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TITLE: EFFECTS OF METHYL BUTYL KETONE ON PERIPHERAL NERVES AND ITS MECHANISM OF ACTION.

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16. Abstract (Limit: 200 words)

The effects of methyl-n-butyl-ketone (591786) (MBK) and methyl-ethyl-ketone (78933) (MEK) on the peripheral nerves of rats were studied. Wistar-rats inhaled 60 to 1050 parts per million (ppm) of MBK or 1460 to 4740ppm of MEK for 6 hours per day, 5 days per week for 4 to 6 weeks; rats were exposed to MBK until overt signs of neuropathy were evident. Electrophysiological, biochemical, and morphological examinations were performed. No animals exposed to MBK or MEK developed overt neuropathy by 6 weeks. Clinical signs of neuropathy became noticable at 9 to 10 weeks of MBK exposure. All MBK treated rats developed neuropathy by weeks 11 to 13. No neuropathic effects were seen in rats exposed to MEK. The authors conclude that MBK causes neuropathy while MEK does not.

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The purpose of this project was to study the effects of methyl n-butyl ketone (MBK) and methyl ethyl ketone (MEK) on the peripheral nerves of rats by correlating the results of electrophysiological, biochemical and morphological studies. The major objective was to attempt to elucidate the pathogenesis of peripheral nerve lesions which might occur and to obtain clues how best to control or treat them. This is, therefore, the report of the results of the experiments on Wistar rats using MBK and MEK.

MATERIALS AND METHODS

Wistar rats weighing 100 g to 200 g at the start were used. They were kept in cages in the animal quarters, color-coded, and fed normal Purina Rat Chow and water which was withheld for both control and experimental groups during inhalation exposures. Ample food pellets were supplemented inside the cages of rats which had developed neuropathy. A total number of 225 Wistar rats were used, and 20 of these animals died of intercurrent or post-operative infection, or anesthesia. The individual weights were obtained immediately before and at the beginning of the inhalation exposure and serially thereafter. Initially, both male and female Wistar rats were used. Since there was no convincing evidence that either sex would react differently from each other with the ketones and our preliminary studies at least supported this, and in order to avoid aberrations in some results, e.g. the weight, that might ensue from physiological changes, e.g. pregnancy (although caged separately, both sexes were combined in the same chamber during exposure), only male Wistar rats were used in the subsequent studies. Serial clinical evaluations of all the rats were made throughout the entire experimental period.

Two Fisher gravity convection incubators (Blue M: enameled steel inner walls, inner glass door and nickel-plated shelves) were used as exposure

chambers. The insulating material was removed to facilitate better regulation of internal temperature which was kept at 21°C to 26°C. The internal oxygen and carbon dioxide concentrations, and relative humidity were kept at normal levels similar to external ambient conditions. The animals were kept inside the chambers for 6 hours daily, 5 days a week. The occasionally missed exposure days were made up by increasing the length of exposure time in the succeeding days until the total hours lost were recovered; the usual schedule was then resumed. Initially, the inhalation phase was pursued up to 4 to 6 weeks for MBK and MEK, but subsequently MBK exposure was continued until all the animals showed overt signs of neuropathy.

The air-ketone mixture was obtained by bubbling pure air into a gas washing bottle containing commercial grades of either MBK or MEK. The saturated air-ketone mixture was further diluted by pure air through a pair of flowmeters to obtain the desired concentration and which was then introduced through teflon tubing into the incubators.

In order that we might have a better understanding about how to set our flowmeter controls for the proper dilution of the ketones, we made certain theoretical considerations. With a vapor pressure of 10 mm at 20°C for MBK and 90.6 mm at 25°C for MEK, the theoretical saturation would be about 13,200 ppm for MBK at 20°C and 120,000 ppm for MEK at 25°C. Moreover, by knowing the flow rate of air bubbling through these ketones, and of the second diluting air, the concentration of each ketone in parts per million (ppm) could be estimated. For instance, if the flow rate (#1) of air bubbling through the gas washing bottle with the ketone was 538 ml/min, and the flow rate (#2) of the diluting air was 4850 ml/min, the theoretical concentration being delivered into the chamber would be about 1460 ppm. These computations were based on the following formula:

$$\frac{\frac{\text{Vapor Pressure (e.g. at 20°C}}{760} \times \text{Flow Rate \#1}}{\text{Flow Rate \#2}} \times 1{,}000{,}000 = \text{Theoretical Concentration (ppm)}$$

Initially, the actual concentrations of the ketones in the inhalation chambers were determined using radioactive labeled material. Through fragile and cumbersome one-way connections saturated air-radioactive ketone was obtained by bubbling air through known quantities of radioactive material, which was then diluted further with air as described above for the main set-up. We allowed 5 to 10 minutes for the radioactive material to equilibrate inside the exposure chamber, based on the known capacity of the chamber (about 18,000 ml^3) and the total flow rates at the time. Known amounts of air were drawn from different parts of the exposure chambers with an airtight syringe containing known quantity of scintillating counting solution. After shaking the fluid inside the syringe, both air and fluid were emptied into a vial with counting solution for further "trapping" of the radioactive ketone. With this method we obtained a fairly good correlation with the predicted concentration and showed that there was no significant difference in concentrations in various parts of the exposure chambers. Separate groups of animals were exposed to each ketone; no groups of rats were exposed to a mixture of both MEK and MBK. The concentrations used for each experiment were: 1) MBK 60 ppm, and MEK 2150 ppm for 6 weeks; 2) MBK 100 ppm, and MEK 4740 ppm for 4 weeks; and 3) MBK 1050 ppm and 1460 ppm for 5 weeks. Because of the inherent difficulties of the above technique (and changes in the circumstances then), gas liquid chromatography (GLC; HP-7160A) and SKC chemical detector tubes specific for ketones were used for final and definitive experiments.

In the GLC technique, MBK trapping was accomplished by bubbling the

air sample through a tube with a known amount of acetone which had been pre-cooled in dry ice. During the recalibration process we observed that the mean (± SEM, (SD)) MBK saturation was only 6506 ± 400 ppm (SD, 1060 ppm); for some undetermined reason this value was lower than what was obtained initially with the previous method. Nevertheless, we were able to test the predicting value of the above formula (Table 1).

The sampling technique for the GLC was satisfactory for the inlet of the exposure chambers, with an average concentration of 2027 ± 54 ppm (SD, 541 ppm). However, the method employed in sampling from within the chamber was not too satisfactory. Therefore, for this purpose, we used the SKC chemical detector kit for ketones. The ketone was trapped inside a calibrated disposable glass tubing in which one end was inserted into the chamber and the other connected to a hand pump. A chemical indicator inside the calibrated tubing gave a yellow color for ketones which could be quantitated with an error of ± 20%. Outside the exhaust hood where the chambers were located the ambient air was negative for ketones by this technique while exposure was in progress. The average concentration within the chambers was 1480 ± 12 ppm (SD, 165 ppm) for the final experiments.

