Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7071–7075, October 1985
Medical Sciences

Membrane lipid changes in laminectomized and traumatized cat spinal cord

(icosanoids/laminectomy)

PAUL DEMEDIUK*, ROYAL D. SAUNDERS*, DOUGLAS K. ANDERSON†, EUGENE D. MEANS†, AND LLOYD A. HORROCKS*‡

*Department of Physiological Chemistry, The Ohio State University, 1645 Neil Avenue, 214C Hamilton Hall, Columbus, OH 43210; and †V. A. Medical Center, Neurology Service, 3200 Vine Street, Cincinnati, OH 45220

Communicated by Ralph T. Holman, June 17, 1985

ABSTRACT **Free fatty acid (FFA), diacylglycerol ($acyl_2$-Gro), icosanoid, phospholipid, and cholesterol levels were measured in samples of cat spinal cord (L2) that were frozen *in situ* (*i*) with vertebrae intact, (*ii*) at various times after laminectomy, and (*iii*) at various times after laminectomy with compression trauma to the spinal cord. Tissue samples either were grossly dissected into gray and white portions prior to FFA and $acyl_2$Gro analysis or were used whole for the other lipid types. Gray matter total FFA and $acyl_2$Gro values were abnormally high in samples frozen with vertebrae intact and in those frozen 10 min after laminectomy. This indicates that the surgical procedures resulted in some perturbation of spinal cord lipid metabolism. If the experimental animals were allowed to recover for 90 min after laminectomy, the gray matter FFA and $acyl_2$Gro levels were greatly reduced. Compression of the spinal cord with a 170-g weight for 1, 3, or 5 min (following 90 min of recovery after laminectomy) caused significant elevations of total FFA, $acyl_2$Gro, icosanoids, and phosphatidic acid and significant decreases in ethanolamine plasmalogens and cholesterol. Among the total FFA, arachidonic acid was found to have the largest relative increase. Comparisons of gray and white matter demonstrate that, in general, changes in white matter FFA and $acyl_2$Gro were similar to those seen in gray matter. However, the increases in white matter levels of FFA and $acyl_2$Gro were delayed, occurring after the elevations in gray matter. For some FFA (e.g., arachidonate), the rise in white matter occurred as gray matter levels were decreasing. This suggests that the initial alteration in spinal cord lipid metabolism after trauma was in gray matter but, with time, spread radially into white matter.**

Following blunt traumatic injury, the mammalian spinal cord undergoes a progressive series of autodestructive pathological changes, the severity of which depends upon the magnitude of the trauma delivered (1, 2). A significant loss of motor function occurs in severe cases as a result of hemorrhagic necrosis of central gray matter that begins within minutes of the injury and the degeneration of long tracts of the white matter that is evident within 24–36 hr after injury. At present, little can be done clinically to alleviate the tissue destruction and restore the losses in function that occur after spinal cord trauma. Although the histopathology of spinal cord trauma has been extensively documented (1, 2), development of a more effective clinical treatment has been handicapped by our lack of understanding of the biochemical events that initiate this irreversible autodestructive process. Recently, attention has been focused on the cell membrane as a possible site for early molecular damage following trauma. Perhaps the earliest posttraumatic cellular events involve activation of membrane hydrolytic lipases. Some of the products of lipid hydrolysis, such as free fatty acids (FFA), lysophospholipids, and diacylglycerols ($acyl_2$Gro) are known to perturb the integrity of biomembranes (3–6). The action of these compounds may then render the remaining constituent membrane lipids more susceptible to peroxidative attack as well as acting to reduce the activities of critical membrane enzymes such as Na^+,K^+-ATPase (7–9). Within the FFA fraction, liberated arachidonic acid may act as a substrate for metabolism to biologically active prostaglandins, thromboxanes, and leukotrienes (icosanoids) (10). Arachidonic acid has also been implicated in the edema production and swelling seen after brain injury (11, 12).

Under normal conditions, the concentrations of FFA, $acyl_2$Gro, and icosanoids in brain tissue are very low (10, 13). However, certain pathological conditions, such as hypoglycemia, status epilepticus, hypoxia, ischemia, and cold injury, cause the levels of these compounds to rise dramatically (14–21). No comparable values for FFA and $acyl_2$Gro in either normal or traumatized spinal cord are currently available. Only one report of spinal cord prostaglandin levels has been published (22). Therefore, the purpose of the present study was 2-fold: (*i*) to determine the levels of FFA, $acyl_2$Gro, icosanoids, phospholipids, and cholesterol in normal feline spinal cord and (*ii*) to examine the effect of laminectomy and compression trauma on the spinal cord tissue levels of lipolytic products.

