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Triethyltin Toxicity as a Model for Degenerative Disorders

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GERREN, R. A., D. E. GROSWALD AND M. W. LUTTGES. *Triethyltin toxicity as a model for degenerative disorders.* PHARMAC. BIOCHEM. BEHAV. 5(3) 299–307, 1976. – Triethyltin (TET) toxicity in mice was examined as a model for certain degenerative disorders. Spontaneous and elicited behavioral tests, electrophysiological measures and nervous system protein characterizations were used to study anomalies resulting from TET treatments. TET animals exhibited lowered spontaneous locomotor activity levels, increased sciatic nerve excitation threshold and conduction velocities, and increased power levels in the slower frequency components of their electroencephalograms. Performance in an active avoidance task and nervous system protein compositions were not found to be altered as a consequence of TET treatments. The results suggest that the gross ultrastructural changes commonly seen in TET intoxication are not primarily responsible for the observed neurophysiological changes. Possible sites of action of TET, in both the peripheral and central nervous systems, that would produce these neurophysiological changes and the relationship of these changes to the behavioral symptoms are discussed.

Triethyltin Degenerative disorders Central nervous system Sodium azide Peripheral nervous system Spontaneous locomotor activity Simple active avoidance

SEVERAL promising experimental models of nervous system demyelinating diseases have recently received attention. One of the more useful models depends upon the toxic consequences of the organo-metallic compound, triethyltin [27]. Changes in ultrastructure, resulting from triethyltin (TET) intoxication [3, 14, 28, 29] include widespread vacuolization and splitting at the intraperiod line of central nervous system (CNS) myelin. Other changes include the accumulation of both water and sodium in myelin clefts, and the enlargement of astrocytes in CNS grey matter.

Previously, TET treatments were believed to have no pathologic effects on the peripheral nervous system [25]. This apparent resistance of PNS tissues to TET toxicity was even suggested as a useful criterion for discriminating between CNS and PNS tissues. Recently, however, evidence has been obtained for intramyelinic vacuolization in spinal nerve roots of animals chronically treated with TET [10]. The vacuolization was limited in extent to the outer lamella of myelin sheaths associated with large diameter fibers, and no edema was obvious. Although the observed PNS effects were quite modest as compared to the CNS effects of TET, questions still arise regarding the specificity of TET toxicity. Alternatively, questions might be raised regarding the PNS or CNS identity of spinal nerve roots.

Known morphological [22] and chemical [11, 31, 32] differences between PNS and CNS myelin might possibly account for differential TET sensitivity of CNS and PNS tissues. In addition, the TET-induced loss of myelin [8] may depend on specific myelin protein actions [26,27] or lipid actions [27]. It is already known that TET can inhibit structurally associated enzymes such as mitochondrial ATPase [7,30] and that TET can uncouple oxidative phosphorylation [1, 2, 5]. Other moderate alterations in membrane structure have also been reported in connection with TET toxicity [5,29].

Although TET treatments have been shown to have highly toxic qualities, little has been done to examine the behavioral and functional consequences of such treatments at a systemic level. Previous studies have focused only upon gross alterations such as induced stupor, paralysis or death [14,29]. It seems that without more exhaustive tests, the validity of TET toxicity as a model for demyelinating disorders of the nervous system remains tenuous.

The present study focuses upon some of the behavioral and neural changes produced by acute and chronic TET toxicity. Possible protein changes in CNS and PNS tissues has been examined electrophoretically. While subtle but reliable alterations were observed in behavior and in neural conduction, electrophoretic separation of nervous system proteins failed to reveal any specific deletions or additions. These results are discussed in regard to TET toxicity as a model system of demyelinating diseases of the nervous system.

METHOD

Animals

Both male and female adult HS mice [20] were used in these studies. Mice used in chronic TET administration studies were housed 4 to a cage (2 TET and 2 control mice). Mice used in acute TET studies were drawn

randomly from group cages housing 10 mice each. Except during testing, all mice were provided ad lib access to food and water.

Procedure

Behavioral tests. Two types of behavioral tests were used. The first quantified spontaneous locomotor activity exhibited by mice during a daily 5 min test period. The apparatus consisted of four small (18 cm sq × 10 cm deep) opaque Plexiglas boxes, each being an individual test unit. The floor of each box was made up of nine, 5 cm sq metal plates connected alternately to the positive or common side of a low voltage DC power supply. As a mouse bridged any two adjacent squares a small amount of current passed to close a circuit and register one unit on a counter. Four mice (2 TET and 2 control) were tested simultaneously, each in an individual unit.

The daily experimental procedure involved placing each mouse in a test unit for 15–30 sec for adaptation. The unit was then activated for a 5 min period. Immediately after testing, each mouse was removed, weighed and injected intraperitoneally with either TET (2 mg/kg body weight; triethyltin bromide in 30% ETOH) or equivalent volumes of fluid without TET. The procedure was repeated daily for 27 consecutive days with 24 mice routinely tested approximately 24 hr after the last injection.

The second behavioral task tested for the ability of mice to learn a simple active avoidance. The apparatus consisted of four individual automated wheel-turn boxes as described previously [4]. During daily 30 min periods mice were given 50 trials of training to learn to avoid footshock. Avoidance was accomplished by turning small wheels when a tone, signaling the imminent onset of shock (1 mA), was sounded. Each time footshock was successfully avoided a correct trial was registered. In addition, the total amount of wheel-turn activity was recorded. Both TET and control mice were tested for 3 consecutive days following the cessation of daily injections.

