X-Linked Ichthyosis: Increased Blood Cholesterol Sulfate and Electrophoretic Mobility of Low-Density Lipoprotein

Abstract. *Plasma cholesterol sulfate concentration is increased in patients with recessive X-linked ichthyosis, a disease in which steroid sulfatase activity is absent. In these patients, cholesterol sulfate is found primarily in the low-density lipoprotein fraction of plasma, and the electrophoretic mobility of these lipoproteins is greatly increased.*

Patients with recessive X-linked ichthyosis (RXLI), one of the commoner hereditary forms of scaly skin, lack steroid sulfatase, an enzyme that removes the sulfate group from the 3-position of sterols and steroids (*1*). Absence of activity of this enzyme has been detected in leukocytes, hair bulbs, stratum corneum, and cultured fibroblasts and keratinocytes of these patients (*1–3*). Except for the consistently found cutaneous scaling and the clinically insignificant corneal opacities, no other consequences of the enzyme defect are known.

We observed that low-density lipoproteins (LDL) from patients with RXLI have abnormally rapid anodic electrophoretic mobility, a finding that could be explained by increased electronegativity imparted by binding of a sulfated sterol. We therefore measured sulfated steroids and sterols in whole plasma and lipoprotein fractions of affected persons.

Blood was obtained in the morning after overnight fasting from ten RXLI patients belonging to seven different families, and from three obligate carriers of the trait (mothers or daughters of patients), three patients with ichthyosis vulgaris, one with psoriasis, and 12 normal controls. Clinical categorization was confirmed by measurement of steroid sulfatase activity in peripheral blood leukocytes (*2*). Blood was treated with EDTA, and the plasma was refrigerated for up to 5 days until lipoprotein electrophoresis was performed (*4*) or was stored at −20°C until sterol sulfates were measured. The relative electrophoretic migration (R_F) of LDL was expressed as the distance from the origin to the peak of the β band divided by the distance from the origin to the end of the α (high-density lipoprotein) band. Concentrations of cholesterol sulfate and dehydroepiandrosterone sulfate were determined by capillary gas chromatography (*5, 6*). Plasma LDL was separated from very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL) by ultracentrifugation (*7*).

The LDL of all patients with RXLI migrated faster than that of any other subject studied (Fig. 1). The R_F of the LDL was unchanged after ultracentrifugal isolation.

All patients with RXLI had plasma cholesterol sulfate concentrations that were markedly higher than those of normal persons, female carriers, and patients with several other types of scaly skin, the mean increase being 17-fold. In contrast, there was considerable overlap between the levels of dehydroepiandrosterone sulfate in patients with RXLI and the other subjects (Fig. 2). The mean and range were higher in affected persons, especially when the three patients in their third and fourth decades were compared with four normal men of the same ages; but the differences in the whole group were not statistically significant, a finding similar to that reported for another series of patients (*8*).

No consistently abnormal concentration of plasma total cholesterol, triglycerides, or lipoproteins was found in patients with RXLI. The LDL isolated by ultracentrifugation from three of the patients contained an average of 70 percent of the total cholesterol sulfate present in plasma. No enrichment in cholesterol sulfate was found in VLDL or HDL when compared with levels in a normal subject. Dehydroepiandrosterone sulfate and other steroid sulfates were not detected in the isolated lipoprotein fractions.

Cholesterol sulfate was identified first several decades ago and is present in many tissues (*9*), although its function is unclear. Several investigators have reported normal plasma concentrations similar to those we observed (*10, 11*). It is elevated in the stratum corneum of RXLI patients (*12*). One patient with multiple sulfatase deficiency, in whom the activity of steroid sulfatase and other sulfatases was diminished, had elevated plasma and tissue levels of cholesterol sulfate (*13*). Cholesterol sulfate can be bound tightly to membranes, at least those of erythrocytes (*14*). When infused, most of it appears to be taken up by the liver, spleen, and intestine (*15*), and much of a radioactive tracer dose is converted to free and esterified cholesterol (*10*). Liver, skin, lung, and kidney can add sulfate to the free cholesterol (*15*). Cholesterol sulfate can be transformed to sulfated steroid hormones without desulfation (*16*), but it is uncertain whether plasma cholesterol sulfate normally is a quantitatively significant precursor of steroid hormones (*15*). It has been suggested that, at least in the fetus, sulfated steroid hormones are de-

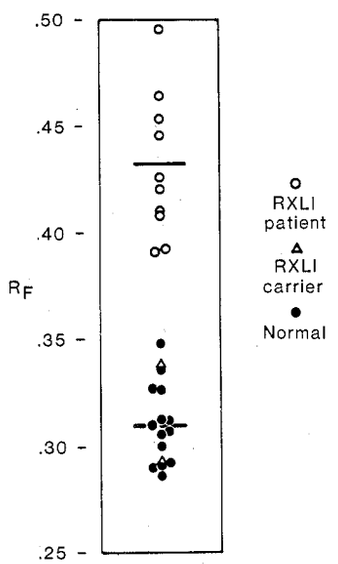

Fig. 1. Agarose gel electrophoretic mobility of plasma low-density lipoproteins. Upper horizontal line denotes the averages for patients with RXLI (mean ± standard deviation = 0.43 ± 0.03) and lower horizontal line, that for all others, including carriers of the trait and "normal" subjects with no skin disease or with ichthyosis vulgaris or psoriasis (0.32 ± 0.03).

