Lipid composition of rat brain myelin in triethyl tin-induced edema

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ABSTRACT Chronic triethyl tin intoxication was induced in young adult rats by oral feeding of triethyl tin sulfate. Progressively severe brain edema developed during the 3-month experimental period. The yield of myelin from the brains of the experimental animals decreased to almost half normal per brain, but the isolated myelin appeared morphologically normal. The analysis of whole brain showed corresponding decreases in proteolipid protein and total lipid, particularly galactolipids. The proportions of the major constituents of isolated myelin (chloroform-methanol-insoluble residue, proteolipid protein, and total lipid) were unchanged despite the low yield. However, the proportion of cholesterol increased from 16 to 21% dry weight, and that of total galactolipid decreased from 21 to 15% as the yield of myelin decreased. This decrease of total galactolipid was mainly due to the decrease in cerebroside. Total phospholipid remained constant initially but showed a slight decrease toward the end of the experiment, due mostly to decreased ethanolamine phospholipid. There was no preferential loss or preservation of phosphatidylethanolamine. The fatty acid composition of sulfatide showed statistically significant shifts to less long-chain fatty acids and less monoenoic acids, but cerebroside and sphingomyelin did not show significant changes in the fatty acid composition. There was no increase in esterified cholesterol. These findings generally support our hypothesis of nonspecific chemical abnormalities of the myelin sheath undergoing secondary degeneration.

In an acute experiment, a single intraperitoneal injection of triethyl tin sulfate produced acute and transient brain edema. There were slight decreases in the yield of myelin, but no detectable changes in the chemical composition.

SUPPLEMENTARY KEY WORDS cholesterol • galactolipid • cerebroside • sulfatide • sphingomyelin • fatty acid • demyelination

Previous investigations in our laboratory on the composition of myelin, isolated from brains of patients with various neurological disorders, indicated that there is a set of chemical abnormalities which are common in all instances of nonspecific breakdown of the myelin sheath (1). These abnormalities are: (a) low yield of myelin, which, however, retains the normal protein-lipid ratio; (b) substantial increase of cholesterol and decrease of galactolipids, particularly cerebroside; and (c) slight to moderate decrease of ethanolamine phospholipids. Despite these chemical abnormalities, the myelin sheath appears to retain the normal physical and morphological characteristics. Accumulation of esterified cholesterol in white matter may or may not occur. We have observed myelin with this type of chemical abnormality in subacute sclerosing panencephalitis (2), spongy degeneration of the brain (3), Gm1-gangliosidosis and infantile Gm2-gangliosidosis (Tay-Sachs disease) (4), Niemann-Pick disease (5), and Schilder’s disease (6). We postulated on the basis of these observations that, except for myelin breakdown due to specific underlying metabolic derangements such as in metachromatic leukodystrophy (7, 8) or Refsum’s disease (9), the myelin sheath follows a stereotyped biochemical process of breakdown, regardless of the nature of the assaults which initiate the breakdown. The data reported by Gerstl and coworkers on the chemical composition of myelin in sudanophilic leukodystrophy (10) are also consistent with this hypothesis.

In order to test this hypothesis, it would be desirable to produce breakdown of myelin experimentally by a variety of means under controlled conditions, and sequentially study the composition of myelin. This report describes the result of our first such attempt, in which chronic triethyl tin intoxication was chosen to produce destruction of myelin in young adult rat brain.

MATERIALS AND METHODS

Experimental Design

A. Chronic Experiment. Randomly bred Sprague-
Dawley rats, weighing 250–300 g, were used. Triethyl tin sulfate (a gift from Organisch Chemisch Inst., Utrecht, The Netherlands) was dissolved in drinking water, initially at a concentration of 5 mg/liter. Rats were maintained on this drinking water, with solid food given ad lib., for 15 days. The concentration of triethyl tin sulfate was then raised to 10 mg/liter. At this high triethyl tin content, clinical signs progressed appreciably. From the 30th day, the concentrations of 5 and 10 mg/liter were alternated at 5-day intervals to prevent the death of the experimental animals.