ELECTROPHYSIOLOGY

The technique has been described previously (1 - 4). Briefly, both control and experimental rats were anesthetized before the electrophysiological study with intraperitoneally administered pentobarbital sodium (0.01 to 0.04 mg/g body weight, repeated in smaller doses during the study as necessary). One sciatic nerve was surgically exposed and electrically stimulated at the sciatic notch (proximal) and at the popliteal fossa (distal). The threshold and supramaximal responses of the triceps surae muscle were recorded. The following parameters were determined quantitatively: current at threshold and supramaximal stimulation, proximal and distal

latencies (motor nerve conduction velocity (MNCV) = distance between the two points of stimulation divided by the difference of latencies) and the amplitude of the evoked muscle action potential (EMAP). Throughout the conduction study, the tissue temperature was maintained at 35°C to 36°C with a thermoregulating unit.

In serial studies, the nerve contralateral to the initial one examined was used; only one conduction study was performed on each nerve. Results of studies of the controls and of the experimental animals were pooled and compared by Student t test for statistical significance.

BIOCHEMISTRY

The nerves of control and experimental animals were pooled and homogenized as previously described (1,5). Analysis was performed in duplicate aliquots. Myelin was prepared by a minor modification of the method of Norton and Poduslo (6). Sulfatide was assayed by colorimetric method of Kean (7) and unesterified cholesterol by the colorimetric method of Rudel and Morris (8).

Myelin protein was determined by the method of Lowrey et al (9) after overnight incubation in 0.5 N NaOH at 37°C. Whole nerve collagen was estimated by multiplying the nerve hydroxyproline content (10) by a factor of seven which was derived by assuming that nerve collagen is one-half basement membrane with a weight ratio of hydroxyproline to total amino acids of about 1:6, and one-half interstitial tissue with a weight ratio of hydroxyproline to total amino acids of about 1:8 (11). Total nerve content of amino acids was determined by the ninhydrin method (12).

MORPHOLOGY

Routine postmortem examination of the central nervous system was performed on two rats exposed to MBK for 15 weeks showing moderate to severe

clinical signs of neuropathy, and on two controls. The animals were euthanized by sodium pentobarbital anesthesia followed by perfusion with glutaraldehyde in one comparable pair and formalin in the other pair. Sections of the cerebral cortex, hippocampus, diencephalon, cerebellum, medulla, and cervical, thoracic and lumbar spinal cord were obtained and stained with H & E, Nissl, and Myelin stains.

The sciatic and posterior tibial nerves, and the intramuscular nerve twigs to the gastrocnemius of 12 controls and 17 MBK rats were carefully dissected under a dissecting microscope and then fixed in situ with 3.6% buffered glutaraldehyde in phosphate buffer for 10 minutes. After excision, the nerves were further fixed in glutaraldehyde, and then washed in 0.1 M phosphate buffer at a pH of 7.5. The nerves were then post-fixed with osmium tetroxide for 2 hours, dehydrated, embedded in epoxy, hardened and sectioned onto glass slides for light, phase and electron microscopy (13-14). Additional segments were placed in glycerin for single fiber teasing.

RESULTS

CLINICAL

In the first experiment, 10 controls (5 males and 5 females) were weighed, baseline electrophysiological studies were performed, and then the animals were sacrificed in order to obtain tissue samples for baseline biochemical and morphological studies. Three other groups of Wistar rats were identified numbering 10 per group with both sexes equally represented in each group: one group was exposed to 60 ppm of MBK, another to 2150 ppm of MEK, and the last served as parallel control. The animals exposed to MBK or MEK did not develop overt signs of neuropathy after 6 weeks on inhalation exposure. All the control and experimental animals gained weight steadily, and

the group mean weights were not significantly different from one another (Table 2). The mean weights of the male and female rats in the experimental group did not differ significantly from the controls. The difference in body weights between the male and female rats was due to the high initial weights of the males and not due to sex specific effect of MBK or MEK.

Similar negative clinical results were obtained using 90 ppm of MBK and 4740 ppm of MEK for 4 weeks. No further experiments were performed with MEK. Only male rats were used in this and subsequent experiments.

In the third experiment, 2 concentrations of MBK (1050 ppm and 1460 ppm) were studied for 5 weeks. Several control and experimental animals succumbed to an intercurrent infection. Of those that survived (Table 3), no overt clinical signs of neuropathy were apparent at 5 weeks. Although the weights of the experimental animals were not significantly different from the controls, the former seemed to have either lost some weight, or gained less than the latter. Moreover, the experimental animals were visibly smaller than the controls. The intercurrent illness in this group might have altered the actual weight difference between the controls and experimental groups, since, as will be shown subsequently, the weight difference between the control and experimental groups has been shown several times to be statistically different at about 5 weeks of exposure or earlier. Because the MNCV of both these experimental groups were decreased (see below) without clinical signs of neuropathy, the immediate needs at this point dictated that serial studies be performed until all exposed animals became symptomatic, and then again until all symptomatic animals have become completely asymptomatic.

In the fourth experiments 4 groups of rats were exposed to MBK (1480 ppm) in two serial batches. The development of overt signs of neuropathy during the exposure phase, and the ultimate recovery from these signs were consistent in all groups.

The initial objective change was the diminished gain in weight of the MBK group compared to the controls (Fig. 1), which was significant as early as the second week of exposure (Fig. 2) ($p<.05$). This difference in weight between the control and MBK animals progressively became more prominent as the controls gained more weight and the MBK rats gained less weight with continued exposure until the 15th week. The MBK rats were visibly smaller than the controls at 5 weeks of exposure, correlating with the decrease of gain in weight in the former. The emaciation of the MBK rats progressed steadily during the exposure phase of the experiment with the muscle wasting becoming more manifest in both hindlimbs in the last 4 to 5 weeks of exposure when persistent overt signs of neuropathy were also present. During the immediate post-exposure (recovery) phase, the MBK animals actually lost weight (Fig. 1,2), which could not be attributed to the effects of surgery for electrophysiological testing since the controls were only minimally affected by the procedure and since the unoperated MBK animals showed the same pattern as the operated group. This weight loss in the immediate post-exposure phase could only be attributed to an inherent effect of MBK. Thereafter the weights of the MBK rats increased towards normal (Fig. 2) so that the weight difference between the control and MBK groups was no longer significant after the 7th recovery week. The muscle bulk of the hindlimbs progressively increased but was not visibly comparable to the normal controls during surgery until the 15th recovery week.

At the beginning of the inhalation experiments, the rats were somewhat unsteady and drowsy, as if "drunk", when examined soon after termination of exposure at the end of the day. These signs would clear up immediately, and certainly by the next morning the animals were again normal prior to the exposure regimen. One batch of rats developed a "hyperstartle response" to trivial sound stimuli, transiently appearing immediately after each exposure, and altogether disappearing completely after the 10th week or so of exposure. This sign did not occur in the second batch.

The earliest time when clinical signs of neuropathy were barely noticeable in some rats was on the 9th to 10th week of exposure. By the 11th to 13th week, all the MBK rats showed overt signs of neuropathy, and there was worsening of the signs in the rats which developed neuropathy earlier. In all, the clinical signs of neuropathy were progressive until the termination of exposure to MBK on the 15th week; in some progression was more rapid than others. The earliest signs noticeable were impairment of gait and tendency to fall one way while the cage was moved the other way. There was poor paw control in the forelimbs while eating, and in the hindlimbs there was poor positioning of the paws on walking which progressed later on to "heel-walking". Initially, weakness of the proximal muscles of the hindlimbs was suggested by a certain "waddle" in the gait, followed by "hopping" locomotion, and then paraparesis (e.g. difficulty supporting weight with the hindlimbs while attempting to reach up for food). Some animals developed virtually complete paraplegia (dragging the hindlimbs), with overt paresis of the forelimbs. The muscle weakness seemed to be flaccid and worse distally, (e.g., forepaws were floppy on walking, sometimes the rats were stepping with the dorsum or side of the paws). All the animals demonstrated the "tail drop sign", decreased or delayed response to a tail pinch, and some showed diminished or

delayed avoidance reaction to threat.

