cretion of this enzyme for 3 to 4 days, exceeding normal renal lysozyme content.

Endogenous plasma lysozyme levels increased in nephrectomized rats, indicating an extrarenal source and a renal inactivation of this enzyme. Simultaneous measurements of renal clearances of exogenous lysozyme and creatinine demonstrated a decreased reabsorption of lysozyme in Na_2CrO_4, but a normal one in $HgCl_2$ dosed rats. The data imply a decreased tubular reabsorption of lysozyme in the pathogenesis of lysozymuria.

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Cholesterol Esters in Myelin and the Component Fatty Acids.* (31495)

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It is generally held that almost all cholesterol in brain occurs in the free form and that cholesterol esters when present constitute only a small proportion of total cholesterol(1-3). Two to five percent of the total cholesterol was reported to be esterified in human brain in early life(4). No detailed study has been made of the fatty acids of cholesterol esters in brain. O'Brien *et al*(5) have found traces of cholesterol esters in human myelin. Even in the accompanying paper(6) in which the fatty acid and fatty aldehyde composition of various lipid fractions in myelin is described, no such study has been made of cholesterol esters. The present paper reports the isolation and identification of cholesterol esters of bovine myelin and describes the fatty acid composition of these esters.

Materials and methods. Two samples of bovine myelin were prepared by a procedure previously described(7). From each sample, lipids were extracted, washed, dried(8) and fractionated by thin layer chromatography on Silica gel HR.† The 20 × 20 cm plates were covered with a layer of adsorbent 0.5 mm thick. Fifty milligrams of lipid extract were applied as a streak to each plate. The solvent system was hexane‡:diethyl ether:glacial acetic acid, 73:25:2 (v/v/v)(9). All solvents used in this study except diethyl ether were re-distilled. The fraction which traveled at the same rate as the cholesterol stearate standard was eluted with chloroform:petroleum ether (1:9). The methyl esters of the fatty acids in the cholesterol ester fraction were prepared(10) and analyzed in a dual-column gas-liquid chromatograph. The 6-foot, 1/8-inch stainless steel columns were packed with 15% (w/w) diethylene glycol succinate polyester on acid-washed Chromosorb W.§

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† Obtained from Brinkmann Instruments, Inc., Westbury, N. Y.

‡ Hexane is Skellysolve B obtained from Skelly Oil Co., Kansas City, Mo.

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TABLE I. Infrared Spectra of Free Cholesterol and Cholesterol Ester Fractions.

Chemical group	Wave length microns	Cholesterol	
		Free	Ester
-OH stretching vibration	2.95	+	—
C–CH_3 " "	3.45	+	+
C=O " "	5.75	—	+
C=C " "	6.00	+	+
C=C of unsaturated fatty acid	6.25	—	+
CH_2 bending vibration	6.80	+	+
CH_3 " "	7.25	+	+
=C–O– " "	8.00	—	+
C–O–C vibration of esters	8.60	—	+
–C–O– vibration	9.47, 9.67	+	+
C–OH vibration of secondary cyclic alcohol	10.15	+	—
Δ^5 sterol	11.92, 12.52	+	+

The column temperature was programmed from 155°C to 210°C at a rate of 2°/min. Helium, at a flow rate of 40 ml/min was the carrier gas. A disc integrator-equipped recorder was used to quantitate the peak areas of the fatty acids. Duplicate chromatograms were run on each sample. The fatty acids were identified by the relative retention ratio method (methyl palmitate as 1.00) (11), with fatty acid standards.

In another experiment total sterol esters were isolated by silicic acid-celite (1:1, w/w) column chromatography with chloroform: petroleum ether (1:9) as the eluant(8,12). After drying, the weight of the isolated fraction was determined.

Results and discussion. Cholesterol esters separated by thin layer chromatography or column chromatography constituted 4% or 3.6% respectively of the total lipids by weight. The isolated fraction gave a positive Liebermann-Burchard reaction for cholesterol(13). Infrared spectroscopy (Table I) showed that the fraction contained cholesterol and unsaturated fatty acids which were esterified as shown by the adsorption peak at a wave length of 5.75 μ. There was no adsorption band for the hydroxy group (2.95 μ) usually obtained in the infrared analysis of free cholesterol and no band was found for N-CH_3 indicating the absence of choline-containing phospholipids in this fraction.

The gas liquid chromatographic analyses showed the presence of at least 18 fatty acids with a 14-carbon chain or longer. This variety of fatty acids of cholesterol esters in bovine myelin exceeds that of the same lipid fraction in bovine plasma(14). Table II presents the relative percentages of the fatty acids and the order in which they appeared on the chromatogram. The confirmation of the identities of 18:3 (number of carbons:number of double bonds) and 20:4 fatty acids as well as the positive identification of other unknown fatty acids by other methods must await the availability of samples larger than those presently obtainable from the gas chromatographic effluent. Since Johnson *et al*(1) reported that in cat, dog, and beaver cholesterol esters were present only in white matter, but were present in both white and gray matter of one of the two human samples, studies with myelin should be extended to species other than bovine. Alterations in certain lipid fractions of the brain and in their fatty acid composition in various diseases have been reported recently(15-18). It is possible that brain cholesterol esters and their fatty acid composition may be altered also in pathological conditions.

Summary. Cholesterol esters were isolated from purified bovine myelin by thin layer and column chromatography. Liebermann-Burchard reaction and infrared spectroscopy were used to identify the isolated fraction.

TABLE II. Fatty Acid Composition of Cholesterol Esters of Bovine Myelin.

Fatty acid*	Relative retention ratio†	Relative percentage
14:0		.4
15:0		.2
	.87	.4
16:0		7.3
16:1		.9
17:0		.4
	1.47	1.1
18:0		17.3
18:1		48.6
18:2		.9
18:3		5.6
	3.18	1.4
	3.42	.6
	3.58	1.6
20:4		7.0
	4.76	1.0
	5.27	3.6
	6.32	1.7

* No. of carbons:No. of double bonds.
† Relative retention ratio of the unknown fatty acid (methyl palmitate as 1.00).

Component fatty acids of the cholesterol esters were quantitated by gas-liquid chromatography.

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Autointerference of Rabies Virus in Chick Embryo Fibroblasts.* (31496)

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In our previous study(1), HEP Flury strain of rabies virus(2) was found capable of forming plaques in primary chick embryo fibroblasts, and the titration of infectivity by this plaque technique has replaced the more laborious baby mouse(2) and one-day egg LD titrations(3). While applying this plaque assay to materials containing a high concentration of virus, we noticed that monolayers receiving an undiluted seed virus suspension formed no plaques. Our investigation of the nature of this phenomenon led to the finding that interferon production was responsible for it. These results are reported here.

Materials and methods. Diluent. The virus diluent was yolk-saline(1), which was 0.01 M phosphate buffered saline of pH 7.2 containing fresh egg-yolk at 0.1%. Just prior to use, penicillin and streptomycin were added at 500 μ/ml and 100 γ/ml, respectively.

Viruses. Standard 7-day egg passage of HEP Flury strain of rabies virus(2) was used at the 259th to 263rd passage levels. Brains of infected embryos were emulsified to make a 20% emulsion in yolk-saline, to serve as seed virus. In one case, the same strain serially passaged in chick embryo fibroblasts(4) was tested; a pool of culture fluids constituted a seed. Small plaque mutant of Western equine encephalitis (WEE) virus(5) and Sindbis virus were at 15th and 51st chick embryo cell passages, respectively, when used. Dairen I strain of vaccinia virus was received from the National Institute of Health of Japan as

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