The first clinical sign appeared on the second day, when ruffling of the hairs was noted. Weight loss and general weakness were apparent by the 7th day, and mild paraparesis began on the 12th day. Weight loss, loss of appetite, and general weakness persisted during the entire experimental period of 3 months. Paraparesis progressed slowly, but never reached complete paralysis of the hind limbs. Urinary or fecal incontinence did not occur. Rats were killed in pairs on the 10th, 17th, 24th, 31st, 45th, 60th, 74th, and 87th day after the triethyl tin feeding began.

B. Acute Experiment. In one experiment a total of 30 rats received intraperitoneal injections of 0.5% saline solution of triethyl tin sulfate at a dose of 5 mg/kg of body weight. Within 15 min after injection, acute neurological symptoms, such as stupor or coma, appeared, but all the rats survived the acute phase and recovered within 12 hr. Subsequently, the rats were kept on the diet and water ad lib., without triethyl tin. In groups of three they were killed 3, 7, 14, 33, and 65 days after injection. Except for the initial acute symptoms, no detectable neurological signs appeared during the entire experimental period.

Morphology
Rats were killed by decapitation, and the brains were removed immediately, together with cerebellum, brain stem, and medulla oblongata. The brain was weighed, and a coronal section, approximately 2 mm thick, was excised from the central portion of the brain, weighed, and then fixed in 10% buffered formalin. This coronal section was used for histological examination to ascertain the presence of chronic and progressive brain edema. Paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin for general examination, and with the Weil stain for the myelin sheath. Frozen sections were cut from the formalin-fixed tissue and stained with Oil Red O for the examination of sudanophilic material.

A small portion of isolated myelin was taken from every preparation, just before the lyophilization step, to assess the morphological purity of the fractions by electron microscopic examination. It was fixed in osmium tetroxide, dehydrated through increasing concentrations of ethanol and then propylene oxide, and embedded in Araldite.

Isolation and Analysis of Myelin
The remainder of the brain, after removal of the thin coronal section for histology, was used for the isolation of myelin. Brains were not pooled, and myelin was isolated and analyzed separately for each brain. The procedure of myelin isolation was that of Norton (11). To calculate the yield of myelin per brain, the weight of the final dry myelin preparation was corrected for the amount of tissue removed for histological examination, with the assumption that the yield per wet weight was the same for the coronal section and the remainder of the brain.

The lyophilized myelin preparations were analyzed according to the methods previously described (4). Plasmalogen was measured by the method of Wittenberg, Kory, and Swenson (12). For the analysis of the fatty acid composition of sphingolipids, the total lipid fraction was first treated with mercuric chloride in an acidic condition, followed by alkaline saponification, as described by Abramson, Norton, and Katzman (13). This procedure eliminated most of the glycerophospholipids. Pure cerebroside, sulfatide, and sphingomyelin were then obtained by silicic acid column chromatography (14). The fatty acid composition of purified sphingolipids was determined by gas–liquid chromatography, as previously described (15). Methyl esters of α-hydroxy fatty acids were analyzed by gas–liquid chromatography as trimethylsilyl derivatives.

Attempts were made to estimate the concentration of esterified cholesterol in the brain in the following manner. After the initial sucrose density gradient centrifugation for the isolation of myelin, the myelin band at the interface of 0.32 M and 0.85 M sucrose was removed for further purification of myelin. Then, the fractions above and below the myelin band were combined in a test tube, an equal volume of hexane was added, and the tube was shaken. The hexane phase was transferred to another tube, the remainder was extracted twice more with hexane, and all hexane extracts were combined. The dried hexane phase was chromatographed on silicic acid according to Hornig, Williams, and Hornig (16) to obtain the cholesteryl ester fraction. The amount of cholesteryl ester was determined as free cholesterol (17). Our previous experience had shown that esterified cholesterol, often present in demyelinating conditions, is present mostly as a very light and amorphous “floating fraction” and is not associated with the myelin fraction (2, 4). Preliminary experiments indicated that more than 90% of standard cholesterol or cholesteryl olate, added at the bottom of the tube as a thin, dry film before the addition of sucrose solution and hexane, was extracted.
into the hexane phase with this procedure. Therefore, this procedure should give a reasonable, if not a rigorously quantitative, estimate of cholesteryl ester present in the brain.