After the termination of exposure to MBK on the 15th week, the clinical signs continued to progress for the next 2 to 3 weeks in the immediate post-exposure phase; for the first time paraparesis became evident in some rats, and in others with previous paraparesis, paraplegia ensued at this time. The clinical worsening of the MBK rats in the immediate post-exposure phase correlated with loss of weight (Fig. 1,2). This weight loss in the MBK group was more than what the operated controls showed; the operated controls were clinically normal in the 2nd to 3rd postoperative day. Moreover, the unoperated MBK animals not only showed progression of the clinical signs of neuropathy but also demonstrated weight loss similar to the operated MBK animals, thereby indicating that this phenomenon was probably an inherent part of the disorder caused by MBK and that the role of the effect of surgery was at best minimal.

From the 3rd to the 4th recovery weeks, the MBK rats progressively gained weight (Figs. 1,2) and the clinical signs of neuropathy steadily resolved. By the 9th to 10th week of recovery, half of the MBK group had completely recovered clinically, and the other half showed only minimal motor signs, i.e., mild awkwardness in gait and tendency to hop irrespective of whether or not surgery was performed previously. The muscle bulk of the thigh appeared slightly smaller than the controls during surgery for electrophysiological studies at the 10th post-exposure week. By the 12th to 13th post-exposure week, clinical recovery was complete in all MBK rats, and the muscle bulk during surgery on the 15th week seemed grossly comparable in size to the controls.

ELECTROPHYSIOLOGY

The supramaximal current was 10 to 40 times the threshold current, with

the amount of current delivered to the nerves of control animals comparable with the experimental groups (Tables 4-7).

In the first experiment (Table 4), the MNCV and the EMAP amplitudes of the MBK (60 ppm) and MEK (2150 ppm) rats were not significantly different from the controls. The male and female values for these parameters within, and between, each group were essentially similar, suggesting that neither sex was unduly sensitive to MBK or MEK at the dose and length of time these chemicals were given. A further increase in the dosage of MBK and MEK at slightly less exposure time (Table 5) did not produce any significant change in the EMAP or MNCV from the controls.

At this point, we concluded that further experiments with MEK would be fruitless. Therefore, in the third experiment, we used two higher concentrations of MBK (Table 6). The results indicated that although the EMAP amplitude had not changed significantly from the controls, the MNCV of both MBK groups had decreased significantly ($<.001$). This was our first positive result. Therefore, we aimed at studying animals exposed to adequate dosage of MBK serially as they became symptomatic and later, as they recovered from their neurological deficits.

The results of the serial nerve conduction studies performed on the animals exposed to an ambient MBK concentration of 1480 ± 12 ppm (SD, 165 ppm), until all the rats were symptomatic and later recovered, are summarized in Table 7. The MNCV of the MBK rats clearly decreased from the 5th week of exposure until the 15th week when exposure was terminated (Fig. 3). In the post-exposure (recovery) phase it increased gradually so that by the 15th recovery week it was back to within normal limits. In order to further ensure that the decline in MNCV during the inhalation exposure was unrelated to changes which might have occurred with age in the controls, and to the

differences in weights of the control and MBK animals, the Pearson's r correlation coefficient of the MNCV (56.5 to 77.5 m/sec) and the corresponding weights (108.5 g to 577.5 g) of 69 control animals, studied at several points in time during the exposure phase of MEK and MBK experiments, was 0.2130. Its critical value was not significant at the .05 alpha significance level, indicating that the decreese in MNCV of the MBK animals was due to the pathologic effect of MBK and that any changes in the MNCV of the controls with age did not contribute significantly to the differences between the MNCV of the control group and MBK animals.

The EMAP amplitude decreased slightly later in the course of exposure (Table 7), 5 weeks after the MNCV had decreased. This decrease in EMAP amplitude tended to improve in the recovery phase but was still significantly lower than the controls at a time when the MNCV was within normal limits and when the MBK rats seemingly had shown full clinical improvement from the MBK. This observation suggested one or a combination of the following: 1) permanent loss of entire motor units; 2) full functional re-innervation of the previously denervated muscle fibers had not yet occurred: 3) that re-innervation had occurred but because of the immaturity of the nerve sprouts synchronization was still to occur.

BIOCHEMISTRY

The results of the biochemical studies of the posterior tibial nerves of MBK rats were not significantly different from the controls (Table 8). The normal sulfatide and unesterified cholesterol would indicate that there was no significant demyelination, the normal esterified cholesterol would point to insignificant myelin degeneration, and the normal amount of collagen would mean that there was no significant scarring at 10 and at 15 weeks of exposure. Moreover, in the presence of definite morphologic changes at

these times, these normal data would suggest that the biochemical studies might not have been sensitive enough to distinguish subtle changes which could have resulted from spotty morphological alterations. No biochemical studies were carried out in the rats exposed to MEK since there were no neurological signs and there were no pathological changes in the peripheral nerves.

MORPHOLOGY

Light microscopic examination of sections of the cerebral cortex, hippocampus, diencephalon, cerebellum, medulla, and cervical, thoracic and lumbar spinal cord stained with H & E, Nissl, and myelin stains showed no pathologic changes in 2 symptomatic MBK animals at 15 weeks compared to 2 normal controls.

The sciatic, posterior tibial, and lateral plantar nerves of three animals in each of the control, MBK and MEK groups of the first experiment were sampled. The two micron plastic sections of these nerves were normal by phase microscopy.

The evolution of the histological changes (Fig. 4) in the peripheral nerves of rats exposed to 1480 ppm of MBK were as follows:

A. EXPOSURE PHASE

5 weeks: mild abnormalities were observed equally in the sciatic and tibial nerves which included irregularities and increased inpouchings of myelin sheath, and minor paranodal swelling and splitting of myelin lamellae.

10 weeks: sciatic and tibial nerves still equally involved; above abnormalities persisted with somewhat more myelin wrinkles and occasional giant axons.

15 weeks: sciatic nerve somewhat more affected than tibial nerve; focal and paranodal axonal swelling were more prominent, myelin infolding had increased, and there was mild amount of myelin debris in all nerves.

B. RECOVERY PHASE:

10 weeks: sciatic nerve definitely more involved than tibial nerve and pathological changes were more obvious than 15th week of inhalation; giant axons were grossly more abundant with endoneurial edema in some fascicles with intact perineurium, myelin debris was moderately abundant; for the first time, myelinated fibers were reduced in number - the fiber loss in a given fascicle seemed localized to discrete areas sparing surrounding fibers, and some clusters of tiny myelinated nerves (regenerating nerve sprouts) were scattered.

15 weeks: same as above; the clusters of tiny nerves had increased in number.