MATERIALS AND METHODS

Adult mongrel cats, ranging in weight from 2 to 4 kg, were used throughout this study. All cats were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg). Muscle paralysis was achieved with intravenous succinylcholine chloride (1 mg/kg). In all animals, the vertebral column was exposed in the upper lumbar region and laminectomies were performed as described (23, 24). During timed periods after laminectomy and/or compression injury, the surgery site was covered with 0.9% NaCl warmed to 37°C. Trauma was induced by placing a 170-g weight, extradurally, on the spinal cord for a prescribed length of time (23). The spinal cord in the L1–L3 region was frozen *in situ* with liquid nitrogen for all samples (25). Under continuous liquid nitrogen irrigation, ≈2 cm of spinal cord at the L2 site was maximally exposed and removed intact as described (25).

Animals were placed in one of four different experimental groups. Group 1 consisted of animals in which the vertebral column was exposed and the underlying spinal cord was frozen through the intact bone. In group 2, laminectomies were performed and the cats were allowed to recover for either 10, 60, 90, or 120 min before freezing. Group 3 animals

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FFA, free fatty acid(s); $acyl_2$Gro, diacylglycerol(s).

‡To whom reprint requests should be addressed.

were laminectomized and allowed to recover for 90 min, and then the spinal cord was compressed with a 170-g weight for either 1, 3, or 5 min. In this group, the spinal cords were frozen immediately upon termination of compression. In group 4, after laminectomy with 90 min of recovery, the spinal cords were compressed with 170 g for 5 min, but the spinal cords were not frozen until either 5, 15, or 30 min after compression had been terminated.

Each dura-free frozen sample of spinal cord was prepared for lipid extraction as follows. All work was done in a −20°C stand-up freezer. First, the samples were thoroughly cleaned of meninges and blood by scraping with a scalpel. Samples then were either used as is or were cross-sectioned into three segments, and each segment was cored with a flat-ended 14-gauge needle, producing samples that were either predominantly gray matter (central core) or predominantly white matter (outer area). For FFA and acyl$_2$Gro analysis, total lipids were extracted from the separated gray and white matter with a two-phase chloroform/methanol/water system, 3:6:4 (vol/vol) (14). The final lower phase was removed, evaporated under N_2, and redissolved in 0.5 ml of chloroform/methanol, 1:1 (vol:vol). A small aliquot was taken from each sample and used for the determination of total lipid phosphorus (26). Total FFA and acyl$_2$Gro were separated by TLC with petroleum ether/diethyl ether/acetic acid, 110:90:4 (vol/vol), as the developing solvent. Lipid bands were visualized with 2-*p*-toluidinylnapthalene-6-sulfonate under ultraviolet light (27). The FFA and acyl$_2$Gro bands were scraped into screw-cap test tubes and esterified in 3.0 ml of 0.38 M H_2SO_4 in methanol/toluene, 1:1 (vol:vol), at 60°C for 4 hr. The fatty acid methylesters were separated and quantified by GLC using a Shimadzu GC-8A gas chromatograph. Alltech CS-10 (10% on 100/120 mesh Chrom WAW) was used as the stationary phase with N_2 as the carrier gas at an operating temperature of 190°C. Chromatograms were recorded and peak areas were calculated by using a Nelson Analytical 760 intelligent interface, a Hewlett-Packard 85 desk-top computer, and Nelson Analytical software.

For prostaglandin, phospholipid, and cholesterol analyses, total lipids were extracted with hexane/2-propanol, 3:2 (vol/vol) (28, 29), and 20-mg samples were separated on 1.0-g Unisil silicic acid columns. Lipids were eluted with 100 ml each of the following solvents: chloroform (neutral lipids), methyl formate (prostaglandins and cerebrosides), acetone (remaining glycolipids), and methanol (phospholipids). Cholesterol was quantitated by the method of Bowman and Wolf (30) and prostaglandins were measured by radioimmunoassay (31). Phospholipids were separated by reactional two-dimensional TLC (32), visualized by iodine vapors, the spots were scraped, and phosphorus was quantitated by the method of Rouser *et al.* (26).

In all cases, significance levels of observed differences were determined by analysis of variance. Differences between individual groups were determined by Duncan's multiple-range test. The minimum level of significance was set at 0.05.