**Analysis of Whole Brain**

A separate experiment was carried out to correlate the yield of myelin and the changes in the composition of whole brain. A group of Sprague-Dawley rats, weighing 250–300 g, were given drinking water containing triethyl tin sulfate, 10 mg/liter, as before. The concentration of the compound was kept constant throughout the experiment. At 1 and 2 months after the beginning of the triethyl tin feeding, experimental and control rats were killed in pairs. The brains were removed and divided longitudinally into the right and left sides, and each half was weighed separately. One half of the brain was used to isolate myelin, as described above. The other half was used for the analysis according to our standard procedures (4), with attention to the characteristic myelin constituents, such as chloroform-methanol-soluble protein and lipid constituents, particularly galactolipids. The results were calculated for the whole brain, assuming that there was no left-to-right asymmetry for either the yield of myelin or the chemical composition.

**RESULTS**

**Morphological Changes**

In the chronic experiment, mild edema was present in white matter on the 10th day. There was a slow progression of edema during the entire experimental period (Fig. 1). With the myelin stain, there was a noticeable decrease in the amount of stainable myelin after 30 days of triethyl tin sulfate feeding, but, due to the presence of severe edema, a quantitative estimate of the decrease of myelin was not possible. On the other hand, similarly severe brain edema was noted 3 days after the single injection of triethyl tin sulfate in the acute experiment. The edema, however, subsided during the course of the experiment, and the pallor of the myelin sheath was not observed. The sudanophilic droplets, common in non-specific myelin breakdown, were not found in either the acute or chronic experiment.

The electron microscopic examinations of isolated myelin indicated that all of the fractions were extremely pure morphologically, consisting only of myelin lamellae with virtually no detectable contaminants. The myelin isolated from the experimental animals appeared identical to that from controls. These findings were constant throughout the experimental period, despite the significant decrease in the yield of myelin in the chronic experiment.

**Brain Weight and Yield of Myelin**

The weight of the brain increased, consistent with the degree of histologically demonstrable edema (Fig. 2). In the acute experiment, the brain weight increased sharply during the first 10 days, reaching approximately 25% over normal. Then, it declined thereafter. In the chronic experiment, there was a gradual increase in the brain weight, which did not reach the maximum until 2 months after the beginning of the triethyl tin feeding. The increase in the brain weight approached 30% over the normal values.

The yield of myelin per brain did not vary significantly in the acute experiment, despite the presence of acute brain edema (Fig. 3). Considering the normal increment during this period, this finding probably indicates a slight reduction in the yield of myelin from the acute experimental animals. In contrast, there was a gradual and significant decrease in the yield of myelin in the chronic experiment. The curve of the myelin yield in the chronic experiment was roughly the inverse of the brain weight curve. At the height of edema, the yield of myelin was almost half of the normal yield.

The decreased yield per brain must represent either actual loss of myelin from the brain or changes in the physical properties of a significant portion of myelin, which then could no longer be isolated by the procedure devised for normal myelin.