DISCUSSION

When the project began in the latter part of 1974, the published material about MBK and MEK was meager and it did not tell the whole story. Since then, several clinical and experimental studies have appeared. The results of our studies confirm the report of others that MBK causes neuropathy (15-19) and that MEK alone does not (18,19). One group (19) reported that when MEK (1125 ppm) was combined with MBK (225 ppm) at a ratio of 5:1 and given continuously by inhalation to rats, clinical paralysis appeared earlier than when MBK was used alone, and the histological changes were also enhanced. Another group (18) gave 150 mg/kg body weight of a 9:1 mixture of MEK and MBK to cats twice daily by subcutaneous injection and showed that the animals did not develop clinical neuropathy and that the morphological changes in the nerve appeared proportional to the MBK concentration used, although the possibility that MEK might have enhanced MBK toxicity was not entirely ruled out.

In 1974, Mendell et al (16) showed that several species of experimental animals exposed continuously to MBK via inhalation route developed peripheral neuropathy. The interval from the onset of exposure to the appearance of clinical neuropathy varied with the species used; the interval was 4 to 5 weeks in chickens, 5 to 8 weeks in cats and 11 to 12 weeks in rats. They further showed that nerve conduction velocity in the cats decreased from an average of 115 to 50 m/sec, that changes compatible with denervation were obtained in EMG of some muscles, and that histologically, there were focal axonal swelling associated with thin myelin, accumulation of neurofilaments with loss of neurotubules in the swollen axon, and focal areas of paranodal demyelination with the myelin breakdown products frequently observed in the Schwann cell cytoplasm. Essentially similar histological changes were observed by Duckett et al (15) using the sciatic

nerves of rats exposed intermittently to 200 ppm of MBK for 6 weeks by inhalation.

In 1975, Spencer et al (17) systematically studied both peripheral and central nervous system of severely symptomatic rats exposed to 1300 ppm of MBK by inhalation for 4 months given 6 hours daily and 5 days weekly. They showed morphologically that the most prominent nerve fiber abnormality in these rats was axonal dilatation with localized fiber swelling which occurred anywhere along the nerve fiber, the paranodal swelling being associated with secondary myelin retraction; there were occasional ovoid formation and evidence of advanced stage of degeneration in other fibers. These changes were most apparent in the intramuscular and distal portions of all peripheral nerves although scattered changes could be seen anywhere along the nerve up to the level of the posterior root ganglia. In the central nervous system similar changes were observed initially in the distal segments of nerve tracts, spreading proximally at later stages (17,18); occasional anterior horn cells were abnormal in the lumboscral spinal cord (17). Because of the characteristic spatial-temporal distribution of this disease which produced symmetrical signs, they designated this condition as "central-peripheral distal axonopathy" (18).

In 1976, Saida et al (19) showed by quantitative techniques that the neuropathological signs preceeded the clinical signs by several weeks. The earliest changes were the increase in the number of neurofilaments and the inpouchings of the myelin sheath; the former was associated with a decrease in neurotubules, and the neurofilaments increased with time, leading to the development of axonal swelling and secondary thinning of myelin sheath. The results of their study did not reveal any predilection of the early changes at the axon terminal, and the neuromuscular junction showed abnormalities only after a full spectrum of changes had been

observed in the main nerve trunk, nerve roots and intramuscular nerves. They did not observe any changes, including chromatolysis, in the anterior horn cells and dorsal root ganglion. However, the suggestion that the increased neurofilaments with decreased neurotubules which appeared initially in the main nerve trunk and later in the distal nerve twigs (19) and the implications of the term "central-peripheral distal axonopathy" may not be mutually exclusive, that is, both may represent epiphenomena of a disturbed axonal transport mechanism with MBK (20-25) but this remains to be demonstrated. Thus, the ultimate mechanism that leads to the development of MBK neuropathy is still unsettled.

In the last few years, MBK has gained notoriety as a cause of peripheral neuropathy in man as a result of an outbreak of this condition in 1973 in a coated fabrics factory in Ohio (26). MBK neuropathy has been reported since then among spray painters (27). The clinical picture and course, decrease in nerve conduction velocity and "giant axonal" change, are fairly similar in MBK neuropathy as in the neuropathy due to glue sniffing (28-33) variably named with such exotic terms as "giant axonal" (32) or "huffer's" (29) neuropathy. The various adhesive compounds on the market are usually made up of several agents in various proportions but the two most significant components which are thought to produce the neurotoxic effects of glue are toluene and n-hexane (28,30). Toluene predominantly causes variable picture of central nervous system degeneration (34,35); there is only one case reported with neuropathy attributed to toluene (36). On the other hand, n-hexane causes a picture which is predominantly due to its peripheral neurotoxic effects even though there may be concomitant central nervous changes (37); as the peripheral neuropathy recovers, hyper-reflexia and spasticity may then become manifest for the first time (31), and these signs are probably due to the degenerative

effects of n-hexane in the central nervous system where regeneration is less likely to occur.

MBK and n-hexane may have something in common in the production of a neuropathy which appear to have similar clinical and pathological features. Spencer and Schaumburg (38) have shown that the major water soluble metabolite of MBK in experimental animals, 2, 5-hexanedione, produces identical neurotoxic effects as MBK. The chemical agent 2, 5-hexanedione is also a major metabolite of n-hexane in guinea pigs (39). Thus, MBK and n-hexane neuropathy may have a common metabolic origin. In any event, the ultimate biochemical substrate of the pathogenetic mechanism of MBK toxicity remains to be determined.

There is no question, therefore, about the neurotoxicity of MBK. The morphological changes have been intensively investigated and although there might have been differences in methods, experimental designs, morphological parameters and experimental animals used, several consistent points have emerged: 1) that the morphological changes antedate the development of clinical signs, 2) that the appearance of clinical signs are determined by the inverse relationship of the dose and the length of time of exposure, and 3) that the common denominator is the development of "giant axonal" neuropathy. Although there is considerable information about the morphological changes during the period of intoxication (15-19), and except for the single cat which was allowed to recover for 4-1/2 months (19), the evolution of the morphological changes during the recovery phase has not yet been studied systematically. It would seem that information gained during the recovery phase could give more precise meaning for the changes observed in human nerve biopsies which are ordinarily obtained several weeks or months after discontinuation of the intoxicating material, and could explain some of the differences observed such as predominance of

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axonal loss in some reports and of "giant axonal" change in others, etc. (15-19,28,30-33).

Another parameter which has not been investigated systematically is the evolution of the electrophysiological abnormality during the intoxication and recovery phases, especially in relation to the evolution of the clinical and morphological pictures. This method could be helpful in giving objective assessment of the clinical course and possibly some insight as to the relative involvement of the axon, myelin sheath, or both. To date there has not been any systematic biochemical study of the nervous system in MBK neuropathy; however, our results did not give any clue as to the basic biochemical mechanism of the disorder.

Our results clearly showed the following: MBK was neurotoxic to the peripheral nerves and MEK was not; both electrophysiological and morphological changes antedated the appearance of clinical signs of neuropathy; the MNCV progressively decreased until the end of inhalation exposure and and then gradually normalized in parallel with the regression of clinical signs; EMAP amplitude decreased at a later date than MNCV and showed only partial recovery; the clinical signs worsened transiently for 2 to 3 weeks in the initial post-intoxication period before steady recovery took place; and the morphological changes increased during the intoxication phase and progressed some more during the recovery phase reaching its maximum change on the 10th recovery week, at a time when the animals were only minimally affected clinically and the MNCV had shown some improvement; the biochemical studies were normal. Since the biochemical studies were normal in the clinically involved animals it was considered that further investigation, i.e., in vivo radioactive MBK incorporation, would not be scientifically fruitful.

