rived instead from LDL-transported cholesterol (*17*). Dehydroepiandrosterone sulfate is a major secretory product of the adrenal gland and is believed to be desulfated peripherally. Hence it is not clear why plasma and urinary dehydroepiandrosterone levels (*18*) are not elevated consistently in RXLI patients. Sulfated estrogens are believed to be excreted in bile, desulfated by intestinal bacteria, and reabsorbed and conjugated in the intestinal mucosa as glucuronides (*19*). Such desulfation by intestinal bacteria might prevent the accumulation of sulfated steroids in RXLI patients or, alternatively, steroid sulfation might be decreased. In any event, the high cholesterol sulfate concentration indicates that this is a physiologic substrate for steroid sulfatase and that the enzyme deficiency has some direct biochemical consequences in vivo.

Our finding that cholesterol sulfate is increased in the LDL fraction of patients with RXLI suggests a role for LDL in the transport of the sterol in plasma. Further, it is likely that an increased content of cholesterol sulfate is responsible for the increased electrophoretic mobility of LDL in patients with this disease. Whether other properties of LDL or of LDL metabolism are also affected by the increased sulfated sterol content remains to be determined.

The relatively rapid migration of LDL in patients with RXLI should be detectable by routine lipoprotein electrophoresis now performed in many clinical laboratories and hence should provide a diagnostic test that is simpler and more widely available than measurement of steroid sulfatase activity. However, for detection of carriers of the trait, the latter still is necessary (*2*).

Note added in proof: Following submission of this manuscript, Bergner and Shapiro (*20*) increased cholesterol sulfate in plasma and erythrocytes of patients with RXLI.

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6. Dehydroepiandrosterone sulfate and cholesterol sulfate were measured by gas chromatography. The method used for obtaining a sulfate fraction from plasma was essentially that described by O. Jänne, R. Vihko, J. Sjövall, K. Sjövall [*Clin. Chim. Acta* **23**, 405 (1969)]. Briefly, 2 ml of plasma was extracted by sonication with a mixture of 30 ml of acetone and ethanol (1:1). After being dried under reduced pressure, the total lipid extract was fractionated on a 4-g column of Sephadex LH-20 with a solvent system of chloroform and methanol (1:1 by volume, saturated with NaCl). The first 28 ml of eluent contained nonpolar lipid and free steroids, and this fraction was not further studied. The column was then eluted with 50 ml of methanol to recover steroid mono- and disulfates. The methanol eluate was dried, dissolved in 5 ml of water, and desalted (*5*). After addition of internal standards, the steroid sulfate fraction was solvolyzed [S. Burstein and S. Lieberman, *J. Biol. Chem.* **233**, 333 (1958)]. Methyloxime-trimethylsilyl ether derivatives of the liberated steroids were prepared and these were analyzed by capillary gas chromatography (*5*). The quantitative results were corrected for the gas chromatographic response given after solvolysis of known amounts of authentic dehydroepiandrosterone sulfate and cholesterol sulfate. About 95 percent of the [^{3}H]dehydroepiandrosterone sulfate added before extraction was recovered. Gas chromatography–mass spectrometry was carried out to verify identity of the steroid components measured.
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In vivo Carbon-13 Nuclear Magnetic Resonance Studies of Mammals

Abstract. *Natural abundance carbon-13 nuclear magnetic resonances (NMR) from human arm and rat tissues have been observed in vivo. These signals arise primarily from triglycerides in fatty tissue. Carbon-13 NMR was also used to follow, in a living rat, the conversion of C-1–labeled glucose, which was introduced into the stomach, to C-1–labeled liver glycogen. The carbon-13 sensitivity and resolution obtained shows that natural abundance carbon-13 NMR will be valuable in the study of disorders in fat metabolism, and that experiments with substrates labeled with carbon-13 can be used to study carbohydrate metabolism in vivo.*

During the past several years nuclear magnetic resonance (NMR) studies of living cells and perfused organs have shown how metabolic information can be obtained in situ. In ^{13}C NMR studies with labeled substrates metabolic fluxes have been quantitated in a variety of cells and tissues (*1*, *2*). Very recently, high-resolution ^{31}P NMR spectra with good signal-to-noise ratios have been obtained from organs such as the liver and brain in living rats, and also from human arms. These studies have shown that ^{31}P NMR can noninvasively assay the energetic status and the *p*H of the tissue (*3*).

