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Lipid Class and Fatty Acid Composition of Intact Peripheral Nerve and During Wallerian Degeneration

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Abstract

Lipid extracts from normal cat, chicken, and beef sciatic nerve were fractionated into their components by combinations of silicic acid, Florisil, DEAE-cellulose, or silicic acid-silicate column chromatography.

The constituent fatty acids of total lipid extracts and of individual lipid classes were qualitatively and quantitatively determined as their methyl esters by gas chromatography.

These methods were also applied to lipid extracts from cat sciatic nerve undergoing Wallerian degeneration at 8, 16, 32, and 96 days after section and to chicken sciatic nerve undergoing demyelination due to organophosphate poisoning.

All fatty acids were markedly decreased in the total lipids of cat sciatic nerve at 96 days after section and most of these were decreased at 32 days. As early as 8 days after section 16:0, 16:1, 18:2, 20:0, and 20:4 showed decreases, while 18:0, 18:1, 22:1, 22:5, 22:6, and 24:1 did not begin to show decreases until 16 days after section. The decreases in fatty acids were considered to be due to increased catabolism, decreased synthesis, or increased removal of fatty acids from nervous tissue. The fatty acid content of the total lipids of chicken nerve undergoing demyelination resembled that of cat sciatic nerve between 16 and 32 days after section.

Myelin lipids, sphingomyelin, cerebrosides, and phosphatidyl ethanolamine (PE) began to decrease as early as 8 days after section in cat sciatic nerve. Phosphatidyl serine (PS) also decreased at this time. Cholesterol, lecithin, and ethanolamine plasmalogen did not begin to decrease until 16 days after section and phosphatidyl inositol (PI) did not decrease until 32 days after section. Triglycerides decreased markedly at 8 days after section gradually returning to normal by 96 days. This was accompanied by a transient increase in free fatty acids and monoglycerides. Cholesterol esters and lysolecithin increased markedly at 8 days after section and were higher than normal levels even at 96 days after section.

In chicken sciatic nerve undergoing demyelination after organophosphate poisoning, cerebroside was the only myelin lipid which decreased in amt, while cholesterol esters and diglycerides increased.

Sphingomyelin and cerebrosides containing 16:0, 18:0, 18:1, 18:2, 20:0, 22:0, 23:0, 24:0, 24:1 seemed to be most susceptible to degradation or interference in synthesis in degenerating nerve. For the most part, these fatty acids were observed to increase in cholesterol esters, free fatty acids, and, in some instances, triglycerides.

The changes in various lipid classes and their constituent fatty acids are discussed in relation to various cellular changes which accompany degeneration.

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Introduction

THERE ARE DESCRIBED in the literature many detailed studies of the lipid compositions of brain in various animal species and in recent years increasing attention has been focussed on such studies making use of modern methods of lipid chemistry. However, there are relatively few reports on the lipid composition of peripheral nerve. Studies on peripheral nerve are of interest because an experimental peripheral nerve demyelination which follows a reproducible course can be induced in laboratory animals by a relatively simple operative procedure.

Early studies on the lipid composition of peripheral nerve were reported by Falk (1), Randall (2), Schmidt et al. (3), Johnson et al. (4), Brante (5), and a more recent study by McCaman and Robins (6). These investigations all made use of indirect procedures involving hydrolysis of lipids with subsequent colorimetric estimation of water-soluble hydrolysis products. The only reports involving isolation of lipids are those of Webster on the plasmalogen, cephalins, lecithin, sphingomyelin, and lysolecithin content of sciatic nerve (7,8).

Classical studies on the lipid composition of a peripheral nerve undergoing Wallerian degeneration are those of Johnson et al. (9), Burt et al. (10), and of Brante (5). These authors applied the terms "myelin lipids" and "sheath typical lipids," respectively, to describe the group of lipids which decline rapidly in concn in degenerating nerve (cholesterol, cerebroside, sphingomyelin). McCaman and

Robins (6) have more recently confirmed these early findings. Indirect methods were employed in all these investigations.

Attempts to refine the information on the inorganic P^{32} incorporation into the lipids of degenerating peripheral nerve were made by Magee et al. (11) using the early techniques of Dawson (12) and by Miani (13) using the newer method of Dawson (14). These methods involve the hydrolytic deacylation of phospholipids and chromatographic separation and identification of the water-soluble phosphate esters. Rossiter (15) has indicated the need for application of modern direct methods to reinvestigate the lipid composition of peripheral nerve.

Kline et al. (16), Pritchard and Rossiter (17), and Majno and Karnovsky (18) have reported on the increased incorporation of C^{14}-acetate into the fatty acids of degenerating nerve, and, in particular, the phospholipid fatty acids. No differentiation of lipid type or specific fatty acid was made. The fatty acid composition of sciatic nerve lipids has been reported by Baker (19) and by Bartley et al. (20). Only the fatty acids from alkali-liabile lipids were reported and the amide-bound fatty acids of the sphingolipids were not included. No reports have appeared on the fatty acid composition of degenerating nerve although there is an isolated report (21) on the fatty acid composition of degenerating white matter in the brain from a multiple sclerosis patient.

The present investigation was designed to isolate and quantitate the lipid classes of normal peripheral nerve and that undergoing Wallerian degeneration after section and to determine the fatty acid composition of the isolated lipid classes. A preliminary report of this work has been presented (22).

Experimental Procedure

Surgical Technique and Extraction of Tissue

The right sciatic nerve was cut at the level of the greater trochanter of the femur. The proximal stump was retracted and sutured to the overlying muscle to minimize the possibility of regeneration. After varying periods of degeneration, animals were sacrificed by decapitation and the segment of nerve distal to the site of operation was removed. At the same time, a similar length of the left sciatic nerve was removed to serve as a control. Each nerve was stripped of fatty and connective tissue and the nerve was rapidly weighed. Prior to extraction, the nerve was cut into small pieces. In the case of beef nerve, freezing in liquid nitrogen followed by pulverization was helpful to subdivide the tissue.

Small nerve sample (rat, cat, chicken) were extracted with 20 volumes of chloroform-methanol 2/1 in a conical homogenizer (Kontes Glass Co.). Large samples (beef nerve) were more conveniently extracted in a blendor. Subsequent extraction of the residue with chloroform-methanol-HCl (200/100/1) or chloroform-methanol 2/1 saturated with aqueous ammonia failed to extract any additional lipid. In the case of beef nerve lipids prepared as described by Rouser et al. (23), only 0.3% of total lipid was extracted by $CHCl_3$-MeOH saturated with ammonia.

In earlier studies with cat and chicken nerve, the lipid extracts were made to 0.0015 N with respect to $HgCl_2$ and acidified to 0.5 N with HCl in order to convert plasmalogens to free aldehydes and the corresponding lysophosphatide. It is now preferred to carry out such procedures on individual pure lipid fractions.