The clinical worsening immediately after removal of the intoxicating

agent prior to steady recovery is a well known clinical phenomenon in intoxications with several industrial agents (26,28,31). The temporal differences of our result in rats from that of Allen et al (28) in humans might be due to species differences (16). Moreover, Allen et al (28) reported that the electrical phenomena worsened before showing steady improvement; we did not study the MNCV of our rats at the time this phenomenon occurred but subsequently our data is compatible with theirs.

The interesting morphological observation in this study is the progression of the histological changes until the 10th recovery week when about half of the animals had recovered and the other half showed only minimal signs. It is possible that either MBK or its metabolite 2, 5-hexanedione may remain in the tissue for a long time after cessation of the intoxication process thereby maintaining the toxic effect on the nerves. This seems not likely since clinical and electrical improvement were evident at this time. It is conceivable that this delayed and somewhat paradoxical appearance of the severe morphological abnormalities may be somehow related to the disturbed axonal flow mechanisms; nevertheless, this is speculative and remains to be proven. The other important morphological observation is the absence of signs of nerve sprouting during the intoxication phase; only minimal regenerative changes were observed 10 weeks after cessation of MBK exposure when recovery was well under way. The evidence of Saida et al (19) of increasing neurofilaments early in the toxic state with decreasing neurotubules, the evidence of Spencer and Schaumburg (18) for their "central-peripheral distal axonopathy", and our present evidence of lack of regenerative capacity of the nerve during intoxication with MBK would fit very nicely within the concept of "dying-back" phenomenon (40). It would make sense for the nerve fiber to be unable to send off sprouts to reinnervate denervated muscle fibers while the main axon is still exposed to MBK toxicity. Thus, the absence of signs of regenerative capability

while the toxic process is still in progress seems therefore to be an implicit requisite of "dying-back" phenomenon, and our result would be supportive of this concept.

In the ultimate analysis, the available major morphological evidence in MBK neuropathy indicates axonal degeneration. The explanation given for the decrease in nerve conduction velocity in axonal neuropathies has been to invoke the notion of secondary demyelination, or at least a secondary myelin change (41). In this context, the clinical neuropathy ought to be far advanced before the MNCV decreases. However, in MBK neuropathy of rats we obtained an early decrease in MNCV. Nerve conduction velocity decreases when the myelin sheath is altered biochemically or physically (1-4, 42-44) so that internodal capacitance and transverse resistance are modified; paranodal myelin change or demyelination may be more effective in slowing nerve impulses than total internodal demyelination (44). It is possible that the myelin inpouchings and splitting of myelin lamellae early in the course of MBK neuropathy can explain the decreased MNCV. The current evidence is that the early increase of neurofilaments (19) may not be directly related to the alteration of nerve conduction (4). Selective loss of large myelinated fibers (30) may not be an adequate explanation for the decreased MNCV, because when this finding was observed in our recovering rats, the earlier decrease in MNCV had begun to improve, the MNCV was most severely impaired at a time when such loss was not evident, and the MNCV progressively normalized despite the evidence of axonal loss. Whether or not the affected but seemingly intact axon conducted abnormally because of alteration in its ionic content or flow characteristics is only speculative; Baker et al (45) have shown that replacement of internal potassium by glucose resulted in increased internal resistivity of the axon with a resultant decrease in conduction velocity.

Regardless of the explanation for the alteration of the MNCV, it remained to be the closest parallel of the clinical progression and recovery. As for the axonal change, the better electrical correlative was the alteration of the EMAP amplitude. There was some suggestive improvement on the 10th week of recovery but it still remained significantly lower even at 15 weeks when clinical recovery appeared complete.

SUMMARY

MBK produced peripheral neuropathy in rats; MEK did not. Both electrophysiological and morphological parameters, and the decreased weight gain, antedated the appearance of clinical signs of neuropathy. The MNCV progressively decreased until the end of inhalation exposure and then gradually normalized thereafter in parallel with the regression of clinical signs. The EMAP amplitude decreased at a later time than the change in MNCV but the amplitude did not normalize with full clinical recovery. The clinical signs worsened transiently for 2 to 3 weeks in the initial post-intoxication phase before steady recovery occurred. The morphological changes increased during the intoxication phase and progressed some more during the recovery phase reaching its maximum on the 10th recovery week when one-half of the animals were completely recovered and the other half showing only minimal clinical deficits and the MNCV had shown some improvement. The biochemical studies did not show any evidence of demyelination, Wallerian degeneration or fibrosis.

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REFERENCES

1. deJesus PV, Pleasure DE: Hexachlorophene neuropathy. Arch Neurol 29:180-182, 1973.
2. deJesus PV, Clements RS Jr., Winegrad AI: Hypermyoinositolemic polyneuropathy in rats, a possible mechanism for uremic polyneuropathy. J Neurol Sci 21:237-249, 1974.
3. Greene DA, deJesus PV, Winegrad AI: Effects of insulin and dietary myoinsoitol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes. J Clin Invest 55:1326-1336, 1975.
4. Graham DI, deJesus PV, Pleasure DE, Gonatas, NK: Triethyltin sulfate-induced neuropathy in rats; electrophysiologic, morphologic and biochemical studies. Arch Neurol 33:40-48, 1976.
5. Pleasure DE, Towfighi J: Onion-bulb neuropathies. Arch Neurol 27:289-301, 1972.
6. Norton WT, Poduslo SE: Myelination in rat brain: method of myelin isolation. J Neurochem 21:749-757, 1973.
7. Kean EL: Rapid, sensitive spectrophotometric method for quantitative determination of sulfatides. J Lipid Res 9:319-327, 1968.
8. Rudel LL, Morris MD: Determination of cholesterol using o-phthaldehyde. J Lipid Res 14:364-366, 1973.
9. Lowry OH, Rosenbrough NJ, Farr AL, et al: Protein measurement with a folin phenol reagent. J Biol Chem 193:265-275, 1951.
10. Prockop D, Udenfriend S: A specific method for the analysis of hydroxyproline in tissues and urine. Anal Biochem 1:228-239, 1960.
11. Kefalides N: Structure and biosynthesis of basement membrane. Inf Rev Conn Tissue Res 6:63-104, 1973.
12. Spies J: Colorimetric procedures for amino acids. Methods Enzymol 3:467-477, 1957.

13. Asbury AK, Picard EH, Baringer JR: Sensory polyneuritis. Arch Neurol 26:302-312, 1972.

14. Asbury AK, Gale MK, Cox SC, et al: Giant axonal neuropathy - a unique case with segmental neurofilamentous masses. Acta Neuropath (Berl) 20:237-247, 1972.

15. Duckett S, Williams N, Francis S: Peripheral neuropathy associated with inhalation of methyl n-butyl ketone. Experientia 30:1283-1284, 1975.

16. Mendell JR, Saida K, Ganansia MF, et al: Toxic polyneuropathy produced by methyl n-butyl ketone. Science 185:787-789, 1975.

17. Spencer PS, Schaumburg HH, Raleigh RL, et al: Nervous system degeneration produced by the industrial solvent methyl n-butyl ketone. Arch Neurol 32:219-222, 1975.