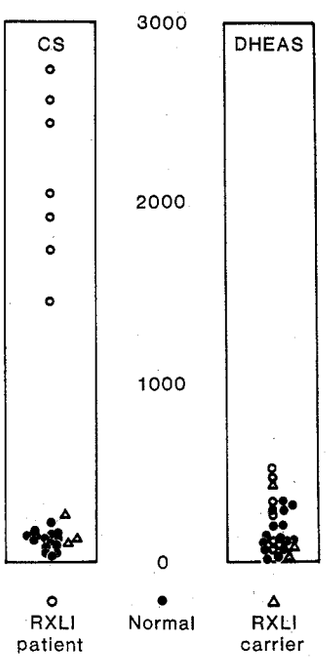

Fig. 2. Plasma concentrations (in micrograms per 100 milliliters) of cholesterol sulfate (*CS*) and dehydroepiandrosterone (*DHEAS*) in patients with RXLI, in carriers of the trait, and in "normal" subjects, including those with no skin disease and those with ichthyosis vulgaris or psoriasis.

0036-8075/81/1106-0659$01.00/0

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.

ERVIN H. EPSTEIN, JR.*
Department of Dermatology, San Francisco General Hospital Medical Center, and University of California, San Francisco 94110
RONALD M. KRAUSS
Donner Laboratory, University of California, Berkeley 94720, and Department of Medicine, University of California, San Francisco
CEDRIC H. L. SHACKLETON
Department of Laboratory Medicine, University of California, San Francisco, and Biomedical Mass Spectrometry Resource, Space Sciences Laboratory, University of California, Berkeley

References and Notes

1. A. C. Jöbsis, C. Y. van Duuren, G. P. de Vries, J. G. Koppe, Y. Rijken, G. M. J. van Kempen, W. P. de Groot, *Ned. Tijdschr. Geneeskd.* **120**, 1980 (1976); L. J. Shapiro, R. Weiss, M. M. Buxman, J. Vidgoff, R. L. Dimond, J. A. Roller, R. S. Wells, *Lancet* **1978-II**, 756 (1978).
2. E. H. Epstein, Jr., and M. E. Leventhal, *J. Clin. Invest.* **67**, 1257 (1981).
3. H. P. Baden, P. A. Hooker, J. Kubilus, A. Tarascio, *Pediatr. Res.* **14**, 1347 (1980); J. Dancis, V. Jansen, J. Hutzler, *ibid.*, p. 521; J. Kubilus, A. J. Tarascio, H. P. Baden, *Am. J. Hum. Genet.* **31**, 50 (1979).
4. F. T. Hatch, F. T. Lindgren, G. L. Adamson, L. C. Jensen, A. W. Wong, R. I. Levy, *J. Lab. Clin. Med.* **81**, 946 (1973).
5. C. H. L. Shackelton and J. O. Whitney, *Clin. Chim. Acta* **107**, 231 (1980).
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.
7. F. T. Lindgren, L. C. Jensen, F. T. Hatch, in *Blood Lipids and Lipoproteins*, G. J. Nelson, Ed. (Wiley-Interscience, New York, 1972), pp. 181–274.
8. A. Ruokonen, A. Oikarinen, R. Palatsi, I. Huhtaniemi, *Br. J. Dermatol.* **103**, 245 (1980).
9. M. Iwamori, H. W. Moser, Y. Kishimoto, *Biochim. Biophys. Acta* **441**, 268 (1976).
10. E. Gurpide, K. D. Roberts, M. T. Welch, L. Bandy, S. Lieberman, *Biochemistry* **5**, 3352 (1966).
11. G. Bleau, A. Chapdelaine, K. D. Roberts, *Can. J. Biochem.* **50**, 277 (1972).
12. M. L. Williams and P. M. Elias, *Clin. Res.* **29**, 26A (1981).
13. J. V. Murphy, H. J. Wolfe, E. A. Balazs, H. W. Moser, in *Lipid Storage Diseases. Enzymatic Defects and Clinical Implications*, J. Bernsohn and H. J. Grossman, Eds. (Academic Press, New York, 1971), pp. 67–110.
14. G. Lalumière *et al.*, *Biochim. Biophys. Acta* **394**, 120 (1975).
15. R. B. Hochberg, S. Ladany, S. Lieberman, *Endocrinology* **94**, 207 (1974).
16. R. B. Hochberg, S. Ladany, M. Welch, S. Leiberman, *Biochemistry* **13**, 1938 (1974).
17. C. R. Parker, Jr., E. R. Simpson, D. W. Bilheimer, K. Leveno, B. R. Carr, P. C. MacDonald, *Science* **208**, 512 (1980).
18. E. H. Epstein, Jr., R. M. Krauss, C. H. L. Shackelton, unpublished data.
19. H.-A. Unnérus, *Acta Endocrinol. (Copenhagen) Suppl.* **226**, 7 (1979).
20. E. A. Bergner and L. J. Shapiro, *J. Clin. Endocrinol. Metab.* **53**, 221 (1981).
21. Supported in part by NIH grants AM 28069, HL 18574, and RR 00719. We thank J. M. Bonifas and D. Duncan for technical assistance.
* Send requests for reprints to Room 269, Building 100, San Francisco General Hospital Medical Center, 1001 Potrero Avenue, San Francisco, Calif. 94110.

6 April 1981; revised 29 June 1981

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

0036-8075/81/1106-0660$01.00/0