With small nerve samples lipid extracts were partitioned against 0.2 times their volume of 0.9% NaCl (24). No loss of lipid could be detected after this extraction. Losses in gangliosides might be expected (25). However, some investigators (26) have failed to find gangliosides in peripheral nerve. Under acidic conditions, no other lipids passed into the saline-methanol phase. Under neutral conditions and after incubation of nerve homogenates in an aqueous system, 0.2% of the free fatty acids may be carried into this phase bound to protein. With large volumes of extracts such as are obtained from beef nerve, it is preferable to remove nonlipid material on a cellulose column (27). The chloroform phase was filtered and dried over solid anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator under vacuum. Beef nerve lipid extracts were processed as described by Rouser et al. (23).

All solvents used were redistilled and deoxygenated before use by gassing with prepurified nitrogen having no more than 6 ppm of oxygen. A few seconds of cavitation with a sonicator is useful to degas solvents prior to nitrogenation.

Fractionation of Lipids

Silicic acid prepared by the method of Horning et al. (28) was used for the initial fractionations of cat and chicken nerve lipids. Since the neutral lipids compose half to three fourths of the lipids of these tissues, respectively, this was a fortunate choice. Even with beef nerve lipid which contains only a third of its total lipid as neutral lipid, enormous volumes of solvent were necessary to remove neutral lipid from DEAE-cellulose columns prior to fractionation of the phospholipids and glycolipids. For the complete elution of neutral lipids of beef nerve from silicic acid only half the volume of solvent was required as was necessary with DEAE-cellulose containing the same lipid load.

Column chromatography was carried out in 1.8 × 20 cm columns of the type described by Hirsch and Ahrens (29). The following fractionation scheme was used for cat and chicken nerve. Lipids were applied to the column in hexane. Free aldehyde was eluted with 150 ml hexane, cholesterol ester with 150 ml 1% ether in hexane, triglycerides with 500 ml 4% ether in hexane, free fatty acids with 200 ml 8% ether in hexane, cholesterol (which may contain some overlap of free fatty acids) with 600 ml 15% ether in hexane, diglyceride with 200 ml 30% ether in hexane, monoglyceride with 200 ml 80% ether in hexane. The column was then washed with 150 ml $CHCl_3$. A mixture of cerebrosides, sulfatides, phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), and phosphatidyl inositol (PI) was eluted with 600 ml 20% MeOH in $CHCl_3$. In earlier studies, a portion of this eluate was chromatographed on a Florisil column as described by Kishimoto and Radin (30) for the separation of cerebrosides. The remaining components of this fraction were then separated on a silicic acid-silicate column (27). This mixture is now more conveniently separated on a DEAE-cellulose column in which cerebrosides are eluted with 7 column volumes of $CHCl_3$/MeOH 9:1, PE with 8 column volumes of $CHCl_3$/MeOH 7:3, phosphatidyl inositol with $CHCl_3$/HAc 3/1 + 0.05M ammonium acetate, phosphatidyl serine with the same solvent + 0.1M ammonium acetate, and sulfatide with the same solvent + 0.2M ammonium acetate (23).

The silicic acid column was then treated with 600 ml 25% MeOH in $CHCl_3$ which elutes lysophospha-

TABLE I
Relative Fatty Acid Composition of Total Lipids from Normal Sciatic Nerve
Total lipid extract methylated and fatty acid methyl esters separated by GLC (details in text)

Fatty acid	Normal nerve (relative %)[a] Rat	Cat	Chicken
12:0	....	0.6	0.03
14:0	1.0	3.5	1.8
14:1	....	0.7	0.4
15:0	....	1.2	0.3
16:0	23.9	14.9	29.8
16:1	3.2	6.6	6.4
17:0	1.1	0.9	0.5
17:1	1.3	2.3	0.1
18:0	8.3	9.6	6.3
18:1	45.0	23.0	39.6
18:2	11.4	10.5	6.6
18:3	.	1.4	.
20:0	1.0	0.8	0.5
20:1	....	3.1	0.4
20:4	....	2.8	0.1
22:0	2.7	0.6	2.3
22:1	....	....	0.2
22:5	.	1.4	0.2
22:6	0.1	4.0	0.1
23:0			0.5
24:0	1.3	2.3	2.8
24:1	0.8	2.8	2.1

[a] Values are given for trace components which are less than 1% of the total only when these were noted to undergo changes in degenerating nerve.

tidyl ethanolamine. The column was stripped with MeOH and the eluate applied to a silicic acid-silicate column (27) for the separation of lecithin and sphingomyelin.

Column eluates were collected as 10-ml fractions and analyzed for cholesterol by the method of Hanel and Dam (31), glycerol by the method of Van Handel and Zilversmit (32), ester bonds by the method of Snyder and Stevens (33), phosphorus by the method of Bartlett (34), and relative amts of ninhydrin positive substances visualized by the method of Rouser (35). Aldehyde was estimated by the method of Gray and McFarlane (36) and carbohydrate by an α-naphthol procedure (37). Recoveries of lipid from columns averaged 99%.

Aliquots of fractions were monitored by paper chromatography using silicic acid-impregnated paper and solvent systems and conditions described by Rouser (38). This monitoring procedure is now usually carried out by thin-layer chromatography (TLC) using chloroform/methanol/water 65/25/4 for phospholipids and glycolipids and hexane/ether/acetic acid 80/20/1 for neutral lipids. This procedure offers the additional convenience of quantitation if the sulfuric acid/dichromate charring reaction is used in combination with a densitometer (39).

TABLE II
Fatty Acid Content of Total Lipids from Normal and Degenerating Sciatic Nerve
Total lipid extract methylated and fatty acid methyl esters separated by GLC (details in text). Underlined figures are significantly different from values expressed for normal nerve.

Fatty acid	Mg of fatty acid/nerve[a] Cat (days after section) 0	8	16	32	96	Chicken Normal	Degenerating[b]
12:0	0.8	1.5	9.7	0.6	0.1	0.04	0.08
14:0	5.0	4.2	9.0	2.5	2.6	2.1	0.5
14:1	1.0	2.6	2.3	0.3	0.2	0.5	0.07
15:0	1.7	3.2	2.8	0.3	0.2	0.4	0.5
16:0	21.5	13.2	10.9	13.1	8.6	34.9	18.9
16:1	9.5	4.3	6.5	3.0	5.6	7.5	1.6
17:0	1.3	1.6	1.2	0.2	0.1	0.6	0.2
17:1	3.3	2.2	2.2	0.4	0.3	0.1	0.1
18:0	13.8	12.7	6.4	5.2	3.0	7.4	4.5
18:1	33.1	28.2	21.2	19.1	12.6	46.3	24.5
18:2	15.1	6.6	5.5	3.2	6.0	7.7	4.2
18:3	2.0	1.5	0.6	2.9	3.8	....	....
20:0	1.2	0.3	0.7	2.9	0.1	0.6	0.2
20:1	4.5	5.0	3.1	0	0.2	0.5	0.3
20:4	4.0	2.6	2.6	2.4	1.9	0.1	0.2
22:0	0.8	1.8	0.9	0.5	0.2	2.7	1.4
22:1	2.3	2.2	1.2	0	0.5	0.2	0.01
22:5	2.0	3.1	1.0	0.5	0.9	0.2	0.01
22:6	5.8	4.9	2.7	1.5	2.7	0.1	0.2
24:0	3.3	3.9	2.2	0.2	0.2	3.3	1.6
24:1	4.0	4.2	1.8	0.2	0.2	2.5	0.8

[a] Values not given for trace components where fatty acid is less than 1% of total and did not undergo significant change.
[b] Organo-phohphate poisoned.