18. Spencer PS, Schaumburg HH: Feline nervous system response to chronic intoxication with commercial grades of methyl n-butyl ketone, methyl isobutyl ketone, and methyl ethyl ketone. Toxicol Appl Pharmacol 37:301-311, 1976.

19. Saida K, Mendell JR, Weiss HS: Peripheral nerve changes induced by methyl n-butyl ketone and potentiation by methyl ethyl ketone. J Neuropath Exp Neurol 35:207-225, 1976.

20. Weiss P: Self-renewal and proximo-distal convection in nerve fibers, In E. Gutmann and P. Hnik (editors): The Effect of Use and Disuse on Neuromuscular Functions. Publishing House Czech Acad Sci, Prague 1963 pp 171-183.

21. Pleasure DE, Mishler KC, Engle WK: Axonal transport of proteins in experimental neuropathies. Science 166:524-525, 1969.

22. Jeffrey PL, James KAC, Kidman AD, et al: The flow of mitochondria in chicken sciatic nerve. J Neurobiol 3:199-208, 1972.

23. Ochs S: Rate of fast axoplasmic transport in mammalian nerve fibers. J Physiol 227:627-645, 1972.

24. Green LS, Donoso A, Heller-Bettinger IE, et al: Axonal transport disturbances in vincristine-induced peripheral neuropathy. Ann Neurol 1:255-262, 1977.

25. Koenig H: Acute axonal dystrophy caused by fluorocitrate: the role of mitochondrial swelling. Science 164:310-312, 1969.

26. Allen N, Mendell JR, Billmaier DJ, et al: Toxic polyneuropathy due to methyl n-butyl ketone, an industrial outbreak. Arch Neurol 32:209-218, 1975.

27. Mallov JS: MBK neuropathy among spray painters. JAMA 235:1455-1457, 1976.

28. Goto I, Matsumura M, Inoue N, et al: Toxic polyneuropathy due to glue sniffing. J Neurol Neurosurg Psychiatry 37:848-853, 1974.

29. Prockop LD, Alt M, Tison J: "Huffer's" neuropathy. JAMA 229:1083-1084, 1974.

30. Shirabe T, Tsuda T, Terao A, et al: Toxic polyneuropathy due to glue sniffing. Report of two cases with a light and electron-microscopic study of the peripheral nerves and muscles. J Neurol Sci: 21:101-113, 1974.

31. Korobkin R, Asbury AK, Sumner AJ, et al: Glue sniffing neuropathy. Arch Neurol 32:158-152, 1975.

32. Davenport JG, Farrell DF, Sumi SM: 'Giant axonal neuropathy' caused by industrial chemicals. Neurology 26:919-923, 1976.

33. Towfigi J, Gonatas NK, Pleasure D: Glue sniffers neuropathy. Neurology 26:238-243, 1976.

34. Knox JW, Nelson JR: Permanent encephalopathy from toluene inhalation. N Engl J Med 275:1494-1496, 1966.

35. Grabski DA: Toluene sniffing producing cerebellar degeneration. Am J Psychiatry 118:461-462, 1961.

36. Henser M: Toluolsucht. Med Klin 63:1888-1890, 1968.

37. Schaumburg HH, Spencer PS: Degeneration in central and peripheral nervous systems produced by pure n-hexane: an experimental study. Brain 99:183-192, 1976.

38. Spencer PS, Schaumburg HH: Experimental neuropathy produced by 2,5-hexanedione - a major metabolite of the neurotoxic industrial solvent methyl n-butyl ketone. J Neurol Neurosurg Psychiatry 38:771-775, 1975.

39. DiVincenzo GD, Kaplan CJ, Dedinas J: Characterization of the metabolites of methyl n-butyl ketone, methyl iso-butyl ketone, and methyl ethyl ketone in guinea pig serum and their clearance. Toxicol Appl Pharmacol 36: 511-522, 1976.

40. Bradley WG: Disorders of Peripheral Nerves. Blackwell Scientific Publications, Oxford 1974.

41. Thomas PK: The morphological basis for alterations in nerve conduction in peripheral neuropathy. Proc Roy Soc Med 64:295-298, 1971.

42. Eliasson SG: Properties of isolated nerve fibers from alloxanized rats. J Neurol Neurosurg Psychiatry 32:525-529, 1969.

43. Rasminsky M, Sears TA: Internodal conduction in undissected demyelinated nerve fibers. J Physiol 227:323-350, 1972.

44. Koles ZJ, Rasminsky M: A computer simulation of conduction in demyelinated nerve fibers. J Physiol 227:351-364, 1972.

45. Baker PF, Hodgkin AL, Shaw TI: The effects of changes in internal ionic concentrations on the electrical properties of perfused giant axons. J Physiol 164:355-374, 1962.