RESULTS

Spinal cord was frozen through an intact vertebral column in an attempt to establish "normal" or control values for FFA and acyl$_2$Gro in the feline spinal cord. Levels of these lipolytic products in spinal cord tissue handled in this fashion were substantially higher than those reported for normal brain (13), with laminectomy causing a further elevation in the levels of FFA and acyl$_2$Gro (data not shown). Laminectomy-induced changes also occurred in white matter FFA and acyl$_2$Gro (data not shown). In both gray and white matter, the elevations in FFA and acyl$_2$Gro were transient after laminectomy, falling to levels approximating those seen in brain within 90 min after laminectomy (data not shown). Consequently, we have used the FFA, acyl$_2$Gro, icosanoid, phospholipid, and cholesterol values from this time period (i.e., laminectomy with 90-min recovery) as controls, and all animals subjected to compression trauma underwent a 90-min recovery period after laminectomy before trauma was initiated.

Compression trauma for 1 min caused significant 4.2-fold and 4.6-fold increases in gray matter total FFA and acyl$_2$Gro levels, respectively (Table 1). Increasing the compression time from 3 to 5 min resulted in a further elevation in spinal cord levels of total FFA and acyl$_2$Gro. Individual FFA and acyl$_2$Gro acyl groups were also determined. The only species that were present in significant quantities (≥1%) were the 16:0, 18:0, 18:1, 18:2, 20:4 (arachidonate), 22:5ω3, and 22:6 fatty acids. All of the individual FFA analyzed were significantly elevated by compression trauma (data not shown). However, the largest relative increases occurred in arachidonic acid (Fig. 1). Similarly, all gray matter acyl$_2$Gro acyl groups were elevated following compression trauma (data not shown). Total FFA and acyl$_2$Gro were also significantly

Table 1. Levels of total FFA and acyl$_2$Gro in white and gray matter from traumatized cat spinal cord

Experimental group	Gray matter		White matter	
	Total FFA	Total acyl$_2$Gro fatty acids	Total FFA	Total acyl$_2$Gro fatty acids
Laminectomy + 90 min	4.64 ± 0.81	3.24 ± 0.76	3.15 ± 0.32	1.84 ± 0.13
Compression				
1 min	19.58 ± 1.68*	14.99 ± 1.55*	6.86 ± 0.21*	4.68 ± 1.20*
3 min	39.08 ± 0.23*†	24.04 ± 1.91*†	7.88 ± 0.64*	6.24 ± 1.29*†
5 min	31.18 ± 0.86*†	26.09 ± 1.55*†	8.40 ± 0.60*†	6.94 ± 1.09*†
+ 5-min wait	32.88 ± 1.58*†	24.99 ± 1.49*†	5.59 ± 1.34*‡§	6.06 ± 0.90*
+ 15-min wait	31.42 ± 2.92*†‡§¶	9.32 ± 1.04*†‡§¶	13.54 ± 1.34*†‡§¶	9.03 ± 0.85*†‡§¶
+ 30-min wait	20.55 ± 2.03*†‡§¶‖	9.75 ± 1.26*†‡§¶	13.62 ± 1.46*†‡§¶	9.56 ± 0.64*†‡§¶

Values (mean ± SD) are expressed as nmol/μmol of total lipid phosphorus. $n = 4$ for all groups. Significance level: $P \leq 0.05$.
*Different from laminectomy + 90 min.
†Different from 1-min compression.
‡Different from 3-min compression.
§Different from 5-min compression.
¶Different from 5-min compression + 5-min wait.
‖Different from 5-min compression + 15-min wait.

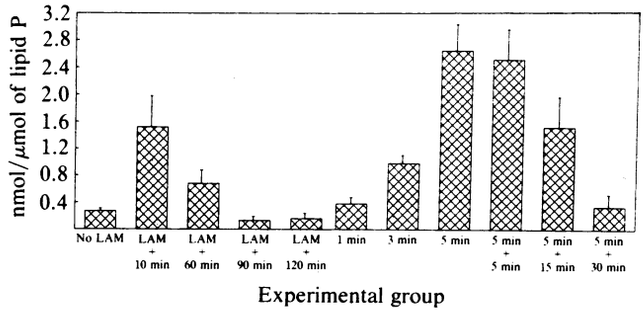

FIG. 1. Free arachidonate from gray matter of cat spinal cord. LAM indicates laminectomy at L2, with the numbers being the time periods in minutes allowed for recovery before freezing. The injury groups include 1 min of 170-g compression trauma, 3 min of compression, 5 min of compression, and 5 min of compression after which the weight was removed and the cord was frozen after an additional 5, 15, and 30 min. Values are means ± SD of four separate determinations (n = 4).

elevated in white matter by either 1, 3, or 5 min of compression trauma, although the magnitude of these increases was less than that seen in gray matter after the same period of compression (Table 1).