Methylation and Gas Chromatography

Total lipid extracts of pooled lipid fractions from column eluates were transmethylated under prepurified nitrogen in culture tubes with screw caps and Teflon liners at 65C in 5 ml methanol with 0.5 ml conc HCl and 0.2 ml of 2,2-dimethoxypropane. Methylations were carried out for 2 hr in the case of neutral lipids and 4 hr in the case of phospholipids. The methyl esters of fatty acids were extracted with n-hexane, washed with water, dried and stored under nitrogen. It is useful to check the methylation procedure with authentic quantitatively prepared standards and to check the completeness of methylation by TLC. The amt of methyl esters may be determined gravimetrically or by the hydroxamate procedure (33).

The methyl esters were chromatographed on the Barber-Colman instrument with an argon ionization detector using two types of column. Identities of methyl esters were established by comparing the retention times with those of authentic standards at different temps and with those of the identified methyl esters from menhaden oil (40). Representative columns used were 6 ft 9% Apiezon L on 60–80 mesh siliconized Chromosorb W at 215C and 36 lb/sq in. or 15% diethylene glycol succinate polyester on 80–100 mesh siliconized Chromosorb W at 175C and 25 lb/sq in. Detector linearity and relative detector response for different mol wts were checked on prepared quantitative mixtures from Applied Science Laboratories or on standards supplied by the National Heart Institute.

Methyl esters were finally saponified in 1 ml 1N KOH in methanol, reextracted and rechromatographed in order to correct for nonmethyl ester peaks such as the dimethyl acetals of aldehydes (41).

Peak areas were estimated by triangulation. Samples of all solvents before and after passing through silicic acid and in corresponding volumes to those actually used for extraction and elution were carried through the entire procedure to minimize artifacts appearing on gas chromatographs from minor impurities.

Results and Discussion

Total Fatty Acids

Table I shows the relative fatty acid composition of total lipids from normal sciatic nerve for rat, cat, and chicken nerve. Rat nerve contains relatively more 16:0 (palmitate) and 18:1 (oleate) than cat nerve, while cat nerve contains relatively more 14:0 (myristate), 16:1 (palmitoleate), 24:0 (lignocerate), and 24:1 (nervonate). The species difference in the latter two acids may be related to the lower content of sphingomyelin in rat nerve (3). Baker (19) reported that sciatic nerve lipids contained little or no fatty acids of chain length greater than 20 carbons. In the studies reported by Baker, the mildly alkaline conditions used would reveal only ester-linked fatty acids but not the longer chain amide-linked fatty acids characteristic of cerebroside and sphingomyelin.

Chicken nerve appeared to be similiar to cat nerve in its content of palmitate and oleate, but similar to cat nerve in its content of palmitoleate, lignocerate, and nervonate. The species difference in the relative

TABLE III
Lipid Composition of Normal Cat, Chicken, and Beef Sciatic Nerve

Fraction	*Source*	Mg/100 mg total lipid		
		Cat nerve	Chicken nerve	Beef nerve
Neutral lipids	$CHCl_3$ eluate of silicic acid or DEAE cellulose	54.7	78.2	35.2
Lechithin + Sphingomyelin	MeOH eluate of silicic acid	17.8	6.1	32.7
Cerebrosides	$CHCl_3$/MeOH 4/1 eluate from silicic acid followed by $CHCl_3$/MeOH 9/1 eluate from DEAE cellulose or $CHCl_3$/MeOH 4/1 + 1.5% H_2O from silicic acid–silicate	7.2	6.7	5.4
Phosphatidyl ethanolamine	$CHCl_3$/MeOH 4/1 eluate from silicic acid followed by $CHCl_3$/MeOH 9/1 eluate from DEAE cellulose or $CHCl_3$/MeOH 4/1 eluate from silicic acid–silicate	12.7	4.7	7.9
Phosphatidyl serine + phosphatidyl inositol	$CHCl_3$/MeOH 4/1 eluate from silicic acid and $CHCl_3$/MeOH 1/4 eluate from silicic acid or $CHCl_3$/MeOH/NH_3/NH_4AC elution from DEAE cellulose	10.1	1.7	7.1
Sulfatide	$CHCl_3$/MeOH 4/1 eluate from silicic acid followed by $CHCl_3$/MeOH/NH_3/NH_4AC eluate from DEAE cellulose	....	0.5	2.4

fatty acid composition of the peripheral nerve lipids may be related to a different natural diet of these species (42–44).

Tables expressing the relative fatty acid composition were useful for comparisons between species. However, misleading results arise when relative fatty acid compositions are compared under conditions when the total amt of fatty acids is changing, e.g., degenerating nerve. The fact that the relative contribution of a fatty acid to the total fatty acids remains unchanged during degeneration may mask the fact that it is decreasing at the same rate as the total fatty acids.

Table II shows the fatty acid content of the total lipid extract from normal and sectioned cat sciatic nerve and normal and organophosphate poisoned chicken sciatic nerve.

In lipid extracts of cat sciatic nerve all the fatty acids were decreased to a greater or lesser extent at 96 days after section. Principal differences between groups of fatty acids occurred in relation to the time when decreases began. As early as 8 days after section, 16:0, 16:1, 18:2, 20:0 and 20:4 show decreases, while 18:0, 18:1, 22:1, 22:5, 22:6, and 24:1 did not begin to show decreases until 16 days after section. By 32 days after section, all fatty acids decreased except 12:0, 18:3, 20:0, and 22:0 and even 12:0 and 22:0 showed trends at this time. These decreases in fatty acids may be due to increased catabolism, decreased synthesis, or increased removal from nervous tissue. There appear to be differences in susceptibility of different fatty acids. The transient increases of 14:1 and 22:0 at 8 days after section and of 12:0, 14:0, 14:1 at 16 days after section may be related to contributions from invading cells or may represent an accumulation of short chain intermediates in a synthetic sequence. This might also account for the restoration of 20:0 to normal at 16 and 32 days. The peculiar decrease of 18:3 at 16 days and restoration to normal levels even at 96 days may have been related to dilution of this fatty acid at 16 days by lipids from invading cells not containing this acid.