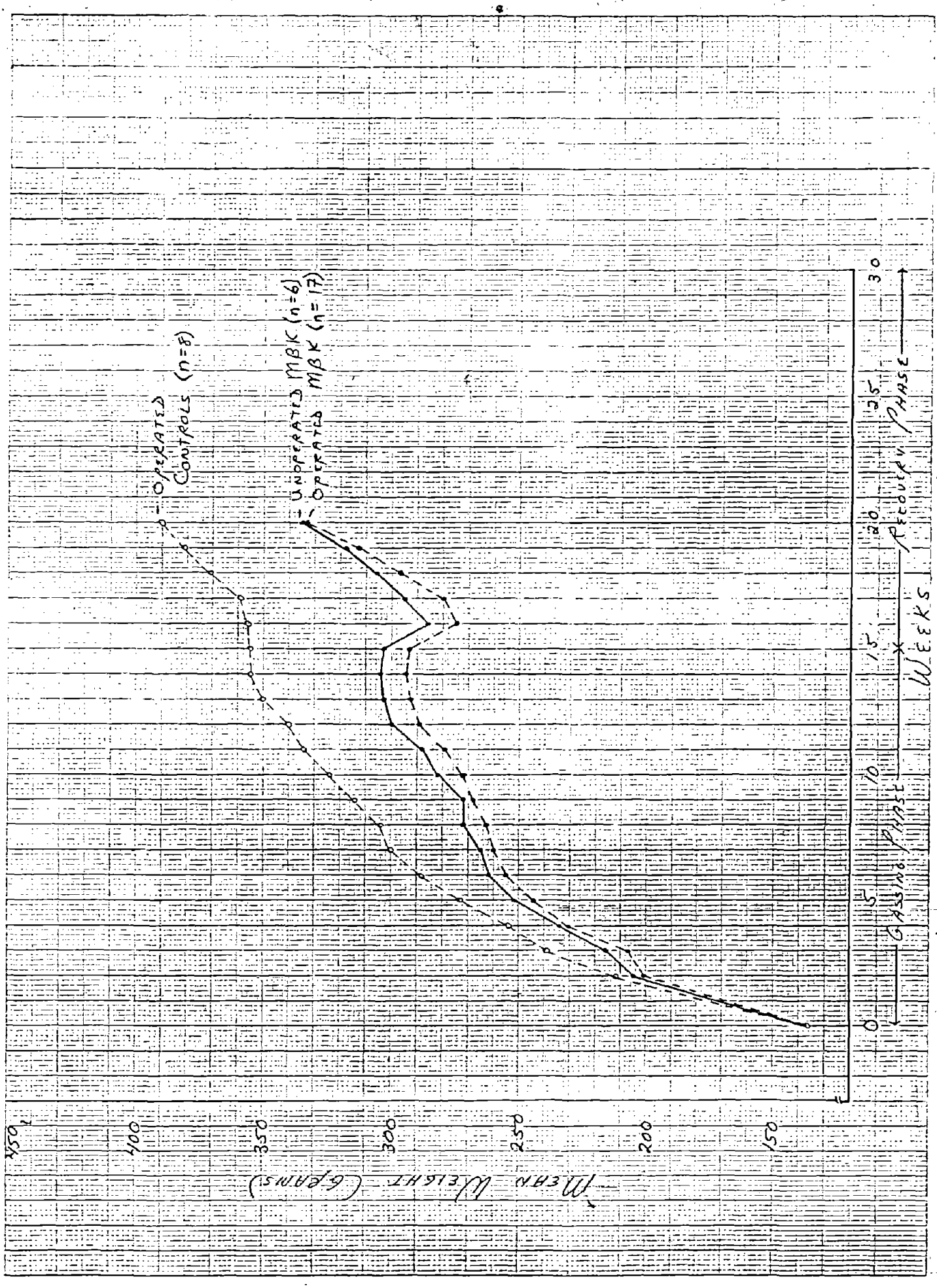

FIG. 1. MEAN WEIGHTS (GRAMS) OF OPERATED CONTROLS, + OPERATED + UNOPERATED MBK RATS.

Millimeters to the Centimeter

FIG 3 MNCV (m/sec) FOR MDK AND CONTROL RATS AT 5 WEEK INTERVALS DURING CROSSING AND RECOVERY PHASES.

10 Millimeters (the Centimeter

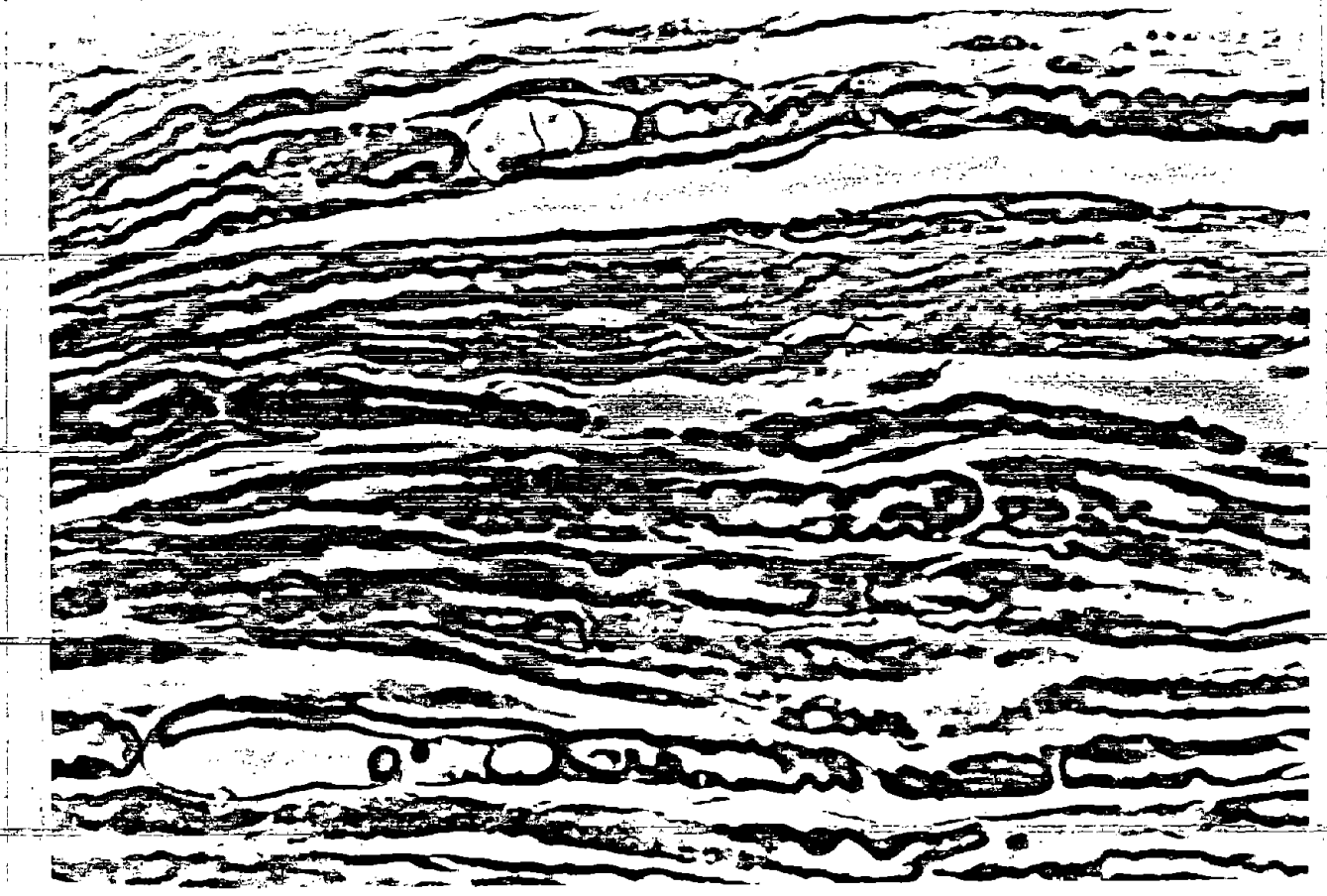

Fig. 4 Electron microscopic section of tibial nerve of MBK animal.

Table 1. Comparison of Predicted Theoretical and Actual MBK Values.

	FLOW RATE (ml/min)		CONCENTRATION (ppm)	
	via MBK	diluting air	Theoretical*	Actual (GLC)
1.	263	4850	353	355
2.	263	6950	246	187
3.	538	2860	1224	1851
4.	538	4850	722	1286
5.	538	6950	504	953
6.	1156	2860	2628	2707
7.	1156	4850	1551	1881
8.	1156	6950	1082	1338

* Used average of 6,506 ppm for formula (see text)

Table 2. Weights of First Experiment.

A. Total Group:	Controls		MBK (60 ppm)		MEK (2150 ppm)	
1. Start of Experiment	182.7 ± 2.3 (7.3)		184.0 ± 3.3 (10.6)		186.0 ± 2.6 (8.3)	
2. After 6 Weeks	300.3 ± 14.3 (45.2)		311.7 ± 15.4 (49.0)		308.0 ± 15.5 (49.0)	
B. According to Sex:	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1. Start of Experiment	297.2 ± 7.1 (15.8)	233.4 ± 8.3 (16.6)	292.1 ± 5.4 (12.1)	222.7 ± 4.0 (8.8)	279.0 ± 10.3 (23.1)	230.1 ± 8.7 (19.6)
2. After 6 Weeks	338.4 ± 10.9 (24.4)	265.1 ± 10.3 (20.7)	353.7 ± 9.2 (20.6)	270.0 ± 10.5 (23.5)	345.9 ± 14.8 (33.2)	270.4 ± 12.2 (27.2)

NB: Mean ± SEM (±SD)

Table 3. Third Experiment - Mean Weights

Experimental Groups	n	Group Mean Weights (g)	
		at Onset	at 5 Weeks
A. Controls	9	150.6 ± 2.6, 8.3	328.6 ± 21.2, 60.0
B. MBK 1050 ppm	8	148.4 ± 2.8, 9.6	292.0 ± 13.7, 36.2
C. MBK 1460 ppm	9	146.7 ± 3.7, 12.9	291.0 ± 12.4, 37.1

NB: Mean ± SEM, SD

Table 4. MBK and MEK Experiment for 6 Weeks - Electrophysiological Studies.