Neurophysiological tests. Following behavioral testing the same mice were subjected to neurophysiological examinations of peripheral nerve conduction. The mice were first weighed and anesthetized (chloral hydrate, 500 mg/kg body weight), and then placed upon a water-heated aluminum plate in order to maintain body temperature (38.5°C). The sciatic nerve on one side was carefully exposed. Bipolar stimulating and recording electrodes were placed on the nerve surface approximately 6–8 mm apart. Stimulating electrodes (bipolar stainless steel) were placed proximal to the spinal cord and the recording electrodes (bipolar tungsten), distally. The exposed sciatic nerve was covered with mineral oil immediately following electrode placement in order to maintain nerve temperature, and to prevent dessication. Stimuli (0.3 msec, 1 stimulus per 3.5 sec) were produced by a square wave generator (E-H Research Laboratories, Inc. Mdl. 131, modified) and nerve signals were obtained by differential preamplification (Argonaut Mdl. L042). The nerve responses were either averaged (Princeton Applied Research, Waveform Eductor) and recorded (Sanborn Mdl. 150) or recorded directly on film (DuMont Kymographic Camera). Stimulation levels were varied systematically from threshold to maximal levels as determined empirically.

The above test procedure was also employed for mice receiving single TET injections 65 to 75 min before testing. Mice were given either TET (8 mg/kg) or equal volumes of injection vehicle without TET 1 hr prior to being anesthetized (400 mg/kg and 500 mg/kg chloral hydrate for TET and control mice, respectively). Testing progressed as already described. In addition, a group of both TET and control mice were tested after the sciatic nerve had been severed from spinal cord influences. Several other mice were also tested after receiving sodium azide (18 mg/kg) injections instead of TET or control injections. Except for the noted alterations, tests were conducted in the usual manner.

Additional mice were surgically implanted with indwelling cortical electrodes according to a method described earlier [16]. Approximately two weeks later these mice were given either TET (8 mg/kg) or vehicle control injections. Spontaneous and visual evoked electroencephalographic (EEG) activities were sampled and recorded (Sanborn Mdl. 150) throughout the following 2 hr postinjection period and again 24 hr later. Spontaneous EEG activity was also recorded on magnetic tape for power spectral analysis. Using a counterbalanced testing design, the same mice were tested two weeks later using opposite treatments. Control mice received TET injections and conversely, TET mice received control injections. Spontaneous and evoked EEG activity was evaluated as in initial tests. The effects of repeated tests were not significant so the results were combined according to treatment and without regard to test order.

Biochemical tests. The sciatic nerves as well as various subcellular fractions of brain were subjected to high-resolution, polyacrylamide gel electrophoresis [12]. The combined materials of six complete sciatic nerves were homogenized in chloroform-methanol (2:1), allowed to extract overnight, and recovered by the addition of 0.5 volumes of water [17]. The precepitate was washed twice with acetone and solubilized in sodium dodecyl sulfate (SDS) sample buffer [12]. The protein content of the SDS soluble extract was measured colorimetrically [15] using bovine serum albumin as a standard. Subcellular fractions were prepared from homogenized brain tissue in a manner previously described [13]. Purified nuclei, myelin, P_2, 100,000 xg precipitates, 100,000 xg supernatant, and pH 5 precipitates were prepared. Each fraction was delipidated with two washes of acetone and solubilized in the above described fashion.

The samples (≅40 μg of protein in each) were subjected to electrophoresis on an exponential-linear concentration gradient (18.75% → 7.5%) polyacrylamide slab gel [12]. Following simultaneous fixing (50% trichloroacetic acid) and staining (0.2% Coomassie blue), protein bands were quantitated microdensitometrically from photographic negatives.

RESULTS

Following the injection of large dosages (8–10 mg/kg) of TET, mice exhibited several characteristic alterations in behavior. Within 15 to 30 min after TET administration the mice exhibited poor hindlimb tonus and control, and locomotor activity was severely impaired. At about 45 min after injection these effects began to diminish. Between 60–90 min after injection the mice became inactive and lethargic. During this time the mice were difficult to arouse and difficult to elicit locomotor activity from. Within a few hours the treated mice recovered from the gross acute

TABLE 1

THE EFFECTS OF CHRONIC TET TOXICITY ON MOUSE BODY WEIGHT.

Group	Initial Weight	Final Weight	% Weight Loss
Control Mice	27.83 ± 2.17 g	27.66 ± 1.65 g	0.61%
TET Mice	27.00 ± 1.71 g	25.47 ± 1.87 g	5.67%

Both initial and final average body weights ± standard deviations are provided for TET and control mice. Initial weights were recorded prior to the start of daily injections of either TET or injection vehicle without TET. Final body weights were recorded on Day 27, 24 hr following the final injection. Weight loss is given as percent initial body weight.

effects of TET and were indistinguishable from control mice.