**Fate of Missing Myelin**

The analysis of whole brain provided a partial answer concerning the missing myelin (Table 1). At both 1- and 2-month periods there were substantial decreases in the yield of myelin from the experimental animals. In whole brain, substantial decreases were found in proteolipid protein and galactolipids, while cholesterol and phospholipid showed little changes. The selective reduction in the constituents that are highly localized in the myelin sheath strongly suggests actual loss of myelin from the brain, rather than alteration of the physical properties of the myelin sheath. At 1 month there was an average decrease of 19.6 mg brain in the yield of myelin. If we assume that this missing myelin is lost from the whole brain, we can calculate the expected decreases for proteolipid protein, total lipid, and galactolipid to be 4.8, 12.9, and 3.9 mg per brain, respectively. The analysis showed the actual decreases of 6.5, 10.5, and 5.2 mg. Similarly, at the 2-month period there was a decrease of 36 mg per brain in the yield of myelin, giving the expected losses of 8.9, 23.6, and 7.1 mg per brain for proteolipid protein, total lipid, and galactolipids. The actual decreases found were 6.6, 15.0, and 5.2 mg, respectively. These data are consistent with the idea that there were actual breakdown and removal.
of myelin from the brain in the triethyl tin-treated animals. The myelin sheath, however, does not appear to be eliminated from the brain as a unit, as indicated by the almost complete preservation of cholesterol and phospholipid. It appears that when the myelin sheath is broken down, protein and galactolipid are rapidly removed, while the remainder, mostly cholesterol and phospholipid, stays longer within the brain. This finding may also explain the slightly increased turbidity noted during the myelin isolation in the supernatant fraction in experimental animals. It is also clear from these data that not only myelin but also other brain structures are broken down in triethyl tin-induced edema. From the decrease in the yield of myelin alone, decreases of only 0.9 and 1.7 mg per brain would be expected for the chloroform–methanol-insoluble residue at 1- and 2-month periods, whereas the actual decreases were 25.5 and 33 mg.

**Chemical Composition of Myelin**

In the acute experiment the composition of myelin remained normal in the amounts of total lipid, proteolipid protein, chloroform–methanol-insoluble residue, and individual lipids, and in the fatty acid composition of the three sphingolipids, cerebroside, sulfatide, and sphingomyelin. Together with the relatively normal yield of myelin, we can conclude that the acute triethyl tin intoxication, produced by a single injection of the compound, did not appreciably affect the structure and...
metabolism of myelin, at least for the analytical parameters we examined.

In the chronic experiment, however, there were significant changes in the lipid composition of isolated myelin, in addition to the decreased yield. Despite the changing yield, the proportions of the chloroform-methanol-insoluble residue, total lipid, proteolipid protein, and the upper phase solids remained constant throughout the 3-month experimental period, as indicated in Table 2. There were, however, consistent changes in the proportions of the major lipids (Fig. 4). Cholesterol increased during the entire experimental period, from 16 to 21% dry weight. On the other hand, the total galactolipid decreased during the first 1.5 to 2 months, from approximately 21 to 15% dry weight, and then gradually increased again, up to 19%. This change in total galactolipid was primarily due to the change in the amount of cerebroside; sulfatide remained relatively constant during the experiment. The total phospholipid remained unchanged during the first 2 months and then showed a slight decrease toward the end. Serine phospholipid, monophosphoinositide, and sphingomyelin did not change at all, and the small decrease in the total phospholipid was due to the decrease of ethanolamine phospholipid and lecithin, particularly the former. There was no preferential loss or preservation of phosphatidylethanolamine, which constituted 73–85% of total ethanolamine phospholipid, with an average of 79% and a normal value of 81%.

The fatty acid compositions of the three sphingolipids, cerebroside, sulfatide, and sphingomyelin, did not show any readily recognizable changes. Therefore, the data are given as means for the control and experimental groups, with standard deviations, rather than as individual values (Tables 3 and 4). Individual fatty acids, both unsubstituted and α-hydroxy, did not show any appreciable changes in any of the sphingolipids. Since the questions of long-chain fatty acid deficiency and the saturation of monoenoic fatty acids are often raised in relation to demyelination, the ratio of short-chain to long-chain fatty acids and the ratio of monoenoic to saturated fatty acids were examined. Only the unsubstituted fatty acids of sulfatide showed statistically significant changes. There were slight decreases in the proportion of long-chain fatty acids (0.10 > P > 0.05) and in the proportion of monoenoic fatty acids (0.05 > P > 0.02) in the sulfatide of myelin in the experimental group. The ratios of short-chain vs. long-chain and
FIG. 3. Yield of myelin from triethyl tin-intoxicated rat brains. There was little decrease in the yield of myelin in the acute experiment (X), in which rats received single intraperitoneal injections of triethyl tin sulfate. The yield of myelin decreased in the chronic experiment (○), consistent with the development of chronic brain edema, to nearly half of the normal yield.