In this report we show how ^{13}C NMR can be used in the study of whole animals. Two different preliminary experiments are presented. In one we show that the natural abundance ^{13}C in fats give well-resolved NMR spectra which contain information about the amount, composition, and structures of the endogenous triglycerides. In the second we show that incorporation of ^{13}C-labeled glucose into liver glycogen can be followed in vivo with ^{13}C NMR.

We acquired the 20.2-MHz ^{13}C NMR spectra by using a surface coil (*4*) with a superconducting magnet having a magnetic field of 1.89 T and a 20-cm bore diameter. With this large diameter it was possible to make measurements on human arms and on intact living animals. High-resolution ^{13}C NMR spectra require decoupling by irradiating the proton resonances. In conducting samples it is necessary to prevent excessive heating

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due to absorption of the 80.3-MHz decoupling radio-frequency field (*5*). With the decoupler gated on only during the free induction decay, no heating was observed in phantom samples (150 m*M* KCl solutions), nor were deleterious effects noticed in rats. Nevertheless, the potential risks to humans presented by ^{1}H decoupling are not completely understood, and so the ^{13}C spectrum of a human arm presented herein was not decoupled.

The natural abundance ^{13}C spectra of rat head, rat abdomen, rat hind leg, and human arm have resonances that can be assigned to triglycerides and lipids (Fig. 1). These lines are generally less than 50 Hz wide but in some instances appear broader because of overlapping resonances. The rather narrow lines are indicative of rapid molecular motion, and the resonances therefore arise from fats in triglyceride droplets and mobile parts of membranes. By measuring the intensities of the glycerol, choline, and fatty acid resonances, the relative contributions of triglycerides and membranes can be assessed (*6*). The composition of the fatty acids can be determined from the intensities of the CH_2 resonances near 30 parts per million (ppm) [which are partially resolved because different CH_2 positions in the acyl chains have different shifts (*7*)], from the methyl groups near 14 ppm, the double-bonded carbons near 130 ppm, and the carboxyl carbons near 170 ppm. In addition, there is a peak at 158 ppm in the rat head spectrum (Fig. 1a) which can be assigned to the arginine ζ carbon, presumably a constituent of the myelin basic protein in the brain (*8*).

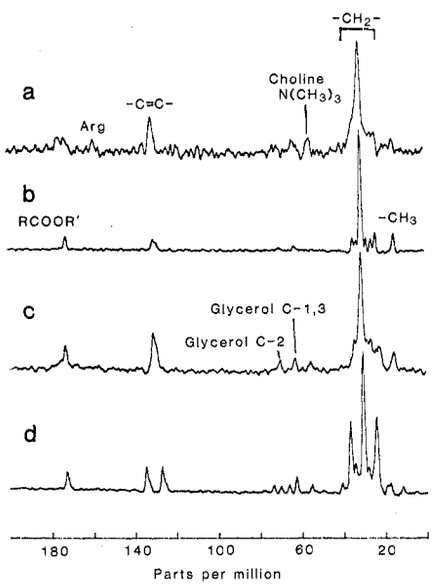

Fig. 1. In vivo 20.2-MHz natural abundance ^{13}C NMR spectra of mammalian tissue. In acquiring the spectra we used an Oxford Research Systems TMR-32 spectrometer with a surface coil (2.5 cm in diameter) placed against (a) rat head, (b) rat abdomen, (c) rat hind leg, and (d) human arm; B_0 field focusing was not used. Acquisition conditions were as follows: (a) 0.20-second acquisition time, 0.5-second delay, 3600 transients; (b) 0.20-second acquisition time, 2.0-second delay, 452 transients; (c) 0.20-second acquisition time, 0.50-second delay, 900 transients; and (d) 0.10-second acquisition time, 0.5-second delay, 600 transients. The pulse widths used gave close to optimum signal-to-noise ratios per unit time. We accomplished ^{1}H decoupling by transmitting, during the free induction decay, a noise-modulated 80.3-MHz radio-frequency field from a 5-cm half saddle coil, which was perpendicular to the surface coil and 4 cm from its center. The spectrum in (d) was not decoupled. The spectra were processed with the use of standard resolution enhancement and digital filtering techniques. During acquisition of the signals from rat tissue, the subject was immobilized in deep surgical anesthesia. The less excitable human subject did not require anesthesia.