Mice receiving repeated daily injections of modest TET dosages (2 mg/kg) exhibited very mild and transient behavioral alterations following each injection. In fact, such TET treated mice were not easily or reliably distinguished from control mice. Within a few days after the initial injection, these mice were chronically less active than cagemate controls. When handled, however, the TET mice were very responsive and hyperactive as compared to control mice. Without the stimulation of handling, the TET mice reverted to relative inactivity.

A record of mouse weight indicated that TET mice lost an average of 5.7% of their initial body weight during the course of 27 successive daily injections (Table 1). In contrast, control mice injected over the same test period exhibited negligible body weight changes.

With the exception of the above observations, TET and control mice appeared very similar throughout the course of chronic toxicity tests.

Behavioral Tests

Chronic TET toxicity induced by daily injections yielded mice which exhibited much less spontaneous locomotor activity than control injected mice (Fig. 1). On the first test day both TET and control mice exhibited the same ($p>0.05$) levels of spontaneous locomotor activity. Both groups showed a transient decrease in activity within the first six test days. After this both groups exhibited transient increases in activity, although only the control mice exhibited significant ($p<0.05$) increases. Following this transient increase, the activity levels of both groups began to decrease continually throughout the remainder of the testing. By the last test day saline mice exhibited activity levels only 10.5% below those exhibited on the first test day, whereas TET mice exhibited levels 57.8% below those exhibited initially. The difference in spontaneous locomotor activity of TET compared to control mice became significant ($p<0.05$) on the sixth test day and highly significant ($p<0.01$) on the ninth test day. Toward the end of spontaneous locomotor activity testing (18–24 days) the difference between TET and control animals was most pronounced ($p<0.005$).

Without any additional injections (2 days after the last injection) the same mice were tested in an active avoidance task. The average number of correct trials obtained by each group is presented for each of three training days in Table 2. Both groups of mice appear to learn the task with equal facility. The TET mice achieved slightly but insignificantly ($p>0.05$) higher numbers of correct trials on each test day. The total number of wheel turns were also recorded thus making it possible to evaluate total amount of task-associated activity and response effectiveness (correct trials ÷ number of wheel turns$^{1/2}$). Although both TET and control mice behaved quite differently in spontaneous behavioral tasks, the two groups of mice performed in an indistinguishable manner in a shock-motivated behavioral task.

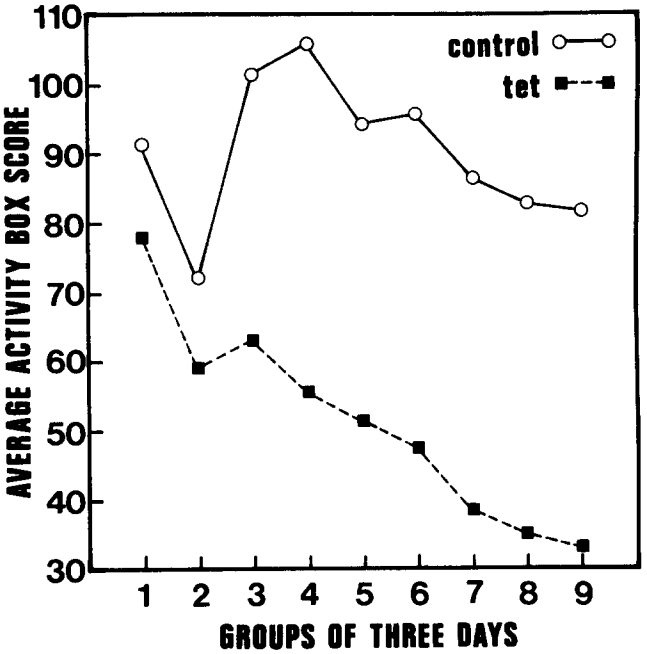

FIG. 1. The effect of chronic TET toxicity on the spontaneous locomotor activity exhibited by mice. Values of spontaneous locomotor activity for groups of mice receiving daily injections of either TET or alcoholic injection vehicle are plotted versus time. Each point represents the activity box scores of all TET or control mice combined and averaged over consecutive three day periods.

TABLE 2

THE EFFECTS OF TET TOXICITY ON SIMPLE AVOIDANCE CONDITIONING IN MICE.

Group	Day	Correct Trials	Wheel Turns$^{1/2}$	Response Effectiveness
Control Mice	1	16.2 ± 10.9	27.9 ± 9.7	0.58
	2	32.3 ± 6.6	28.3 ± 10.6	1.14
	3	35.6 ± 8.0	28.6 ± 10.6	1.24
TET Mice	1	22.5 ± 10.9	28.8 ± 8.7	0.78
	2	36.4 ± 10.3	31.5 ± 11.4	1.16
	3	40.2 ± 8.7	27.6 ± 10.8	1.46

Average number of correct trials ± standard deviations and average number of wheel turns$^{1/2}$ ± standard deviations were obtained from TET and control animals over a three day period of active avoidance training. The square root transformation of wheel turns was used to minimize the variance. Response effectiveness was obtained by dividing the correct trials by the number of wheel turns$^{1/2}$.