FIG. 4. Changes in the lipid composition of isolated myelin in chronic triethyl tin intoxication. The increase of cholesterol and the decrease of cerebroside were the most conspicuous changes.
Logical sudanophilia, the loss of myelin in chronic amounts of esterified cholesterol.

The concentration of esterified cholesterol fluctuated in most samples between 2.5 and 5.5 μg per brain (total range, 1.3–7.6). Consistent with the absence of histological sudanophilia, the loss of myelin in chronic triethyl tin edema is not accompanied by increased amounts of esterified cholesterol.

### TABLE 1 Composition of Rat Whole Brain in Chronic Triethyl Tin Intoxication

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Controls</th>
<th>Experimental</th>
<th>Average Net Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of myelin</td>
<td>50.9, 48.7</td>
<td>31.2, 29.2</td>
<td>-39</td>
</tr>
<tr>
<td>Chloroform-methanol-insoluble residue</td>
<td>200, 204</td>
<td>176, 177</td>
<td>-13</td>
</tr>
<tr>
<td>Proteolipid protein</td>
<td>19.7, 15.4</td>
<td>12.1, 10.0</td>
<td>-37</td>
</tr>
<tr>
<td>Upper phase solids</td>
<td>29.6, 29.6</td>
<td>30.6, 27.4</td>
<td>-2</td>
</tr>
<tr>
<td>Total lipid</td>
<td>141, 139</td>
<td>130, 129</td>
<td>-7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>31.2, 33.1</td>
<td>33.3, 32.2</td>
<td>+2</td>
</tr>
<tr>
<td>Total phospholipid</td>
<td>77.9, 82.4</td>
<td>82.4, 80.0</td>
<td>+1</td>
</tr>
<tr>
<td>Total galactolipid</td>
<td>19.5, 19.5</td>
<td>14.3, 14.4</td>
<td>-26</td>
</tr>
<tr>
<td>Cerebroside</td>
<td>14.2, 15.1</td>
<td>11.1, 10.6</td>
<td>-26</td>
</tr>
<tr>
<td>Sulfatide</td>
<td>5.3, 4.4</td>
<td>3.2, 3.8</td>
<td>-27</td>
</tr>
</tbody>
</table>

Two experimental and two control rat brains were analyzed for the yield of myelin and for the lipid composition at each experimental period. Each analytical value in this table represents a single rat. Whenever possible, determinations were carried out in duplicate.

### TABLE 2 Major Constituents of Myelin in Rats with Chronic Triethyl Tin Intoxication

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Control (n = 3)</th>
<th>Experimental (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform-methanol-insoluble residue</td>
<td>4.7 ± 2.0</td>
<td>5.7 ± 1.6</td>
</tr>
<tr>
<td>Total lipid</td>
<td>65.6 ± 3.5</td>
<td>65.4 ± 3.2</td>
</tr>
<tr>
<td>Proteolipid protein</td>
<td>24.7 ± 4.2</td>
<td>26.0 ± 3.2</td>
</tr>
<tr>
<td>Upper phase solids</td>
<td>4.0 ± 1.7</td>
<td>2.7 ± 0.6</td>
</tr>
</tbody>
</table>

Values are expressed as average % dry wt ± SD. There was no consistent direction of change for any of the major constituents throughout the duration of triethyl tin feeding.

### DISCUSSION

Little breakdown of the myelin sheath occurred in the acute experiment, in which rats received single intraperitoneal injections of triethyl tin sulfate, despite the severe acute brain edema. Therefore, the acute experiment was an unsatisfactory model for the study of the chemical changes of the myelin sheath undergoing degeneration. Due to the nature of the analytical procedures, this result does not exclude the possibility of small focal myelin breakdown.