Recovery from 5 min of compression trauma resulted in a different pattern of FFA and acyl$_2$Gro in both gray and white matter. Thirty minutes after termination of 5 min of compression, gray matter total FFA had declined ≈35% (Table 1), with the largest decrease occurring in arachidonic acid (Fig. 1). By 15 min after termination of compression, gray matter total acyl$_2$Gro levels had fallen 65%, remaining at this level for at least 30 min after removing the weight (Table 1). In contrast, white matter total FFA and acyl$_2$Gro were increased significantly at 15 and 30 min after termination of 5 min of compression (Table 1), with the largest relative increase occurring in arachidonate levels (Fig. 2).

During 1 and 5 min of compression injury, there were no significant increases in the amounts of icosanoids (Fig. 3), although the FFA levels increased markedly after injury. Data are expressed as pmol of icosanoid per μmol of sphingomyelin. If the wet weight is used as the denominator, wide variations are seen in the amounts of phosphorus and cholesterol (65.5–104.5 and 87.5–141.4 μmol/g of wet weight, respectively), whereas the ratio of cholesterol to phosphorus remains relatively constant (1.33 ± 0.08, n = 10). The fluctuations in the wet weight may be caused by edema occurring in the tissue following injury (33). Therefore, the results are expressed as pmol/μmol of sphingomyelin because sphingomyelin is a membrane component with a slow rate of turnover (34, 35).

Prostaglandins E_2 and $F_{2\alpha}$ were elevated 24- and 10-fold, respectively, at 5 min after 5 min of injury. At 30 min, the

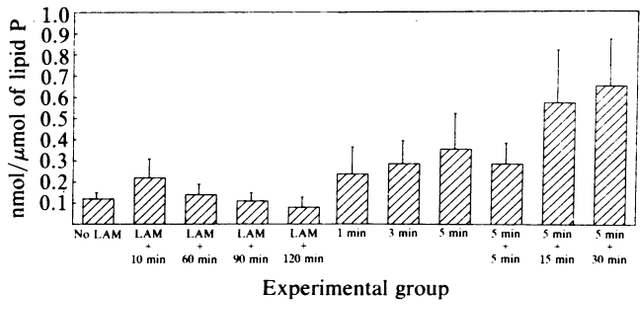

FIG. 2. Free arachidonate from white matter of cat spinal cord. Experiment as described in the legend to Fig. 1. n = 4 for all groups; values are means ± SD.

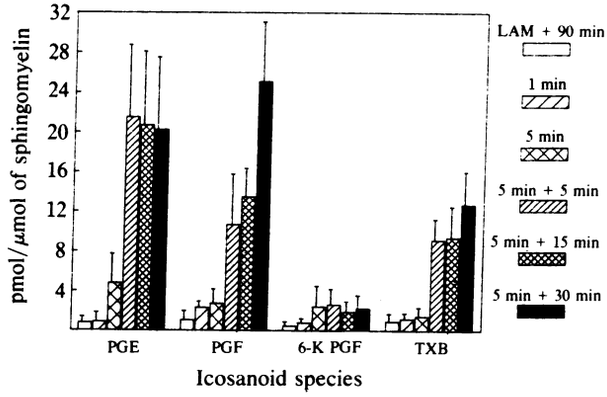

FIG. 3. Protaglandin, prostacyclin, and thromboxane levels of whole cat spinal cord samples. PGE, prostaglandin PGE_2; PGF, prostaglandin $F_{2\alpha}$; 6-K PGF, 6-ketoprostaglandin $F_{2\alpha}$, a stable metabolite of prostacyclin; TXB, thromboxane B_2, a stable metabolite of thromboxane A_2. Injury as described in the legend to Fig. 1. n = 4 for all groups; values are means ± SD.

prostaglandin $F_{2\alpha}$ levels were further increased 24-fold above control levels. Thromboxane levels were increased 10-fold at 5 min after 5 min of compression and 14-fold after 30 min. There was no significant increase in 6-ketoprostaglandin $F_{1\alpha}$ up to 30 min after injury.

The content of ethanolamine plasmalogens was decreased 10% after 1 min of compression and 20% at 30 min after 5 min of compression (Table 2). The levels of phosphatidic acid were increased 50% after 5 min of compression and 150% after 30 min. There were no significant changes in the content of other phospholipids. The cholesterol content decreased 10% after 1 min of compression trauma but did not change any further at later times.