For ^{13}C NMR to be useful in studies of other pathways, it is necessary to develop procedures for introducing ^{13}C-labeled components into metabolic pools. In our procedure we used ^{13}C-labeled glucose introduced into the stomach by intubation. To improve the probability that the injected glucose would be stored in the liver as glycogen, we reduced the endogenous unlabeled glycogen by not feeding the rat prior to experimentation. The ^{13}C spectra in Fig. 2 were obtained with the surface coil placed adjacent to the liver after intubation of 100 mg of D-[1-^{13}C]glucose. The signals in Fig. 2a at 96.8 and 92.3 ppm, which are not present in the unlabeled rat (Fig. 1b) and are not present 14 hours after intubation (Fig. 2g), are the C-1 resonances from the β and α anomers of glucose. These glucose signals may arise from the stomach or the intestinal tract as well as from the liver, because of the limited spatial selectivity of the surface coil. The selectivity might be improved if B_0 (external magnetic field) field focusing (*11*) were used in conjunction with the surface coil. Figure 2b clearly includes an additional resonance at 101 ppm, which has been assigned to the C-1 carbons of glycogen (*12*). Glycogen is present at high concentration only in the liver, so that by 75 minutes an appreciable fraction of the added glucose has been transported to the liver and stored as glycogen. In subsequent spectra (Fig. 2, c through f) the glycogen signal grew as the glucose signals decayed. After 14 hours, during which the rat was not fed, the glucose and glycogen signals had disappeared, presumably because the glycogen was mobilized.

An analysis of the sensitivity and resolution available from ^{13}C label experiments is needed to evaluate the potential of this method for metabolic studies. Experiments with ^{13}C NMR are currently being performed on perfused organs at magnetic fields of 8.46 and 4.27 T (*2*); 1.89 T is the largest field currently available for animals larger than rats. The glucose ^{13}C line widths in the 1.89-T rat abdomen spectrum (Fig. 2c) are approximately 20 Hz before the filtering convolution was applied, whereas glucose resonances observed in perfused liver at 8.46 T are approximately 40 Hz wide prior to filtering (*13*). Comparisons of the resolution obtainable with perfused liver at high field and with whole animals at 1.89 T are very indirect but suggest that the resolution obtainable at 1.89 T may not be reduced linearly with the field

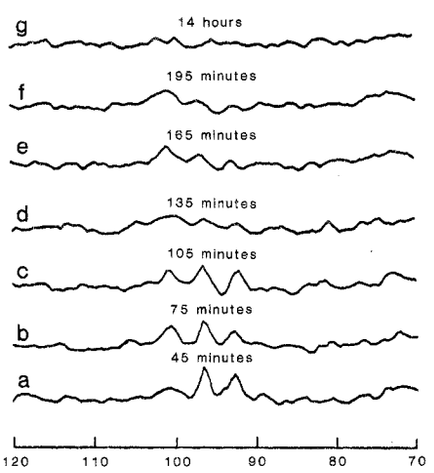

Fig. 2. In vivo rat abdomen ^{13}C spectra after feeding D-[1-^{13}C]glucose. The spectra were collected in 0.5-hour blocks as described in Fig. 1, with a 0.1-second acquisition time and a 0.5-second delay. The time between glucose feeding and the spectrum acquisition is given for each spectrum. Standard resolution enhancement and digital filtering techniques were used in the data processing. The signals at 101, 96.8, and 92.3 ppm arise, respectively, from the C-1 carbons of glycogen and the β and α anomers of D-glucose.

because the lines appear to be narrower at the lower field.