The fatty acid content of the total lipid extract from chicken sciatic nerve presented a picture of change which resembled the period intermediate between 16 and 32 days after section in cat sciatic nerve. Interestingly, in chicken sciatic nerve undergoing demyelination, none of the fatty acids were found to increase. However, chicken nerve undergoing demyelination induced by organophosphate poisoning did not show the proliferative cellular changes found in sectioned nerve (45).

Lipid Composition

Table III compares the lipid composition of normal nerve from cat, chicken, and beef sciatic nerve. It is of interest to compare the data for beef nerve with that reported for beef brain (27). Major differences are the relatively larger amt of neutral lipid in beef nerve and the larger amt of lecithin and sphingomyelin. In beef nerve, cerebrosides were about one third and phosphatidyl ethanolamine about one half the level found in beef brain. The distribution of lipid classes in extracts of cat and chicken sciatic nerve differed from that of beef sciatic nerve principally in the relatively larger amt of neutral lipid in cat nerve and even more so in chicken nerve. This is correlated with relatively smaller amts of total phospholipids in cat and chicken nerve than in beef nerve. Interesting variations in the distribution of lipid classes are the relatively higher content of "cephalin" phospholipids (PE, PS, and PI) in cat nerve as compared to the other two species. There is no indication as to whether these species differences in lipid class distribution may be correlated with species differences in myelin composition.

Table IV shows the results of analyses of the lipid class distribution of cat sciatic nerve at various times after section and of chicken nerve undergoing demyelination due to organophosphorus poisoning. In some instances, particularly with 96 days sectioned nerve, it was necessary to pool several nerves to obtain detectable amts of some of the rapidly disappearing lipids.

These results with cat nerve compare favorably with those of Johnson et al. (9) with regard to the increase in cholesterol ester and the decrease in free cholesterol, sphingomyelin, and cerebroside, and the sudden decrease in triglyceride at 8 days. The transient decrease in triglycerides is paralleled by a sudden increase in free fatty acids and in monoglycerides, which suggests a lipase-like action. This phenomenon was also observed in organophosphate poisoned chicken nerve undergoing demyelination. The increase in cholesterol ester was also observed in chicken sciatic nerve where there was no proliferative cell reaction (45) which suggests that invading cells may not in all cases, be responsible for cholesterol esterification.

This remains a topic of controversy since Johnson et al. (9) suggested that fatty acids arising from breakdown of lipids were utilized in the formation of cholesterol esters. Johnson et al. (46) were unable to demonstrate the formation of cholesterol esters when degeneration was allowed to proceed in vitro. This would suggest that the proliferative cell reaction

TABLE IV
Distribution of Lipid Classes in Extracts from Normal and Degenerating Cat and Chicken Sciatic Nerve

	Mg/nerve ± S. E. M.[a]						
	Cat (days after section)[b]					Chicken[c]	
	0	8	16	32	96	Normal	Degenerating[b]
Cholesterol esters	0.03±0.007	2.4±0.5	16.7	11.6±1.6	1.8	1.4	34.8
Triglycerides	50.1±6.2	19.0±4.0	35.7±0.3	26.1±7.1	43.4	149.2	107.5
Free fatty acids	1.2±0.4	2.5±0.3	2.5	0.5±0.1	0.3	14.9	15.1
Cholesterol	23.8±2.3	18.5±0.3	12.2	6.4±1.3	1.5		
Diglycerides	1.6±0.3	2.4±0.3	1.4	1.0±0.1	0.6	7.7	15.0
Monoglycerides	1.0±0.1	2.2±0.5	1.1	0.9±0.1	0.5	9.9	13.2
Ethanolamine plasmalogen[d]	12.2±1.6	10.6±4.7	4.3	2.5±1.0	1.2	9.7	11.2
Phosphatidyl ethanolamine[e]	6.0±0.7	3.5	1.9	0.5	0.4	1.4	3.9
Phosphatidyl inositol	2.2±0.4	2.4±0.5	2.5	0.6±0.1	0.9	....	....
Lecithin	12.8±1.7	17.2±2.5	6.2	5.9±2.7	0.5	6.8	11.6
Sphingomyelin	12.8±0.7	6.4±0.3	5.1	1.7±0.4	0.2	7.4	5.5
Cerebroside	10.4±0.6	6.3	5.1	1.5±0.6	0.2	15.6	4.4
Phosphatidyl serine	12.4±1.3	3.6	1.2	1.3	1.2	4.1	4.4
Lysolecithint	0.03±0.03	1.7±0.5	1.1	0.3±0.1	0.3	4.6	1.4
Total lipid weight	144.0	114.0	101.0	66.0	54.0	234.3	231.2
Fresh nerve weight	730.0	890.0	920.0	1080.0	850.0	....	....
Number of determinations	10	4	2	4	2	2	2

[a] Standard error of the mean. Not given where only two determinations were carried out.
[b] Underlined values are significantly different from normal (p-value less than 0.02).
[c] 2 nerves pooled for each. Results expressed as mg./2 nerve.
[d] Determined as lyso-phophatidyl ethanolamine after mild acid treatment.
[e] Stable to mild acid treatment.
[f] Isolated after treatment with mild acid.

was necessary for cholesterol ester formation. On the other hand, McColl and Weston (47) found that administration of cortisone to prevent the proliferative response had no effect on the lipid changes in degenerating nerve.

In chicken sciatic nerve, the only complex lipid characteristic of myelin which was markedly decreased was cerebroside.

In addition to the myelin lipids described by Johnson et al. (9) which decrease rapidly after sectioning the nerve were ethanolamine plasmalogen, phosphatidyl ethanolamine, and phosphatidyl serine which decrease markedly from 8 to 32 days after section. These lipids were not estimated individually in the earlier studies of Johnson et al. (9) and Brante (5). The rapid decline in ethanolamine plasmalogen and phosphatidyl ethanolamine lend support to the suggestion of Webster (7) and McMurray (48) that these are myelin lipids. This finding had its histochemical precedent in 1932 with Guyon (49) who made use of the Schiff reagent to demonstrate the disappearance of the aldehyde group as myelin was resorbed. Brante (5) described an alkali-stable lipid which was isolated from the "cephalin" fraction and which did not contain choline. He termed this material cephalin B and proposed that it might be a ceramide phosphoric acid. The cephalin B fraction increased markedly during Wallerian degeneration at 8–16 days after section while sphingomyelin was declining. It was suggested that this might be due to a cleavage of choline from sphingomyelin or a failure to incorporate it. Other workers (50–55) have reported on the presence of alkali-stable, mild acid-stable glyceryl ether form of phosphatidylethanolamine which behaved like cephalin B in its properties. In the present study, glyceryl ether forms of phosphatidyl ethanolamine would be reported as phosphatidyl ethanolamine stable to mild acid. There is no indication of the twofold increase observed by Brante (5) although this change could be easily masked by changes in the diacyl form of phosphatidyl ethanolamine. This is supported in studies on the phosphatidyl ethanolamine from beef sciatic nerve (56) which showed that 19.5% was