DISCUSSION

Compression of the feline spinal cord with 170 g for 5 min (which results in extensive tissue necrosis and sensorimotor loss) caused a pronounced elevation in gray matter FFA and acyl$_2$Gro levels. These increases are similar to those seen in postmortem rodent brain tissue or during complete cerebral ischemia (36–39). In white matter, FFA and acyl$_2$Gro also increased as a result of compression trauma, but the magnitude of these changes was considerably less than that seen in gray matter. The time course of alterations after compression in white matter FFA and acyl$_2$Gro was out of phase with that in gray matter. At 15 and 30 min after compression was terminated, total FFA and acyl$_2$Gro were significantly elevated in white matter, whereas in gray matter levels of these lipolysis products were decreasing (*vida supra*). These biochemical data are consistent with histological findings demonstrating that damage to the spinal cord after trauma is seen first in gray matter and then spreads radially outward to white matter (1, 2).

A pronounced transient elevation of spinal cord FFA and acyl$_2$Gro is also caused by surgical exposure of the vertebral column and laminectomy. This finding, along with previous studies demonstrating that laminectomy causes a transient decrease in spinal cord blood flow (24) and Na^+,K^+-ATPase activity[§] as well as increases in tissue levels of ADP and AMP (25) and prostaglandins (22), indicates that these surgical procedures perturb biochemical processes in the spinal cord. These are relevant findings in that laminectomy is a common

[§]Anderson, D. K. & Means, E. D., Acta of the International Symposium: New Frontiers of Biochemistry of Spinal Cord Injury and Paraplegia, Nov. 14, 1983, Venice, Italy, pp. 23–31.

Table 2. Phospholipid and cholesterol concentrations in cat spinal cord after compression injury

Compound	LAM + 90 min	Compression		Compression with waiting period		
		1 min	5 min	5 min + 5 min	5 min + 15 min	5 min + 30 min
PtdOH	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01‡	0.09 ± 0.01*	0.10 ± 0.01*	0.10 ± 0.01*
PtdSer	0.66 ± 0.05	0.65 ± 0.09	0.68 ± 0.04	0.70 ± 0.02	0.75 ± 0.08	0.76 ± 0.10
PtdIns	0.15 ± 0.04	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.03	0.15 ± 0.02	0.15 ± 0.05
PtdCho	0.99 ± 0.12	0.90 ± 0.06	0.93 ± 0.10	0.95 ± 0.02	0.99 ± 0.02	0.95 ± 0.08
PlsEtn	1.90 ± 0.07	1.71 ± 0.05	1.60 ± 0.04†	1.56 ± 0.04†	1.53 ± 0.02*	1.52 ± 0.03*
PtdEtn	0.50 ± 0.05	0.53 ± 0.04	0.48 ± 0.06	0.53 ± 0.03	0.55 ± 0.04	0.49 ± 0.07
Chol	6.89 ± 0.23	6.12 ± 0.41‡	5.71 ± 0.08‡	5.89 ± 0.10‡	5.96 ± 0.06‡	5.89 ± 0.36‡

Results are expressed as mol of phospholipid and cholesterol per mol of sphingomyelin. Values are the means ± SD of four samples. Significance level: $P < 0.05$. PtdOH, phosphatidic acid; PtdSer, phosphatidylserine; PtdIns, phosphatidylinositol; PtdCho, phosphatidylcholine; PlsEtn, ethanolamine plasmalogens; PtdEtn, phosphatidylethanolamine; Chol, cholesterol. LAM + 90 min denotes laminectomy with a 90-min recovery period. Injury groups include 1 min of 170-g compression trauma, 5 min of compression, and 5 min of compression after which the weight was removed and the cord was frozen after an additional 5, 15, and 30 min. Significance level: $P < 0.05$.
*Different from LAM + 90 min, 1 min, and 5 min.
†Different from LAM + 90 min and 1 min.
‡Different from LAM + 90 min.

neurosurgical procedure for the treatment of clinical disorders, such as tumors within the spinal cord or spinal canal (41).

There are a number of potential sources for the FFA and $acyl_2Gro$ that accumulate in spinal cord following blunt trauma. Based upon our data, however, it is not possible at the present time to pinpoint their origins. The decreases seen in spinal cord FFA and $acyl_2Gro$ following the termination of compression may also be due to several mechanisms (42), including equilibration with the FFA in blood and cerebrospinal fluid, oxidation of the fatty acids as an energy source, reacylation into glycerophospholipids, and enzymic and nonenzymatic peroxidation of the polyunsaturated fatty acids (43–47).