Neurophysiological Tests

Following behavioral testing 6 of the chronic TET mice and 6 of the control mice were randomly selected for sciatic nerve recording experiments. The response characteristics of one sciatic nerve in each animal were examined over a wide range of stimulus intensities. Threshold levels as well as maximal response levels were determined. Since the experiments were conducted on anesthetized animals in which low velocity, high threshold responses were suppressed, only 10 msec poststimulus periods of nerve activity were recorded and analyzed. Each response was characterized in terms of the amount of energy displayed in consecutive 500 μsec windows following the delivery of stimulation. A summary of these characterizations for suprathreshold stimuli is presented in Fig. 2 as the mean percentage of total nerve response energy.

During experimentation it was observed that TET nerves required more stimulation current than control nerves before responses were elicited. Subsequent calculations indicated that approximately 20% more current was required. An average of 26.5 μA was required for control compared to 31.5 μA for TET nerves. Because of the small distances involved in doing such recordings in mice, stimulus artifacts are always apparent in nerve response records. In regard to stimulating current, it was noted that in response analysis the first 500 μsec segment (prior to response initiation under recording electrodes) contained verification of the higher currents required to elicit TET sciatic nerve responses. The preresponse segment contained an average of 6.11% of the total energy for TET nerves and only 3.10% for control nerves. Only the trailing edge of the stimulus artifact contributes to this measurement.

The distribution of response energy in the compound action potentials was different for TET and control nerves. More relative response energy appeared earlier in animals treated with TET. Overall, the peak response energy appeared 500 μsec earlier in the sciatic nerves of these animals compared to controls. Measurement of nerve conduction velocities based upon shortest suprathreshold response latencies (largest diameter fibers) revealed velocities of 13.4 m/sec for TET and 12.9 m/sec for control mice. These small differences in suprathreshold response velocity were comparable to the differences which occurred in both threshold and maximal response velocity comparisons. Thus, although the nerves of TET animals exhibited higher thresholds, they also exhibited reliably higher conduction velocities than the nerves of control animals.

In order to further evaluate such differences, the effects of acute TET administration were studied. In this way the direct effects of TET on nerve conduction could be assessed and the possibility that chronic TET effects might be contaminated with residual direct TET effects also could be assessed. First, mice pretreated with either TET or injection vehicle without TET were tested. To avoid CNS effects the sciatic nerves of half the mice in each group were severed from the spinal cord. Because of reports indicating TET inhibition of mitochondrial activity, high doses of sodium azide were given to an additional group of mice prior to sciatic nerve testing (nerves severed next to cord). In all cases a characteristic triphasic compound action potential was elicited by electrical stimulation. The mice pretreated with TET, however, exhibited a 75% increase in their mean response initiation threshold when contrasted with either vehicle control or sodium azide treated mice. This large

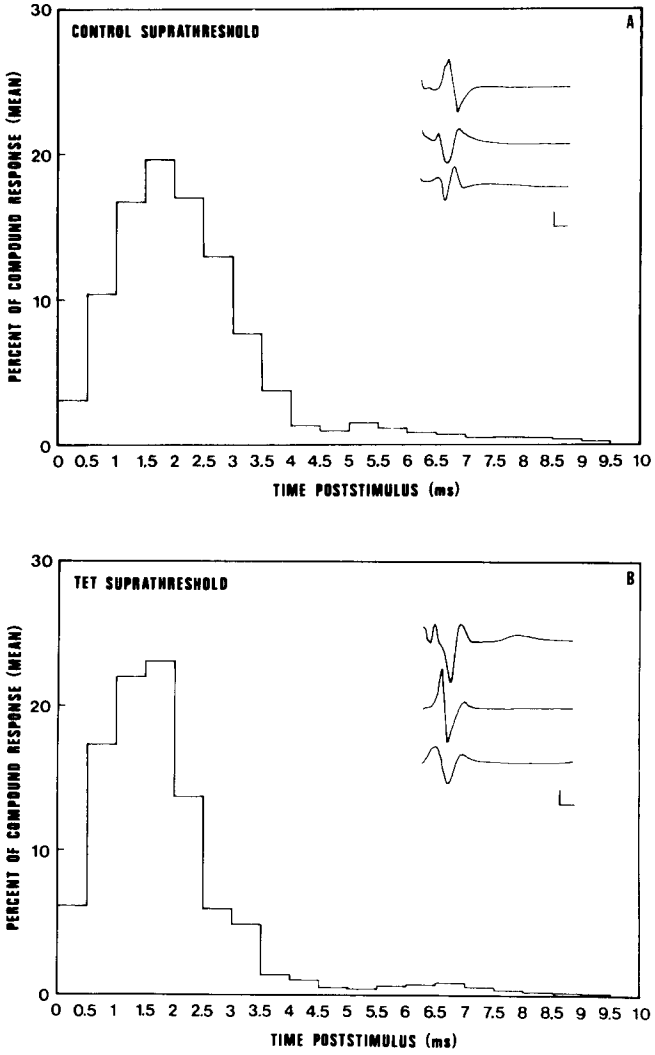

FIG. 2. The effects of chronic TET toxicity on evoked potentials obtained from mouse sciatic nerve. The percent of compound nerve response contained in each consecutive 500 μsec interval immediately following stimulus is plotted for averaged control (a) and TET (b) nerve responses obtained with suprathreshold stimulation levels. Upper right hand corner inserts within each block are representative evoked responses. (Calibrations: horizontal marks represent 1 msec; vertical marks represent 5 mV).

difference in response initiation threshold persisted when nerves had been surgically disconnected from CNS influences. Average large fiber conduction velocities are plotted (Fig. 3) for all pretreatment conditions and for all test conditions including threshold, suprathreshold, and maximal stimulation levels. Both severed and intact sciatic nerves of TET pretreated mice exhibited higher conduction velocities than observed in severed or intact vehicle control nerves. Whereas control nerves, severed or not, had comparable conduction velocities, the severed TET nerves showed notably slower velocities than intact TET nerves across all levels of stimulation. The conduction velocities of severed sciatic nerves of mice pretreated with sodium azide were very similar to the conduction velocities exhibited by the severed nerves of TET pretreated mice.