TABLE V
Fatty Acid Composition of Cholesterol Esters and Free Fatty Acids of Cat Sciatic Nerve[a]

	μg per nerve								
	Cholesterol esters				Free fatty acids				
	Days after section				Days after section				
	8	16	32	96	Normal	8	16	32	96
12:0	26.4	116.9	313.2	....	58.8	115.0	40.0	16.5	13.2
14:0	134.4	2004.0	1148.4	21.6	85.2	245.0	207.5	55.5	37.8
14:1	16.8	467.6	464.0	7.2	38.4	75.0	65.0	14.0	11.7
15:0	480.0	1135.6	649.6	117.0	63.6	255.0	197.5	33.5	18.3
15:1	132.0	1653.3	324.8	111.6	34.8	137.5	195.0	44.0	7.2
16:0	422.4	1419.5	997.6	225.0	177.6	475.0	320.0	46.5	47.7
16:1	110.4	1018.7	336.4	91.8	70.8	150.0	192.5	25.5	21.6
17:0	43.2	2287.9	1183.2	68.4	40.8	122.5	112.5	11.0	10.5
17:1	57.6	434.2	812.0	117.0	28.8	70.0	72.5	27.0	7.8
18:0	326.4	1085.5	498.8	99.0	102.0	177.5	160.0	28.5	12.0
18:1	136.8	1419.5	1612.4	190.8	226.8	500.0	187.5	70.5	28.2
18:2	50.4	283.9	232.0	185.4	79.2	75.0	97.5	22.5	21.9
18:3	7.2	317.3	278.4	37.8	19.2	25.0	45.0	16.0	1.8
20:4	91.2	784.9	324.8	27.0	24.0	90.0	135.0	20.0	7.8
22:0	7.2	150.3	69.6	12.6	54.0	17.5	90.0	6.0	1.5
22:5	16.8	317.3	359.6	45.0	19.2	22.5	32.5	17.0	7.8
22:6	33.6	434.2	777.2	163.8	30.0	12.5	42.5	14.0	22.5
24:0	12.0	150.3	522.0	21.6	12.0	7.5	20.0	19.5	6.6
24:1	9.6	150.3	116.0	14.4	12.0	7.5	32.5	9.5	3.0

[a] In Table V–XI, values represent the product of relative percentage of a fatty acid in a given lipid class and weight of that lipid class per nerve. Values corrected for nonfatty acid portion agree closely with those obtained from injection of a known aliquot of colorimetrically quantitated methyl esters. Each value is the mean of at least four determinations.

TABLE VI
Fatty Acid Composition of Triglycerides and Diglycerides of Cat Sciatic Nerve[a]

	µg/nerve									
	Triglycerides					Diglycerides				
	Days after section					Days after section				
	0	8	16	32	96	0	8	16	32	96
12:0	1603.2	475.0	642.6	704.7	390.6	78.4	36.0	4.2	25.0	19.2
14:0	2655.3	1064.0	3105.9	1409.4	2517.2	113.6	230.4	152.6	75.0	44.4
14:1	601.2	399.0	856.8	391.5	998.2	44.8	33.6	42.0	43.0	6.0
15:0	1252.5	836.0	1749.3	417.6	781.2	134.4	225.6	107.8	71.0	46.2
15:1	901.8	456.0	1892.1	522.0	520.8	62.4	120.0	109.2	52.0	27.0
16:0	9018.0	3344.0	3677.1	3549.6	7464.8	257.6	316.8	179.2	107.0	113.4
16:1	3757.5	836.0	2427.6	1487.7	1605.8	96.0	211.2	106.4	52.0	35.4
17:0	1152.3	475.0	1249.5	417.6	. .	38.4	139.2	57.4	48.0	22.2
18:0	3707.4	1368.0	2427.6	1487.7	2300.2	120.0	194.4	85.4	54.0	37.2
18:1	12024.0	4180.0	5390.7	5976.9	10112.2	206.4	333.6	63.0	130.0	61.2
18:2	5060.0	1159.0	2606.1	1435.5	5598.6	68.8	45.6	35.0	29.0	55.8
18:3	1052.1	190.0	428.4	1853.1	781.2	22.4	50.4	39.2	28.0	9.0
20:0	400.8	133.0	464.1	208.8	1388.8	28.8	4.8	19.6	26.0	7.2
20:1	3406.8	1615.0	2284.8	3915.0		32.0	16.8	23.8	24.0	12.6
20:4	751.5	418.0	963.9	652.5	868.0	51.2	69.6	91.0	22.0	4.2
22:0	951.9	209.0	321.3	678.6	347.2	20.8	2.4	25.2	31.0	10.8
22:1	2104.2	703.0	1071.0	1383.3	390.6	25.6	14.4	29.4	4.0	3.6
22:5	901.8	152.0	499.8	1200.6	434.0	28.8	21.6	32.2	14.0	26.4
22:6	3156.3	437.0	1106.7	1200.6	1779.4	6.4	7.2	12.6	18.0	9.0
24:0	200.4	57.0	357.0	626.4	303.8	28.8	7.2	16.8	36.0	37.2
24:1	250.5	38.0	464.1	391.5	390.6	17.6	40.8	9.8	13.0	9.0

[a] See footnote to Table V.

present as the diacyl form, 77.6% as the plasmalogen form, and 2.8% as the glyceryl ether form.

A curious increase in lysolecithin was noted in cat sciatic nerve after section. The fact that this observation was more prominent at 8 days after section suggests a parallel with monoglycerides rather than with a decrease in lecithin. In fact, a suggestion of an increase in lecithin was noted at 8 days. This may have been related to an increase in available diglyceride precursor. However, it is not clear from this study whether this lysolecithin occurred naturally in degenerating nerve or whether this arose from mild acid treatment of a choline plasmalogen.

Fatty Acids of Individual Lipid Fractions

Tables V through XI show the fatty acid composition of individual lipids from cat sciatic nerve. Principal differences in the composition of any nerve lipid fractions which can be compared to other studies on brain (57) or spinal cord (58) are in the sphingolipids. Cat nerve had a relatively higher content of 20:0, 22:0, 24:1 acids than were reported for brain.