The loss of 20% of the ethanolamine plasmalogens is equivalent to a loss of 7% of the total phospholipids. This change, together with the 10% loss of cholesterol and elevations in FFA and $acyl_2Gro$, may damage cells and affect physiological processes by altering membrane structure and fluidity. Typical control levels of both FFA and $acyl_2Gro$ are ≈0.4 mol% of the pool of total phospholipids. In our model of spinal cord trauma these values increase to ≈4 mol% each, a level at which fatty acids have been shown to perturb both model and biological membranes, as determined by fluorescence polarization, differential scanning calorimetry, and enzymic assays (4, 5, 48, 49). $Acyl_2Gro$ may affect membrane curvature and act to rigidify membranes (50–52). The decreases in the levels of ethanolamine plasmalogens and cholesterol should also have marked effects on membrane fluidity, permeability, and ultrastructure (53–56). These membrane alterations could potentially account for the rapid decrease in extracellular Ca^{2+} that is seen after injury (57, 58).

Physiologically, a number of membrane-related effects of FFA and $acyl_2Gro$ have been observed. For example, FFA inhibit the Na^+-dependent synaptosomal uptake of proline, aspartate, glutamate, and γ-aminobutyric acid and reduce synaptosomal Na^+,K^+-ATPase activity (59). $Acyl_2Gro$ stimulate transepithelial sodium transport in frog skin epithelium (60) and markedly increase phospholipase-catalyzed hydrolysis of phospholipid bilayers (61).

In the context of spinal cord injury, membrane lipid hydrolysis is probably related to the decrease in activity of Na^+,K^+-ATPase that occurs within 5 min after administration of blunt trauma (8, 62). Although FFA have been shown to inhibit this enzyme (7, 63, 64), it is not known if the inhibition is due to direct interactions between FFA and enzyme or due to effects of FFA on the membrane matrix. The release of polyunsaturated fatty acids may be involved in producing the capillary endothelial defects that can be observed within 2 min after injury (33, 65). Intracerebral injections of arachidonic acid in rats selectively destroy the outer leaflet of capillary plasma membranes in white matter (66). Kontos *et al.* (67) attribute such capillary damage to metabolites of arachidonic acid. Arachidonic acid may also play a role in edema formation after spinal trauma. In brain, it has been demonstrated that polyunsaturated fatty acids are active in the induction of cellular and vasogenic edema, both *in vivo* and *in vitro* (11, 68). Vasogenic edema is consistent with observed spinal cord capillary damage (33, 65, 66). Cellular edema is consistent with compromised plasma membrane structure and function.

Arachidonic acid metabolites, with their wide variety of biological activities, almost certainly contribute to the pathophysiological responses of spinal cord. Thromboxane A_2 is a potent vasoconstrictor and promoter of platelet aggregation, whereas prostacyclin is a vasodilator and antiaggregant. The increase in thromboxane synthesis relative to that of prostacyclin may be involved in the decrease in blood flow (68, 69) and platelet aggregation (70) observed following spinal cord injury. Because prostacyclin synthetase is localized in endothelial cell microsomes, the failure of prostacyclin to be synthesized may be explained by the vascular endothelial cell injury observed after trauma (33, 40).

Thus, the primary site of early cellular damage following spinal cord injury appears to be the plasma membrane. Changes in concentrations of FFA, $acyl_2Gro$, cholesterol, and phospholipids of the magnitude that are reported here are sufficient to produce significant perturbations in membrane structure and dynamics, as determined in both model and biological systems. Such perturbations could alter activities of membrane enzymes and change both the active and passive permeability characteristics of the cell membrane. The biologically active icosanoids, produced from released arachidonic acid, could then exacerbate this condition by reducing blood flow and oxygen supply, thereby decreasing the energy available for use in self-repair of damaged cells.

We thank Mr. Thomas Waters for the careful performance of the delicate surgical procedures required for these studies and Dr. Richard Fertel for his consultation and the materials used in the radioimmunoassays. This research was supported in part by Research Grants NS-08291 and NS-10165 and Training Grant NS-07091 from the National Institutes of Health.

1. Osterholm, J. L. (1974) *J. Neurosurg.* **40**, 5–33.
2. De La Torre, J. C. (1982) *Spine* **6**, 315–335.
3. Allan, D., Thomas, P. & Michell, R. H. (1978) *Nature (London)* **276**, 289–290.
4. Usher, R., Epand, R. D. & Papahadjopoulos, D. (1978) *Chem. Phys. Lipids* **22**, 2456–2463.