We have determined the absolute sensitivity available from the TMR-32 spectrometer for a phantom sample composed of 4 ml of lean hamburger meat mixed with 150 m*M* KCl to which 12 mg of 90 percent D-[1-^{13}C]glucose had been added so that the labeled glucose concentration in the "tissue" was 15 m*M*. After optimization of pulse width for an interpulse delay of 1.1 seconds and optimal digital filtering, a signal-to-noise ratio of 20:1 was obtained in 12 minutes. Extrapolating from this value, one would expect to be able to identify (with a signal-to-noise ratio of 4) a ^{13}C-labeled metabolite having a concentration of 3 m*M* in 4 ml of tissue in 12 minutes. Furthermore, the 20-cm bore of the 1.89-T magnet makes it possible to perform experiments on the larger organs of larger animals such as cats, and, in general, the sensitivity is expected to increase with the organ volume because the filling factor, which is quite small in the present experiments, can be increased appreciably. The filling factor can also in some cases be improved if minor surgery is performed to place the coil as close as possible to the organ of interest. However, such procedures are often not desirable. Hence, there will be no difficulty in observing in vivo the more abundant metabolites by ^{13}C NMR, provided that the pools can be fully labeled with ^{13}C.

Despite the similarity of cellular suspensions and perfused organs to in vivo conditions, there may be significant differences between the quantitative metabolic fluxes measured by ^{13}C NMR experiments in cells and perfused organs and in vivo conditions, particularly when there are physiological interactions such as those modulated by hormones. We have shown that the major carbohydrate storage pathway of the rat liver is accessible to ^{13}C NMR studies in vivo where previously it could be studied in a perfused organ. Experiments of this type are needed if we are to better understand the physiological control of this pathway in vivo.

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Electroconvulsive Shock Increases Tyrosine Hydroxylase Activity in the Brain and Adrenal Gland of the Rat

Abstract. *A single application of electroconvulsive shock produced a rapid but short-lasting increase in tyrosine hydroxylase activity above control values in the rat adrenal medulla and striatum. After repeated electroconvulsive shock treatment (once per day for 7 days), tyrosine hydroxylase activity increased significantly in the locus ceruleus, nucleus of the tractus solitarius, hippocampus, cerebellum, and frontal cortex and remained elevated for 4 to 8 days. Adrenal tyrosine hydroxylase activity increased 1 day after the termination of repeated electroconvulsive shock treatments and remained elevated for at least 24 days, possibly reflecting the establishment of a new and higher steady-state level of catecholamine biosynthesis in the adrenal. These findings suggest that the persistent changes in tyrosine hydroxylase activity produced by repeated electroconvulsive shock may be a factor contributing to the long-lasting antidepressant effects of this treatment.*

Electroconvulsive shock (ECS) is the most effective therapy available for the treatment of endogenous depression (*1*), although the precise mechanism underlying the antidepressant effects of ECS remains unclarified. Understanding the antidepressant mechanism of ECS would not only aid in the development of more effective treatments for depression but also might provide insight into the etiology of depression. In order to elucidate the biochemical mechanism of the antidepressant effects of ECS, two crucial factors should be taken into account: (i) the antidepressant effects of ECS appear only after repeated treatments, and (ii) the antidepressant effects of ECS persist for an extended period of time after the termination of treatment. It therefore appears likely that any biochemical change that might explain the mechanism underlying the antidepressant effect of ECS should also persist for a period of time after the termination of a series of ECS treatments (*2*).

A variety of biochemical changes occur in the central nervous system (CNS) after repeated ECS treatment that are not apparent after a single ECS treatment, including an increase in norepinephrine turnover (*3*, *4*), an increase in norepinephrine tissue concentrations (*3*), a decreased affinity for norepinephrine of the high-affinity neuronal uptake system (*5*), a decrease in postsynaptic beta receptor density (*6*), and a decrease in the activity of the norepinephrine-stimulated adenosine 3′,5′-monophosphate

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