The CNS effects of acute TET pretreatments were also

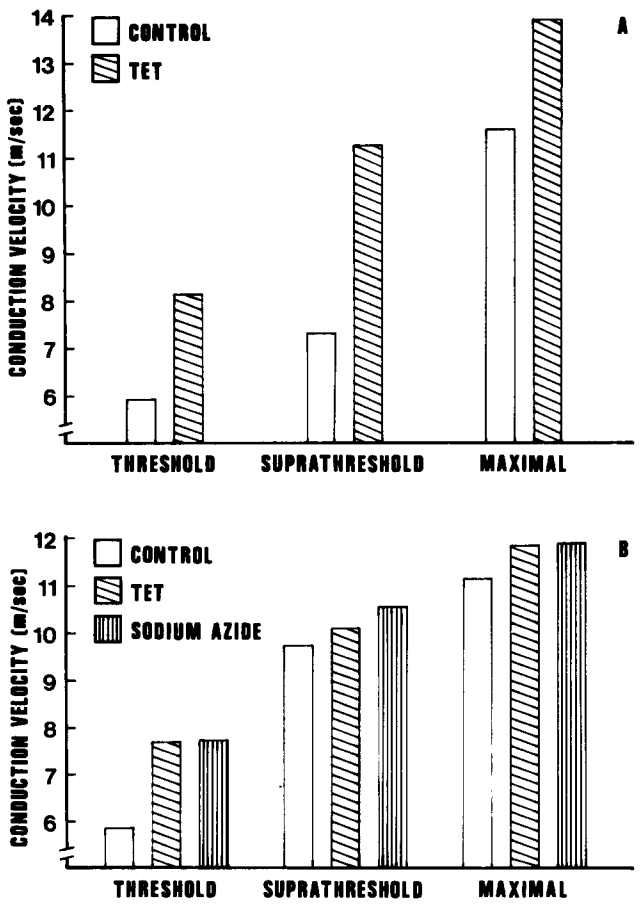

FIG. 3. The effects of TET on mouse sciatic nerve conduction velocities. Average conduction velocities were obtained from mouse sciatic nerves for threshold, suprathreshold and maximal levels of stimulation. Mice were pretreated with either TET, injection vehicle without TET or sodium azide 65 to 75 min before conduction velocity testing. The nerves were either left intact (a) or severed at the spinal cord (b) following electrode placement. All velocities were computed from the time of stimulus onset to that of response initiation. All nerves were kept at slightly lower temperature (36–37°C) than normal body temperature.

evaluated. Half of a group of chronically implanted mice received TET and the other half, injection vehicle without TET, shortly (2 min) before testing. Approximately two weeks later the tests were repeated in the same mice except all mice received the opposite pretreatment. The order of a particular type of pretreatment was counterbalanced and each animal served as its own control. Power spectra constructed from spontaneous EEG activity samples collected at standard periods of time following pretreatments were reliably different for TET as compared to control mice (Fig. 4). Within two minutes after injection the recordings from TET mice exhibited a major power peak at 5 Hz and those from saline mice a peak at 6–7 Hz. In addition, saline mice exhibited a peak of 40 Hz activity not exhibited by TET mice. The lower frequency activity in electroencephalograms of TET mice persisted for at least two hours across repetitive tests conducted every 30 min. When the mice were tested 24 hours later the power spectra of the TET and saline mice electroencephalograms were