5. Klausner, R. D., Kleinfeld, A. M., Hoover, R. P. & Karnovsky, M. J. (1980) *J. Biol. Chem.* **255,** 1286–1295.
6. Lucy, J. A. (1970) *Nature (London)* **227,** 815–817.
7. Ahmed, K. & Thomas, B. S. (1971) *J. Biol. Chem.* **246,** 103–109.
8. Clendenon, N. R., Allen, N., Gordon, W. A. & Bingham, W. G. (1978) *J. Neurosurg.* **49,** 563–568.
9. Hall, E. D. & Braughler, J. M. (1982) *J. Neurosurg.* **57,** 247–253.
10. Wolfe, L. S. (1982) *J. Neurochem.* **38,** 1–14.
11. Chan, P. H. & Fishman, R. A. (1980) *J. Neurochem.* **35,** 1004–1007.
12. Chan, P. H., Kerlan, R. & Fishman, R. A. (1983) *J. Neurochem.* **40,** 309–316.
13. Yoshida, S., Abe, K., Busto, R., Watson, B. D., Kogure, K. & Ginsberg, M. D. (1982) *Brain Res.* **245,** 307–316.
14. Agardh, C. D., Chapman, A. G., Nilsson, B. & Siesjö, B. (1981) *J. Neurochem.* **36,** 490–500.
15. Rodriguez de Turco, E. B., Morelli de Liberti, S. & Bazan, N. G. (1983) *J. Neurochem.* **40,** 252–259.
16. Gardiner, M., Nilsson, B., Rehncrona, S. & Siesjö, B. (1981) *J. Neurochem.* **36,** 1500–1505.
17. Dorman, R. V., Dabrowiecki, Z. & Horrocks, L. A. (1983) *J. Neurochem.* **40,** 276–279.
18. Gaudet, R. J. & Levine, L. (1979) *Biochem. Biophys. Res. Commun.* **86,** 893–901.
19. Shohami, E., Rosenthal, J. & Lavy, S. (1983) *Stroke* **13,** 494–499.
20. Pappius, H. M. & Wolfe, L. S. (1983) *Neurochem. Res.* **8,** 63–72.
21. Ellis, E. F., Wright, K. F., Wei, E. P. & Kontos, H. A. (1981) *J. Neurochem.* **37,** 892–896.
22. Jonsson, H. T. & Daniell, H. B. (1976) *Prostaglandins* **11,** 56–61.
23. Anderson, D. K., Prockop, L. D., Means, E. D. & Hartley, L. E. (1976) *J. Neurosurg.* **44,** 715–722.
24. Anderson, D. K., Nicolosi, G. R., Means, E. D. & Hartley, L. E. (1978) *J. Neurosurg.* **48,** 232–238.
25. Anderson, D. K., Means, E. D. & Waters, T. R. (1980) *J. Neurosurg.* **52,** 494–496.
26. Rouser, G., Fleischer, S. & Yamamoto, A. (1970) *Lipids* **5,** 494–496.
27. Jones, M., Keenan, R. W. & Horowitz, P. (1982) *J. Chromatog.* **237,** 522–524.
28. Saunders, R. D. & Horrocks, L. A. (1984) *Anal. Biochem.* **143,** 71–75.
29. Hara, A. & Radin, N. S. (1978) *Anal. Biochem.* **90,** 420–426.
30. Bowman, R. E. & Wolf, R. C. (1962) *Clin. Chem.* **8,** 302–309.
31. Fertel, R., Yetiv, J. Z., Coleman, M. A., Schwartz, R. D., Greenwald, J. E. & Bianchine, J. R. (1981) *Biochem. Biophys. Res. Commun.* **102,** 1028–1033.
32. Horrocks, L. A. & Sun, G. Y. (1972) in *Research Methods in Neurochemistry*, eds. Marks, N. & Rodnight, R. (Plenum, New York), Vol. 1, pp. 223–231.
33. Goodman, J. H., Bingham, W. G. & Hunt, W. E. (1976) *J. Neurosurg.* **44,** 418–424.
34. Hennacy, D. M. & Horrocks, L. A. (1978) *Bull. Mol. Biol. Med.* **3,** 207–221.
35. DeMedio, G. E., Goracci, G., Horrocks, L. A., Lazarewicz, J. W., Mazzari, S., Porcellati, G., Strosznajder, J. & Trovarelli, G. (1980) *Ital. J. Biochem.* **29,** 412–432.
36. Keough, K. M. W., Macdonald, G. & Thompson, W. (1972) *Biochim. Biophys. Acta* **270,** 337–347.
37. Banschbach, M. W. & Geison, R. L. (1974) *J. Neurochem.* **23,** 875–877.