The chronic triethyl tin intoxication, on the other hand, produced slowly progressive brain edema, which eventually surpassed that of acute intoxication in severity and duration. In these animals there was histologically demonstrable pallor of myelin, although a quantitative estimate of the actual myelin loss was difficult. The yield of myelin substantiated the histological impression by showing a decline to almost half of the normal yield per brain at the height of the edema. To define myelin loss by the decrease in the yield is, by rigorous criteria, only operational. It indicates the degree of the loss of the myelin sheath with normal physical properties, but it does not distinguish between the actual disappearance of the myelin sheath and the presence of abnormal myelin, the physical properties of which have been altered in such a way that it is no longer isolated by the procedure devised for normal myelin. The analysis of whole brain, however, showed decreases of proteolipid protein and galactolipids consistent with the decreased yield of myelin. The myelin sheath is not likely to maintain its morphological integrity when it loses the protein and galactolipid constituents, and the remaining mixture of cholesterol and phospholipid does not, in our opinion, satisfy even the broadest definition of myelin. Therefore, it seems to be justifiable to consider that the low yield of myelin in the experimental animals reflects the actual loss of myelin from the brain.

The myelin isolated from chronic experimental animals appeared morphologically normal under the electron microscope but exhibited abnormal chemical composition. The findings generally substantiated our previously advanced hypothesis of the nonspecific chemical abnormalities of degenerating myelin. As the severity of the edema progressed and the yield of myelin decreased during the first 2 months, cholesterol increased, and total galactolipid, particularly cerebroside, decreased significantly. The moderate decrease of phospholipid, particularly ethanolamine phospholipid, occurred during the last month. These abnormalities were qualitatively the same as those found in the myelin from many neurological diseases, in which myelin degeneration can reasonably be regarded to be secondary and nonspecific (1). The chemical composition of rat myelin is known to change during development, but the adult...
Developmental changes are characterized by the increase in galactolipid and ethanolamine phospholipid, the opposite direction of the changes we found in the present experiment. Also, we found significant changes in the cholesterol content of myelin, which remains constant during normal development. While strict nonspecificity of the myelin breakdown in chronic triethyl tin intoxication may be a matter of debate, because the compound produces an unusual type of brain edema by splitting...
the myelin lamellae at the minor period lines (20), the present data add another instance of degenerating myelin with the same type of chemical abnormalities as in other neurological diseases.

It was somewhat disappointing to find that the relative increase of cholesterol and the decrease of galactolipid did not continue throughout the experimental period. Not only did the cholesterol increase slow down during the latter half of the experiment, but there was actually a small recovery in the total galactolipid content. These findings coincided with the slow increase of the yield of myelin during the same period. After 2 months of triethyl tin feeding, the skulls of the experimental animals became paper-thin. Perhaps the intracranial pressure exerted by the edema did not increase beyond this stage and the rats were able to resume normal myelin formation, which is known to occur in normal rats throughout life (19), resulting in the slow increase in yield. Therefore, toward the end of the experiment we may have been isolating and analyzing myelin which included newly formed myelin with normal chemical composition.

The changes in the fatty acid composition of sphingolipids did not show the drastic abnormality observed in some neurological disorders (8). There were statistically significant decreases in long-chain fatty acids and monoenoic acids in sulfatide, but the fatty acid composition of cerebroside and sphingomyelin did not show significant changes; however, the fatty acids of sphingo-myelin showed unusually wide variations, as reflected by the large standard deviation figures. Even the fatty acid changes in sulfatide were very minor and barely significant, and we do not consider them to be conclusive. This aspect requires further study in a more appropriate experimental model.

The chronic triethyl tin intoxication provided one experimental model to study the changes in the chemical composition of myelin undergoing degeneration. The results were consistent with our hypothesis of the abnormal chemical composition of the degenerating myelin sheath. The observed changes were milder than those seen in some human pathological conditions. Another suitable experimental model would be required to further substantiate our hypothesis, to study the mechanism of cholesterol esterification, and to explore the possible alteration of fatty acids during myelin degeneration.

This investigation was supported by the Inex J. Warriner Memorial Grant for Research on Multiple Sclerosis (670-A-1) from the National Multiple Sclerosis Society, and by research grants NS-08420, NS-05903, and NS-05572 from the U.S. Public Health Service.

Dr. William T. Norton, Albert Einstein College of Medicine, Broux, N.Y., kindly made a copy of his review article (Ref. 19) available before publication.

Manuscript received 23 November 1970; accepted 20 May 1971.

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