Table V shows the changes in the composition of the cholesterol esters and free fatty acids at various periods after section. The free fatty acids reflected the pattern noted with the total fatty acids at 96 days after section in that the amt of all of the free fatty acids showed a decrease from that found in normal nerve. Many of the free fatty acids were also decreased at 32 days after section. Most of the free fatty acids are increased in amt at 8 days and 16 days after section. Since this was not the case with the total fatty acids (Table II), these fatty acids must be arising either from breakdown of specific lipids or from a lack of utilization of these fatty acids for the synthesis of specific lipids. The increased amt of free fatty acids found at 8 days after section could easily be accounted for by the decreased amt of fatty acids in the triglycerides (Table VI). Free fatty acids which decrease at 8 days after section are 22:0, 22:6, 24:0, and 24:1. The only lipid class in which all of these acids increase is the cholesterol esters. If these acids arise from triglycerides, in part, they must be converted to cholesterol esters directly by a transesterification mechanism which does not involve the intervention of free fatty acids.

If it is assumed that free fatty acids present in normal nerve provide the direct major source of fatty acids in cholesterol esters in 8-day sectioned nerve, none of the free fatty acids are present in sufficient quantity to account for the amt of the

TABLE VII
Fatty Acid Composition of Sphingomyelins and Cerebrosides of Cat Sciatic Nerve[a]

	µg per nerve									
	Sphingomyelins					Cerebrosides				
	Days after section					Days after section				
	0	8	16	32	96	0	8	16	32	96
12:0	76.8	115.2	20.4	32.3	21.4	187.2	63.0	66.3	30.0	3.6
14:0	115.2	294.4	239.7	88.4	13.2	135.2	176.4	280.5	60.0	5.4
14:1	38.4	89.6	66.3	22.1	9.8	52.0	50.4	71.4	15.0	1.8
15:0	25.6	153.6	86.7	35.7	15.4	135.2	144.9	158.1	16.5	3.8
15:1	25.6	217.6	91.8	56.1	8.0	72.8	69.3	147.9	18.0	2.4
16:0	1088.0	768.0	805.8	285.6	39.4	478.4	308.7	326.4	103.5	33.6
16:1	115.2	140.8	260.1	35.7	8.0	187.2	157.5	112.2	25.5	5.6
17:0	38.4	108.8	51.0	34.0	5.2	83.2	69.3	112.2	15.0	1.2
17:1	25.6	76.8	61.2	20.4	3.4	52.0	88.2	147.9	27.0	0.8
18:0	1228.8	704.0	504.9	205.7	18.8	665.6	327.6	224.4	105.0	15.2
18:1	819.2	249.6	326.4	100.3	21.4	572.0	396.9	158.1	118.5	44.4
18:2	128.0	89.6	107.1	73.1	9.2	114.4	63.0	117.3	37.5	8.4
18:3	12.8	44.8	15.3	34.0	1.8	10.4	44.1	25.5	13.5	1.0
20:0	857.6	486.4	239.7	69.7	1.2	395.2	201.6	168.3	45.0	1.0
20:1	38.4	19.2	45.9	6.8	7.0	228.8	69.3	25.5	9.0	0.6
20:4	102.4	64.0	51.0	32.3	6.2	145.6	37.8	224.4	12.0	4.0
22:0	1318.4	608.0	382.5	88.4	1.0	946.4	504.0	377.4	112.5	2.2
22:5	. .	19.2	107.1	20.4	4.0	176.8	18.9	35.7	7.5	45.8
22:6	64.0	6.4	51.0	25.5	3.2	20.8	56.7	35.7	34.5	0.2
23:0	179.2		56.1	15.3	....	187.2	44.1	81.6	28.5	0.4
24:0	1817.6	723.2	561.0	90.1	2.4	2631.2	1625.4	1264.8	312.0	14.0
24:1	4390.4	1433.6	958.8	266.9	1.2	2922.4	1474.2	785.4	369.0	8.0

[a] See footnote to Table V.

TABLE VIII
Fatty Acid Composition of Lecithins and Phosphatidyl Inositols of Cat Sciatic Nerve[a]

	μg per nerve									
	Lecithin					Phosphatidyl inositol				
	Days after section					Days after section				
	0	8	16	32	96	0	8	16	32	96
12:0	38.4	395.6	12.4	11.8	20.0	63.8	45.6	82.5	19.8	18.0
14:0	128.0	722.4	179.8	100.3	66.0	112.2	242.4	202.5	36.0	46.8
14:1	25.6	86.0	24.8	17.7	20.0	48.4	64.8	55.0	10.2	9.9
15:0	64.0	275.2	68.2	53.1	29.5	105.6	103.2	115.0	21.0	16.2
15:1	38.4	275.2	37.2	29.5	17.0	96.8	132.0	107.5	21.6	4.5
16:0	4134.4	4730.0	1295.8	1699.2	95.5	426.8	367.2	297.5	97.2	208.8
16:1	358.4	498.8	167.4	194.7	41.5	101.2	108.0	147.5	18.6	37.8
17:0	51.2	292.4	49.6	59.0	8.0	46.2	60.0	27.5	10.8	2.7
17:1	25.6	103.2	49.6	41.3	13.0	19.8	96.0	52.5	16.8	5.4
18:0	1331.2	1943.6	756.4	637.2	32.5	209.0	172.8	320.0	97.2	123.3
18:1	3609.6	2872.4	1233.8	1038.4	47.0	715.0	688.8	607.5	84.0	190.8
18:2	435.2	481.6	328.6	359.9	15.0	85.8	84.0	47.5	40.8	56.7
18:3	9.0	5.2	6.2	123.9	3.5	11.0	7.2	15.0	....	2.7
20:0	89.6	344.0	105.4	76.7	1.5	52.8	14.4	35.0	8.4	2.7
20:1	166.4	258.0	142.6	53.1	13.5	48.4	62.4	62.5	10.2	8.1
20:4	166.4	344.0	272.8	194.7	10.0	37.4	26.4	67.5	24.6	62.1
22:0	115.2	653.6	179.8	129.8	15.5	99.0	21.6	47.5	4.8	3.6
22:1	89.6	103.2	111.6	59.0	....	15.4	12.0	62.5	6.6	9.9
22:5	281.6	68.8	55.8	41.3	21.0	30.8	31.2	22.5	28.8	65.7
22:6	140.8	17.2	117.8	106.2	15.0	26.4	....	40.0	8.4	9.9
23:0	102.4	86.0	55.8	17.7	17.5	15.4	....	7.5	16.8	1.8
24:0	230.4	791.2	341.0	182.9	3.0	28.6	16.8	37.5	6.0	8.1
24:1	998.4	2236.0	644.8	477.9	15.0	6.6	....	72.5	13.2	27.0

[a] See footnote to Table V.

corresponding cholesterol ester formed, with the exception of 12:0, 14:1, 18:1, 18:2, 18:3, 22:0, 22:5 and 24:1. If it is assumed that free fatty acids present in 8-day sectioned nerve are a direct major source of fatty acids in cholesterol esters in 16-day sectioned nerve, even these exceptions are ruled out because of insufficient quantities.

Oleic acid appears to be the only free fatty acid which decreases at 16 days after section and this corresponds to a decrease in the amt of this fatty acid in the total lipid (Table II).