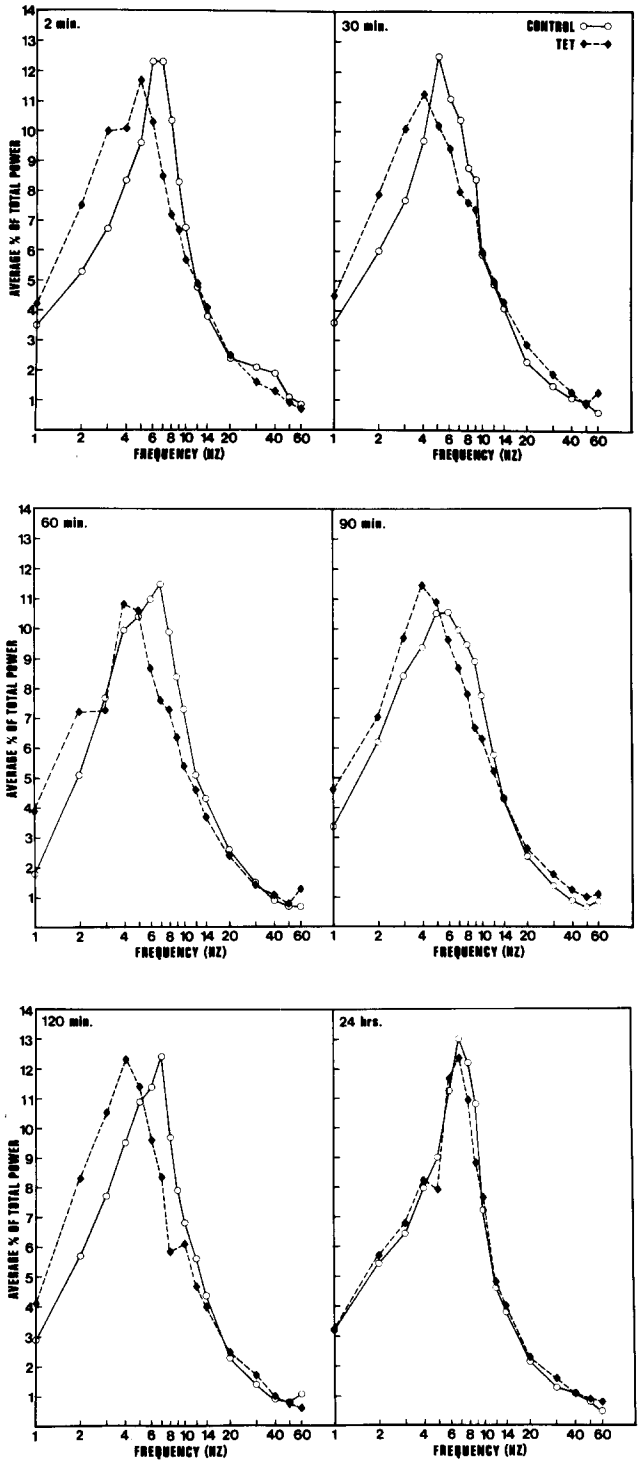

FIG. 4. The effect of acute TET toxicity on EEG frequency components. Power spectra are representative of the EEG activity obtained from mice 2 min, 30 min, 60 min, 90 min, 120 min and 24 hr following the administration of either TET or injection vehicle without TET.

indistinguishable with peak amounts of power at 7 Hz. Since each animal was tested twice and the two test periods were separated by two weeks it was possible to determine that the above reported effects were observed during both test periods and that no residual effects remained from the first to the second test.

During the recording of spontaneous EEG records, stabilimeter measurements were collected as an indication of gross locomotor activity. In all cases except the 24 hour test the TET animals exhibited significantly ($p<0.01$) less activity than control animals. The contribution of this behavioral characteristic to the brain recordings was minimized by brief periods of arousal elicited in all mice just prior to the collection of recordings for power spectral analyses. In the absence of such superimposed arousal the TET mice exhibited a dominance of even lower frequency activity in the brain recordings whereas control injected mice did not.

The averaged evoked responses elicited by light flashes during postinjection periods reflected the lowered frequencies of spontaneous brain recordings. Figure 5 shows some examples of these evoked responses. In all TET cases the earliest response component was elevated and the next component depressed in amplitude when compared to averaged evoked responses collected from control mice. Latencies measured from stimulus to the peak of the earliest response were longer in evoked potentials taken from TET pretreated mice than those taken from control animals.

Electrophoretic Analysis

Immediately after removal from anesthetized mice, the sciatic nerves of both chronically treated TET and control mice were weighed. Table 3 shows that the nerves did not vary significantly in terms of weight per unit length. The TET nerves averaged 0.29 mg per mm length and the control nerves averaged 0.28 mg per mm.

The nerves were digested for SDS-polyacrylamide gel electrophoretic examination, and equal quantities of recovered nerve proteins were resolved on a gradient polyacrylamide slab gel which varied from 7.5% to 18.75% in polyacrylamide concentration from top to bottom (Fig. 6). Subsequent densitometric analysis revealed no difference between the relative protein concentrations of nerves from TET as opposed to control treated mice.

Various subcellular fractions of mouse brain tissue were also subjected to electrophoretic analysis on the same type of slab gels (Fig. 7). Once again no major difference could be detected between samples originating from chronically treated TET as compared to control mice.

Finally, both peripheral nerve and brain tissues obtained from acutely treated TET mice were analyzed. The tissues were obtained at about one hour after mice were treated with lethal doses of TET (20 mg/kg). As might have been expected on the basis of earlier observations, none of the resulting electrophoretic patterns differed from patterns obtained from untreated or control mouse tissues.

