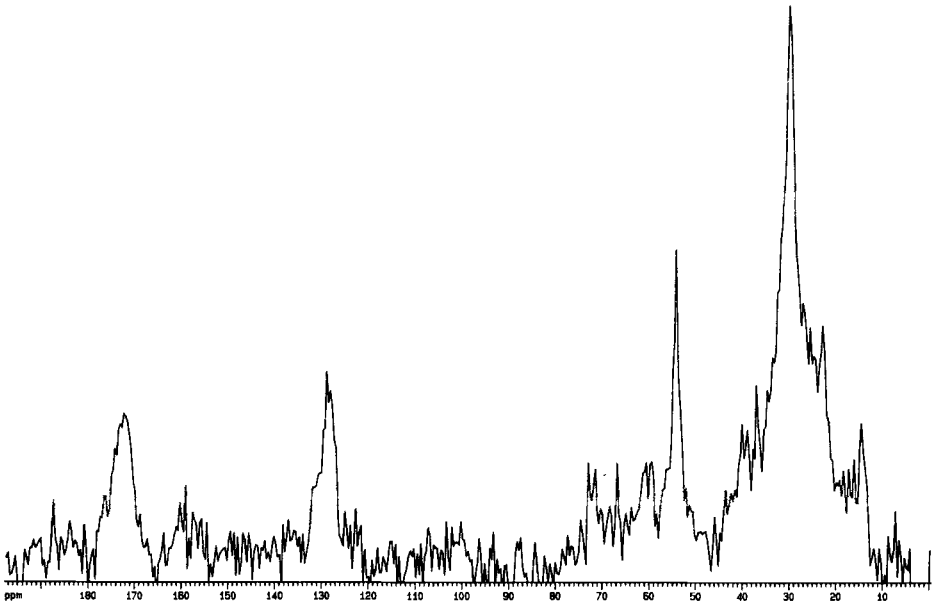

FIG. 7. *In vivo* ^{13}C NMR spectra of rabbit brain after subcutaneous administration of *S*-[^{13}C]methionine. Spectra were obtained as described in the legend to Fig. 1.

pathway of sphingosine leading to ethanolamine, the precursor of choline biosynthesis (*23*).

Our experiment with ^{13}C-labeled methionine suggests that in contradiction to those results of Ansell and Spanner, during myelogenesis neural and glial cells are able to synthesize a vast amount of choline as precursor of phosphatidylcholine and sphingomyelin. Methionine is utilized as the major source of methyl donor for the biosynthesis of choline and subsequently phosphatidylcholine and sphingomyelin, the two main lipid constituents of cell membranes. Despite the high methylase activity in the liver (*19*) the labeled choline formed in the liver is extremely diluted by natural-abundance choline. In addition the normal concentration of choline in the blood is very low (12 nmol/ml). Therefore, labeling of the brain with choline synthesized *de novo* in the liver is highly unlikely. Figure 7 is the spectrum of the brain of a rabbit administered [^{13}C]methionine subcutaneously. The catabolism of choline follows the same kinetics as in ^{13}C-choline-treated rabbits.

Our studies suggest the following conclusions: During maturation of its neuronal and glial tissue the brain utilizes the choline entering the brain via the blood–brain barrier for biosynthesis of the main choline-containing membrane lipids, phosphatidylcholine and sphingomyelin. The brain also utilizes choline synthesized *de novo* by methyl transfer to ethanolamine from methionine which is also delivered via the blood–brain barrier. Choline metabolism remains very active in adult brains; the rapid catabolism of ^{13}C-labeled phospholipids reflects this activity. Choline from the catabolism of membrane phospholipids might considerably contribute to the acetylcholine neurotransmitter pool of the CNS.

REFERENCES

1. P. G. MORRIS, *in* "Nuclear Magnetic Resonance Imaging in Medicine and Biology," pp. 55–67, Oxford Univ. Press (Clarendon), London, 1986.
2. W. HEINDEL, J. BUNKE, S. GLATHE, W. STEINBRICH, AND L. MOLLEVANGER, *J. Comput. Assist. Tomogr.* **12,** 907–916 (1988).
3. W. STOFFEL, B. TUNGGAL, O. ZIERENBERG, E. SCHREIBER, AND E. BINCZEK, *Hoppe Seyler's Z. Physiol. Chem.* **355,** 1367–1380 (1974).
4. W. STOFFEL, O. ZIERENBERG, B. TUNGGAL, AND E. SCHREIBER, *Proc. Natl. Acad. Sci USA* **71,** 3696–3700 (1974).
5. S. FLEISCHER, J. O. MCINTYRE, W STOFFEL, AND B. TUNGGAL, *Biochemistry* **18,** 2420–2429 (1979).
6. H. UTSUMI, B. TUNGGAL, AND W. STOFFEL, *Biochemistry* **19,** 2385–2890 (1980).
7. C. PAHL-WOSTL AND J. SEELIG, *Biol. Chem. Hoppe Seyler's* **368,** 205–214 (1987).
8. C. T. W. MOONEN, R. J. DIMAND, AND K. L. COX, *Magn. Reson. Med.* **6,** 140–157 (1988).
9. I. HANIN, R. MASSARELLI, AND E. COSTA, *in* "Advances in Biochemical Psychopharmacology" (E. Costa and P. Greengard, Eds.), pp. 181–202, Raven Press, New York, 1972.
10. J. J. FREEMAN, R. L. CHOI, AND D. J. JENDEN, *J. Neurochem.* **24,** 729–734 (1975).
11. R. MASSARELLI, S. MYKITA, AND G. SORRENTINO, *in* "Astrocytes" (S. Fedoroff and A. Vernadakis, Eds.), Vol. 2, pp. 155–178, Academic Press, Orlando, FL, 1986.
12. K. B. DALAL AND E. R. EINSTEIN, *Brain Res.* **16,** 441–451 (1969).
13. W. STOFFEL, O. ZIERENBERG, AND B. TUNGGAL, *Hoppe Seyler's Z. Physiol. Chem.* **354,** 1962–1969 (1972).
14. F. B. JUNGALWALA AND R. M. C. DAWSON, *Biochem. J.* **123,** 683–693 (1971).
15. S. L. MILLER AND P. J. MORELL, *J. Neurochem.* **31,** 771–777 (1978).
16. J. M. PASQUINI, R. NAJLE, AND E. R. SOTO, *Brain Res.* **171,** 295–306 (1979).
17. J. S. O'BRIEN AND E. L. SAMPSON, *J. Lipid Res.* **6,** 537–545 (1965).
18. J. M. POPE AND S. EBERL, *Magn. Reson. Imaging* **3,** 389–398 (1985).
19. G. B. ANSELL AND S. SPANNER, *Biochem. J.* **122,** 741–750 (1971).
20. H. KEWITZ AND O. PLEUL, *Proc. Natl. Acad. Sci. USA* **73,** 2181–2185 (1977).
21. R. E. LONDON, S. GABEL, AND A. FUNK, *Biochemistry* **26,** 7166–7172 (1987).
22. R. D. STEELE AND N. J. BENEVENGA, *J. Biol. Chem.* **253,** 7844–7850 (1978).
23. W. STOFFEL, *Chem. Phys. Lipids* **11,** 318–334 (1973).