Two patterns apepared in the fatty acids of the cholesterol esters. Fatty acids which decreased in amt from 16 to 32 days after section appeared to be more rapidly mobilized. Fatty acids which continued to increase at 32 days were 12:0, 17:1, 18:1, 22:5, 22:6 and 24:0 and these appeared to be more slowly mobilized. All of these acids also remained above the levels found at 8 days after section even at 96 days.

The cholesterol esters may not be formed in situ but may be carried in by invading or proliferating cells and the differences in fatty acid composition may be related, in part, to the differential cell population at a given period of degeneration.

Table VI shows the changes in the fatty acid composition of the tri- and diglycerides at various periods after section. All triglyceride fatty acids decreased at 8 days after section. If this is a lipolytic reaction, triglycerides containing 12:0, 14:1, 18:0, 20:0, 20:1, 22:0, 22:1, and 24:0 must be degraded completely to monoglycerides since these diglyceride fatty acids also decreased at 8 days, but increased in the monoglyceride (Table X). However, these fatty acids also increased in lecithin (Table VIII) at 8 days which might be explained if some of these diglycerides were diverted into the synthesis of lecithin according to the pathways elaborated by Kennedy (59). Interestingly enough, of the other glycerophosphatides which might arise from diglycerides (PE, PS, or PI), neither PE nor PS showed increases in these fatty acids at 8 days (Table IX). Only PI showed modest increases in 14:1 and 20:1 at 8 days after section. Triglycerides containing 18:3 were degraded only to diglycerides since this acid increased in the diglycerides and decreased in the monoglycerides. These diglycerides containing 18:3 appeared not to be readily converted to lecithin. When triglyceride resynthesis was established at 16 days after section, 14:0, 14:1, 20:4, 24:1 increased

TABLE IX
Fatty Acid Composition of Phosphatidyl Ethanolamine and Phosphatidyl Serine in Cat Sciatic Nerve[a]

	μg per nerve								
	Phosphatidyl ethanolamine					Phosphatidyl serine			
	Days after section					Days after section			
	0	8	16	32	96	0	8	16	32
12:0	54.0	10.5	41.8	14.0	4.0	136.4	57.6	....	42.9
14:0	264.0	154.0	64.6	17.5	14.8	74.4	194.4	26.4	57.2
14:1	42.0	31.5	17.1	5.0	3.6	49.6	32.4	16.8	15.6
15:0	54.0	119.0	77.9	10.0	4.8	49.6	79.2	24.0	32.5
15:1	96.0	112.0	47.5	10.5	5.6	37.2	93.6	15.6	45.5
16:0	756.0	406.0	222.3	33.5	62.8	446.4	190.8	72.0	123.5
16:1	204.0	63.0	76.0	7.5	9.2	111.6	100.8	33.6	35.1
17:0	348.0	38.5	47.5	3.5	2.0	37.2	57.6	8.4	33.8
17:1	90.0	45.5	70.3	9.5	2.0	24.8	61.2	12.0	23.4
18:0	906.0	553.0	286.9	138.5	54.0	3757.2	820.8	433.2	144.3
18:1	1908.0	1116.5	558.6	114.5	122.4	5071.6	1159.2	255.6	211.9
18:2	204.0	98.0	60.8	12.0	32.8	558.0	93.6	18.0	24.7
18:3	30.0	31.5	13.3	4.5	1.6	161.2	21.6	6.0	9.1
20:0	72.0	42.0	62.7	10.0	1.2	198.4	86.4	13.2	44.2
20:1	120.0	70.0	60.8	10.0	2.0	223.2	90.0	44.4	46.8
20:4	168.0	98.0	58.9	19.0	3.2	359.6	68.4	4.8	11.7
22:0	84.0	49.0	51.3	13.0	13.2	272.8	90.0	28.8	32.5
22:1	60.0	35.0	36.1	....	....	74.4	72.0	43.2	....
22:5	246.0	143.5	13.3	11.5	57.6	198.4	28.8	4.8	24.7
22:6	126.0	73.5	51.3	12.5	4.0	210.8	43.2	12.0	9.1
23:0	96.0	7.0	....	1.5	2.8	136.4	10.8	....	5.2
24:0	78.0	28.0	36.1	4.5	2.0	161.2	68.4	18.0	7.8
24:1	72.0	59.5	36.1	7.5	2.8	111.6	36.0	18.0	31.2

[a] See footnote to Table V.

TABLE X
Fatty Acid Composition of Monoglyceride and Lysolecithin in Cat Sciatic Nerve[a]

	μg per nerve								
	Monoglyceride					Lysolecithin			
	Days after section					Days after section			
	0	8	16	32	96	0	8	16	32
12:0	64.0	114.4	4.4	64.8	13.5	1.1	147.9	26.4	12.3
14:0	119.0	286.0	145.2	102.6	64.0	4.6	158.1	112.2	39.3
14:1	62.0	112.2	29.7	34.2	12.0	1.7	8.5	20.9	23.4
15:0	50.0	158.4	83.6	56.7	28.5	1.7	73.1	57.2	20.1
15:1	37.0	94.6	71.5	45.0	15.0	1.3	105.4	37.4	18.0
16:0	170.0	380.6	145.2	127.8	75.0	5.4	316.2	246.4	55.8
16:1	69.0	149.6	73.7	45.9	40.5	0.9	68.0	75.9	8.1
17:0	30.0	132.0	51.7	24.3	8.0	0.9	79.9	18.7	12.0
17:1	27.0	123.2	27.5	37.8	25.5	0.5	52.7	31.9	14.1
18:0	53.0	158.4	49.5	49.5	29.5	4.4	122.4	97.9	18.3
18:1	62.0	121.0	41.8	103.5	58.0	3.7	205.7	132.0	26.4
18:2	45.0	50.6	16.5	38.7	27.0	1.1	25.5	15.4	11.7
18:3	30.0	2.2	26.4	20.7	8.5	0.5	13.6	7.7	6.9
20:0	17.0	92.4	46.2	18.0	9.5	0.2	57.8	44.0	2.1
20:1	19.0	50.6	13.2	18.9	8.0	0.2	25.5	13.2	7.8
20:4	27.0	55.0	80.3	22.5	10.0	0.3	15.3	27.5	9.3
20:5	25.0	41.8	9.9	18.9	52.5	0.5	....	2.2	2.1
22:0	24.0	57.2	26.4	18.0	5.0	0.9	73.1	24.2	3.3
22:1	6.0	39.6	23.1	37.8	3.5	....	62.9	27.5	2.1
22:5	33.0	15.4	40.7	24.3	14.0	0.1	13.6	29.7	4.5
22:6	33.0	15.4	4.4	13.5	1.5	0.4	27.2	36.3	8.1
23:0	28.0	4.4	20.9	16.2	18.0	0.5	49.3	9.9	1.8
24:0	20.0	59.4	16.5	24.3	8.5	0.8	110.5	18.7	3.3
24:1	24.0	48.4	12.1	16.2	22.0	0.5	54.4	53.9	2.7

[a] See footnote to Table V.

in the triglycerides. This is accompanied by corresponding changes in the di- and monoglycerides.