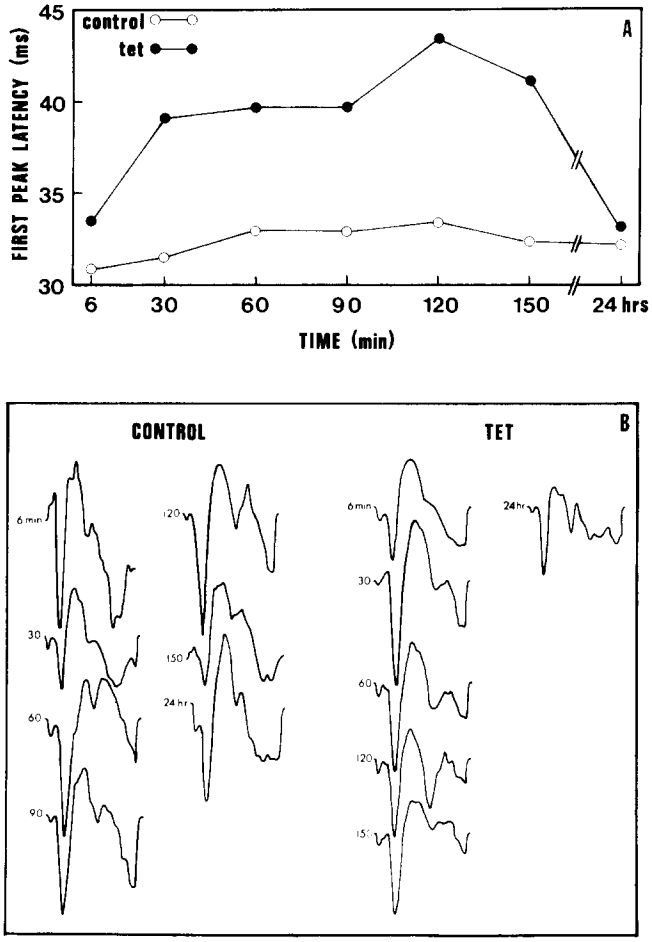

FIG. 5. The effect of acute TET toxicity on visually (light flash) evoked responses recorded from mice implanted with cortical electrodes. a. The latencies, measured from termination of stimulus to first peak, are provided for responses obtained at 6 min, 30 min, 60 min, 90 min, 120 min, 150 min and 24 hr following the administration of either TET or injection vehicle without TET. b. Some representative light flash evoked responses (250 msec window) obtained from a control mouse and a TET mouse.

TABLE 3

THE EFFECTS OF CHRONIC TET ADMINISTRATION ON PERIPHERAL NERVE WEIGHT.

Nerve	Mean Weight	Mean Length	Mean Weight Per Unit Length
Control	2.74 ± 0.68 mg	9.63 ± 1.37 mm	0.28 mg/mm
TET	2.89 ± 0.58 mg	9.88 ± 1.08 mm	0.29 mg/mm

Average weights ± standard deviations and average lengths ± standard deviations are provided for sciatic nerves removed from animals chronically treated with either TET or injection vehicle without TET. These were used to calculate the average weight per unit length of sciatic nerve for each group.

DISCUSSION

Both acute and chronic TET treatments induced significant functional alterations in the mouse nervous system. Mice treated with successive daily doses of TET as well as mice treated with only one dose of TET exhibited overall decreases in spontaneous locomotor activity. The chronic locomotor differences between TET and control mice disappeared during the course of simple avoidance training.

Direct electrical stimulation of sciatic nerves indicated that the nerves of TET-treated mice had higher thresholds

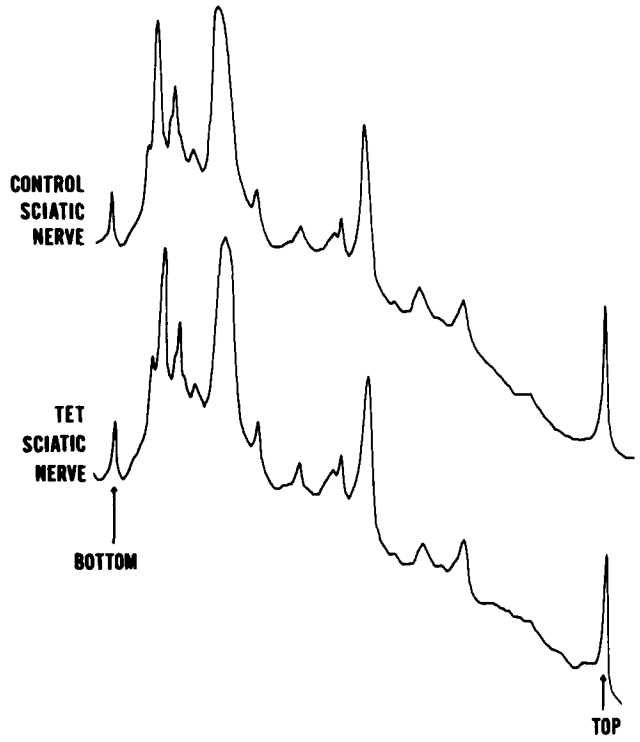

FIG. 6. The effect of chronic TET toxicity on mouse sciatic nerve protein composition. Microdensitometric scan patterns are shown for whole sciatic nerves obtained from mice chronically treated with either TET or injection vehicle without TET.

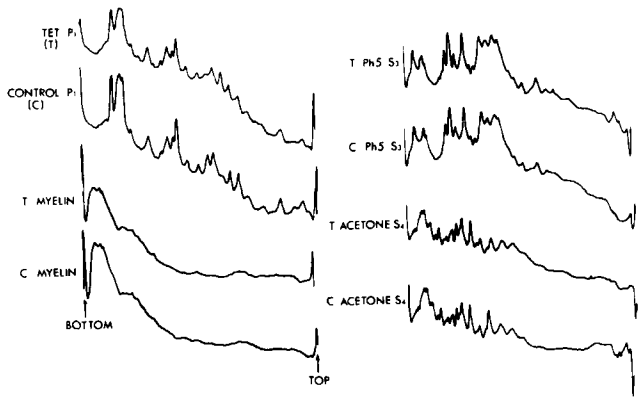

FIG. 7. The effect of chronic TET toxicity on mouse brain protein composition. Microdensitometric scans of electrophoretic protein patterns are shown for various mouse brain subcellular fractions obtained from mice chronically treated with either TET or injection vehicle without TET. The subcellular fractions shown are purified nuclei (P_1), myelin, pH 5 precipitate and soluble cell proteins.

than those of control mice. Once action potentials were initiated, however, conduction velocities were higher for TET as compared to control nerves. Response latencies for cortical evoked responses elicited by light flashes were longer for TET than control mice. Spontaneous electrical activity recorded from cortical electrodes contained significantly more low frequency activity when obtained from TET-treated mice than when obtained from control mice.