Experimental Groups	n	Stimuli TM (mA)	Stimuli SM (mA)	Potential Amplitude (mV)	MNCV (m/sec)
I Controls:					
a) Female Rats	5	0.1 ± 0	1.2 ± .04 (.08)	24.0 ± 4.1 (8.2)	68.9 ± 2.3 (4.6)
b) Male Rats	5	0.1 ± .01 (.03)	1.2 ± .03 (.07)	26.6 ± 2.7 (6.1)	66.6 ± 2.4 (5.4)
c) Both	10	0.1 ± .007 (.02)	1.2 ± .02 (.07)	25.4 ± 2.3 (6.8)	67.6 ± 1.6 (4.9)
II MBK (60 ppm):					
a) Female Rats	5	0.1 ± .01 (.03)	1.1 ± .04 (.09)	23.0 ± 4.2 (9.5)	63.6 ± 1.9 (4.3)
b) Male Rats	5	0.1 ± .02 (.04)	1.1 ± .02 (.04)	21.4 ± 2.4 (5.4)	64.3 ± 1.4 (3.0)
c) Both	10	0.1 ± .01 (.04)	1.1 ± .02 (.07)	22.2 ± 2.3 (7.3)	64.0 ± 1.1 (3.5)
III MEK (2150 ppm):					
a) Female Rats	5	0.1 ± 0	1.1 ± .06 (.14)	26.0 ± 5.5 (12.4)	64.0 ± 1.1 (2.4)
b) Male Rats	5	0.1 ± .02 (.04)	1.2 ± .02 (.05)	24.0 ± 4.4 (9.9)	66.9 ± 2.2 (5.0)
c) Both	10	0.1 ± .01 (.03)	1.1 ± .02 (.07)	25.0 ± 3.4 (10.6)	65.4 ± 1.3 (4.0)

Table 5. MBK and MEK Experiment for 4 Weeks - Electrophysiological Studies.

Experimental Groups	n	Stimuli		Potential	MNCV
		TM (mA)	SM (mA)	Amplitude (mV)	(m/sec)
1. Control	8	0.1 ± .03 (.09)	1.2 ± .02 (.05)	18.2 ± 1.3 (3.7)	65.0 ± 1.8 (5.1)
2. MBK 100 ppm	8	0.1 ± .01 (.02)	1.2 ± .02 (.07)	19.2 ± 1.7 (5.0)	61.8 ± 1.6 (4.7)
3. MEK 4740 ppm	9	0.1 ± .01 (,04)	1.2 ± .01 (.03)	22.9 ± 3.7 (11.0)	61.9 ± 1.2 (3.5)

Table 6. MBK Experiment for 5 Weeks - Electrophysiological Studies.

Experimental Groups	n	Stimuli		Potential	MNCV
		TM (mA)	SM (mA)	Amplitude (mV)	(m/sec)
1. Controls at					
a) Day 0	8	0.1 ± .02 (.05)	1.2 ± .01 (.03)	21.3 ± 3.3 (9.4)	64.3 ± 2.0 (5.7)
b) 5 Weeks	8	0.1 ± .01 (.02)	1.3 ± .02 (.07)	23.3 ± 1.9 (5.2)	65.5 ± 1.4* (3.9)
2. MBK at 5 Weeks					
a) 1460 ppm	9	0.1 ± .01 (.03)	1.2 ± .02 (.07)	21.4 ± 1.7 (5.2)	52.7 ± 2.3* (6.8)
b) 1200 ppm	7	0.1 ± .01 (.02)	1.2 ± .02 (.07)	28.6 ± 2.1 (5.6)	54.2 ± 1.2* (3.1)

* Either MBK value vs control: $p < .001$

Table 7. Cumulative Electrophysiological Data

Experimental Group	n	Stimuli		Potential Amplitude (mV)	MNCV (m/sec)
		TM (mA)	SM (mA)		
A. Controls - Day 0	8	0.12 ± .02 (.05)	1.21 ± .01 (.03)	21.3 ± 3.3 (9.4)	64.3 ± 2.0 (5.7)
B. Gassing Phase					
1. 5th Week					
a) Controls	8	0.11 ± .009 (.02)	1.32 ± .02 (.07)	23.3 ± 1.8 (5.2)	65.5 ± 1.4 (3.9)
b) MBK	9	0.12 ± .009 (.03)	1.22 ± .02 (.07)	21.4 ± 1.7 (5.2)	52.7 ± 2.3 (6.8)
p				NS	<.001
2. 10th Week					
a) Controls	9	0.14 ± .01 (.03)	3.7 ± .09 (.27)	23.0 ± 1.3 (3.9)	68.6 ± 2.4 (7.2)
b) MBK	9	0.10 ± .01 (.05)	3.7 ± .10 (.31)	16.5 ± 2.0 (6.0)	45.0 ± 1.7 (5.0)
p				<.025	<.001
3. 15th Week					
a) Controls	10	0.11 ± .009 (.02)	3.31 ± .22 (.71)	23.8 ± 2.8 (8.8)	66.5 ± 1.2 (3.7)
b) MBK	10	0.12 ± .009 (.03)	3.14 ± .17 (.56)	7.2 ± 1.6 (5.2)	40.9 ± 2.6 (8.2)
p				<.001	<.001
C. Post-Gassing (Recovery) Phase					
1. 5th Week					
a) Controls	9	0.12 ± .01 (.03)	3.36 ± .17 (.52)	27.0 ± 2.9 (8.7)	69.6 ± 1.9 (5.8)
b) MBK	9	0.10 ± .01 (.03)	3.98 ± .11 (.35)	13.0 ± 3.1 (9.2)	47.2 ± 1.4 (4.0)
p				<.01	<.001
2. 10th Week					
a) Controls	9	0.10 ± .02 (.06)	3.40 ± .14 (.43)	21.5 ± 2.6 (7.8)	66.9 ± 1.2 (3.6)
b) MBK	8	0.15 ± .01 (.02)	3.30 ± .10 (.27)	13.4 ± 1.9 (5.0)	51.2 ± 2.1 (5.6)
p				<.025	<.001
3. 15th Week					
a) Controls	9	0.10 ± .02 (.06)	3.20 ± .12 (.36)	25.6 ± 2.1 (6.2)	67.1 ± 1.7 (5.1)
b) MBK	9	0.14 ± .01 (.04)	4.00 ± .24 (.73)	16.3 ± 1.7 (5.1)	62.3 ± 2.4 (7.3)
p				<.005	NS

Table 8. Biochemical Analysis of Posterior Tibial Nerves.

Experimental Group	n	Collagen (% Total Protein)	Unesterified Cholesterol*	Sulfatide*	Esterified Cholesterol*
A. 15 Weeks of Gassing					
1. Control	5	26%	18.7	2.6	2.1
2. MBK	5	25%	24.7	3.8	1.2
B. 10 Weeks of Gassing					
1. Control	5	28%	26.1	4.2	1.4
2. MBK	5	30%	22.5	2.7	2.2

* nM/μM Amino Acids