Table VII shows the fatty acid composition of the myelin lipids, sphingomyelin and cerebrosides, of cat nerve during degeneration. Decreases in some fatty acids were apparent as early as 8 days after section. Similarities between the two lipid classes were the decreases at all time periods of sphingomyelins and cerebrosides containing 16:0, 18:0, 18:1, 18:2, 20:0, 22:0, 23:0, 24:0, and 24:1. Cerebrosides containing 16:1, 20:1 and 22:5 apepared to be less stable during degeneration than did the corresponding sphingomyelins; while sphingomyelins containing 20:4 and 22:6 appeared to be less stable than the corresponding cerebrosides. The early decreases in longer chain fatty acids in both lipid classes were balanced by increases in short chain, odd carbon number, or some polyunsaturated fatty acids which might tend to lower the melting point of these sphingolipids.

Tables VIII, IX, X, and XI show the fatty acid compositions of lecithin and PI, PE and PS, monoglycerides, lyso PE, and lysolecithin, respectively, in cat sciatic nerve at varying periods after section.

TABLE XI
Fatty Acid Composition of Lysophosphatidylethanolamine in Cat Sciatic Nerve[a]

	μg per nerve				
	Days after section				
	0	8	16	32	96
12:0	244.0	190.8	81.7	37.5	42.0
14:0	414.8	837.4	309.6	230.0	45.6
14:1	195.2	328.6	60.2	35.0	19.2
15:0	97.6	371.0	137.6	45.0	16.8
15:1	109.8	455.8	103.2	52.5	18.0
16:0	1207.8	1431.0	537.5	330.0	232.8
16:1	256.2	519.4	253.7	52.5	42.0
17:0	109.8	106.0	86.0	42.5	7.2
17:1	73.2	180.2	94.6	47.5	3.6
18:0	683.2	837.0	387.0	277.5	150.0
18:1	5892.6	2671.2	1247.0	540.0	307.2
18:2	378.2	233.2	107.5	127.5	64.8
18:3	61.0	21.2	21.5	27.5	8.4
20:0	109.8	254.4	55.9	62.5	8.4
20:1	292.8	148.4	98.9	70.0	4.8
20:4	353.8	318.0	240.8	305.0	24.0
20:5	48.8	53.0	17.2	55.0	12.0
22:0	183.0	243.8	90.3	37.5	12.0
22:1	61.0	159.0	94.6	17.5	56.4
22:5	244.0	243.8	90.3	87.5	194.4
22:6	719.8	254.4	81.7	47.5	1.2
23:0	170.8	53.0	34.4	....	6.0
24:0	146.4	339.2	107.5	42.5	2.4
24:1	268.4	402.8	55.9	22.5	1.2

[a] See footnote to Table V.

It would appear that lecithin containing 18:1 and 18:3 was not formed from diglycerides containing these acids since these acids increased in the diglycerides (Table VI) at 8 days after section without a corresponding increase in these fatty acids in lecithin.

In general, the fatty acid changes in the other lipids differed chiefly in the time that decreases were observed. These differences might relate to a difference in susceptibility to degradation of lipids with a specific fatty acid composition, a difference in susceptibility to removal of specific lipids from the tissue, a difference in susceptibility of synthesizing systems for specific lipids, or dilution of a specific lipid class by invasion or proliferation of cells rich in the same lipid class with a different fatty acid composition. This latter explanation might be expected if the observation of an increase in a specific fatty acid in a given lipid class were accompanied by an increase in the fatty acid in the total lipids (e.g., 14:1 and 22:0 in lecithin and the total lipids). At 96 days and, in many cases, at 32 days after section these changes could be related to a decrease in the corresponding fatty acid in the total lipids. The decreases observed in fatty acids in the total lipids could be explained by a decrease in fatty acid synthesis, an increase in fatty acid catabolism, or an increased removal of the fatty acids from the tissue.

Although such experiments are necessarily descriptive and do not offer concrete conclusions about the metabolism of individual fatty acids in nerve during degeneration, they offer many useful leads for metabolic experiments involving the use of isotopically labelled precursors and for the selection of substrates for specific enzyme experiments.

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Human Platelet Lipids and Their Relationship to Blood Coagulation

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Abstract

There is now reasonable agreement on the sequence of physiological and biochemical events leading to fibrin formation, and phospholipids are an important part of this process. The phosphatides are ordinarily provided by platelets, and it appears that a lipoprotein complex is responsible for this activity. The anatomic site of this complex is not known, but evidence is presented that it may be a property of the platelet membrane. Methods for the study of platelet lipids including fatty acids and aldehydes are described, and include silicic acid column and paper chromatography, as well as thin-layer and gas-liquid chromatographic procedures. These are also being utilized in studies of subcellular platelet particles, where only limited amts of biological material are available for study. It is stressed that experimental results obtained from studies on isolated lipids should be interpreted with a certain degree of caution. It is unlikely that they are available as such in in vivo coagulation, and the drastic procedures used for their extraction and isolation may alter their basic physiological properties.

IN RECENT YEARS, the role of lipids in blood coagulation has been better understood, and there appear to be two main reasons for this increase in our knowledge. First, the sequence of protein interactions leading to the formation of a fibrin clot has been further elucidated (1). Secondly, advances in research have enabled us to characterize the specific lipids involved in the coagulation mechanism. Our thinking has probably been further clarified by the realization that there are three physiological events that should be dealt with separately—at least for the time being. They are: a) blood coagulation; b) hemostasis; c) thrombosis. The tendency in the past to think of these as a single entity has led to confusion. For example, an increase in plasma lipids or postprandial lipemia was equated with a "hypercoagulable" state. This was presumed to be directly related to thrombosis. Arterial thrombi histologically resemble hemostatic platelet plugs in that there is a white "head" consisting of relatively intact platelets. Venous thrombi, on the other hand, more closely resemble clots as formed in the test tube. That is, they consist of a mixture of fibrin, entrapped red cells and leukocytes, as well as platelets. These are the so-called "red" thrombi. Thus, it is important to realize that lipids may not play a role in thrombosis and hemostasis, but may only be important for coagulation. These concepts have been discussed in detail in recent reviews of the subject (2–4).

Current Theory of Coagulation

Figure 1 shows the sequence of events leading to the formation of fibrin. Contact with a foreign surface, such as damaged endothelium or the cut edge of a blood vessel appears to activate Hageman factor. This in turn activates PTA and a pattern of biochemical transformations appears to ensue, which involves the activation of a previously inactive coagulation