These behavioral and neurophysiological changes were not associated with subcellular CNS or whole sciatic nerve protein alterations as studied electrophoretically.

Previous studies [3, 14, 28, 29] which report a number of morphological changes cite a time course too long to explain many of the functional alterations observed in the present study. Both behavioral and neurophysiological changes can be observed soon after high, nonlethal doses of TET are administered. Many of these acutely produced functional alterations are similar to the functional alterations typical of chronically treated mice. The presumed consequence of TET on peripheral sciatic nerves also includes vacuolization [10], but again such morphological alterations are poor candidates for acutely produced changes in peripheral nerve thresholds and conduction velocities. It seems more likely that acute TET toxicity alters other physiological systems which affect both short-term neurophysiological measures and long-term morphological indices.

Some recent evidence exists suggesting that TET alters vascular membrane permeability [25,29]. It might be suggested that TET could, therefore, increase extracellular fluid and concomitant sodium concentrations by increasing vascular permeability. Such effects would be consistent with the observed effects of TET on peripheral nerve threshold and conduction velocity. Increased extracellular fluid accumulating from blood vessels immediately adjacent to nerve fasciculi also could account for eventual myelin vacuolization along intraperiod lines.

Both TET and sodium azide have been shown to effectively inhibit oxidative phosphorylation [5]. Since nerves treated with either TET or sodium azide have similar conduction velocities, it seems reasonably to assume that the mechanisms affected when oxidative phosphorylation is inhibited are at least partially responsible for the increase in nerve conduction velocity associated with TET intoxication. The threshold shift observed in TET intoxicated nerves but not in sodium azide intoxicated nerves, however, is probably a consequence of the membrane altering characteristics also believed to be associated with TET.

The present study indicated that the CNS and PNS behave quite differently during TET intoxication when examined neurophysiologically. As described above, the differences in the myelinic structures of the two systems [11, 22, 31, 32] very likely are not responsible for the neurophysiological anomalies present during TET intoxication. These differences are probably best explained by examining the possible sites of action of TET in both the CNS and PNS. Vascular membrane permeability, as described above, and oxidative phosphorylation inhibition are probably two important reasons for PNS neurophysiological changes during TET intoxication. Although CNS tissue also may be effected by these changes, other important sites of action may exist as a consequence of the known complexity of the CNS blood-brain barrier and functional characteristics of synapses.

TET treated animals have previously been used as experimental models of demyelination [27]. Specifically, TET intoxicated animals exhibit a large number of morphological, behavioral and electrical correlates with patients afflicted with Multiple Sclerosis (MS) or Peripheral Neuritis (PN). Morphological changes may be misleading since acute administration of either TET or MS serum, obtained from patients having MS [21], has been shown to alter normal electrical activity in neural tissues. Confusion arises from the fact that serum obtained from patients not having MS

has, in some cases, produced identical alterations of normal electrical activity [26]. EEG records taken from MS patients are composed predominantly of slow wave activity [9,23]. MS patients exhibit longer latencies in both auditory and visually evoked potentials recorded from the cortex [19,24]. These findings are in agreement with the longer CNS latencies and lower EEG frequencies observed in the present TET studies.

The behavioral symptoms of MS which result from the morphological and neurological anomalies are quite extensive [6,23] and include visual disturbances, gait ataxia, dysphagia, and general lack of motivational interest. One unusual aspect of the MS behavioral symptoms is the tendency toward alternating periods of remission and exacerbation. Animals receiving a single, relatively high dose of TET go through what might also be considered, on a brief time scale, an initial exacerbation followed by a short-term remission followed by a second exacerbation [29]. Alternating periods of long-term degenerative effects coupled with short-term physiological disturbances may explain some of the remission-exacerbation cycles common to both TET toxicity and MS.

The fact that TET treated mice exhibit little spontaneous locomotor activity, but perform as well as control animals when presented with an adequate impetus, may be simply a reflection of TET produced illness in the animals. It may, on the other hand, imply the presence of an activating system [18] involvement of the CNS. If increased threshold levels and synaptic decrements are characteristic of paths leading to or paths within the CNS following TET intoxication, some central reticular activating core of the brain may not receive adequate sensory collateral input. The observed reductions in spontaneous locomotor activity seen in TET intoxicated mice may arise from similar decreases in sensory input. It is tempting to suggest that some activating system decrement may be part of the MS etiology. The TET toxicity model appears promising for testing such a hypothesis of MS etiology. If supported, such a hypothesis may lead to more innovative and effective clinical treatments of MS type disorders.

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