38. Bazan, N. G. (1971) *Lipids* **6,** 211–212.
39. Bazan, N. C., Bazan, H. E. P., Kennedy, W. G. & Joel, C. D. (1971) *J. Neurochem.* **18,** 1387–1393.
40. Demopoulos, H. B., Flamm, E. S., Pietronigro, D. D. & Seligman, N. L. (1980) *Acta Physiol. Scand. Suppl.* **492,** 91–119.
41. Gurdjian, E. S. & Thomas, L. M. (1970) *Operative Neurosurgery* (Williams & Wilkins, Baltimore), p. 371–390.
42. Yoshida, S., Inoh, S., Asano, T., Sano, K., Shimasaki, A. & Ueta, N. (1983) *J. Neurochem.* **40,** 1278–1286.
43. Kimes, A. S., Sweeney, D., London, E. D. & Rapoport, S. I. (1983) *Brain Res.* **274,** 291–301.
44. Agardh, C. D., Chapman, A. G., Nilsson, B. & Siesjö, B. K. (1981) *J. Neurochem.* **36,** 490–500.
45. Sun, G. Y. (1977) *Lipids* **12,** 661–665.
46. Gaudet, R. J. & Levine, L. (1979) *Biochem. Biophys. Res. Commun.* **86,** 893–901.
47. Horrocks, L. A., VanRollins, M. & Yates, A. J. (1981) in *The Molecular Basis of Neuropathology*, eds. Davison, A. N. & Thompson, R. H. S. (Arnold, London), pp. 601–630.
48. Karnovsky, M. J., Kleinfeld, A. M., Hoover, R. L. & Klausner, R. D. (1982) *J. Cell Biol.* **94,** 1–6.
49. Hill, D. J., Dawidowicz, E. A., Andrews, M. L. & Karnovsky, M. J. (1983) *J. Cell. Physiol.* **115,** 1–8.
50. Sheetz, M. P. & Singer, S. J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4457–4461.
51. Allan, D., Thomas, P. & Michell, R. H. (1978) *Nature (London)* **276,** 289–290.
52. Ohki, K., Sekiya, T., Takeo, Y. & Nozawa, T. (1982) *Biochim. Biophys. Acta* **693,** 341–350.
53. Vaughn, D. J. & Keough, K. M. (1974) *FEBS Lett.* **47,** 158–161.
54. Demediuk, P., Cowan, D. L. & Moscatelli, E. A. (1983) *Biochim. Biophys. Acta* **730,** 263–270.
55. Houslay, M. D. & Stanley, K. K. (1982) *Dynamics of Biological Membranes* (Wiley, New York), pp. 71–81.
56. Blok, M. C., Van Deenen, L. L. M. & DeGier, J. (1977) *Biochim. Biophys. Acta* **464,** 509–518.
57. Young, W., Yen, V. & Blight, S. (1982) *Brain Res.* **253,** 105–113.
58. Stokes, B. T., Fox, P. & Hollinden, G. (1983) *Exp. Neurol.* **80,** 561–572.
59. Rhoads, D. E., Kaplan, M. A., Petersen, N. A. & Raghupathy, E. (1982) *J. Neurochem.* **38,** 1255–1260.
60. Yorio, T., Torres, S. & Tarapoom, N. (1980) *Lipids* **18,** 96–99.
61. Dawson, R. M. C., Hemington, N. L. & Irvine, R. F. (1983) *Biochem. Biophys. Res. Commun.* **117,** 196–201.
62. Hall, E. D. & Braughler, J. M. (1982) *J. Neurosurg.* **57,** 297–253.
63. Chan, P. H., Kerlan, R. & Fishman, R. (1983) *J. Neurochem.* **40,** 309–316.
64. Swann, A. C. (1984) *Arch. Biochem. Biophys.* **233,** 354–361.
65. Griffiths, I. R. (1978) *Adv. Neurol.* **20,** 415–522.
66. Wakai, S., Aritake, K., Asano, T. & Takakura, K. (1982) *Acta Neuropathol.* **58,** 303–306.
67. Kontos, H. A., Wei, E. P., Povlishock, J. T., Dietrich, W. D., Magiera, C. J. & Ellis, E. F. (1980) *Science* **209,** 1242–1245.
68. Chan, P. H., Fishman, R. A., Caronna, J., Schmidley, J. W., Prioleau, G. & Lee, J. (1983) *Ann. Neurol.* **13,** 625–632.
69. Griffiths, I. R. (1976) *J. Neurol. Sci.* **27,** 247–259.
70. Sandler, A. N. & Tator, C. H. (1976) *J. Neurosurg.* **45,** 638–646.