by the number of chromosomes compared. For instance, small variation between 2 chromosomes or 2 pairs of chromosomes normalized to 200 units would be more easily detected than variation among 23 pairs normalized to 200 units. Sensitivity to variation therefore increases as the total amount of the genome to be analyzed decreases. Details of the statistical methods are given in previous reports (*5*). For chromosome 1, Selles, Marimuthu, and Neurath (*5*) determined that a variation of 2.3 percent from the grand mean would be significant with $P < .05$. The deleted segment in our case in comparison represents approximately a four- to sixfold greater variation than that ultimately detectable.

8. Chromosome lengths are given in units defined as 0.14 μm per unit: normal 8(N_8), 54.14 units; translocated 8(T_8), 58.16 units; normal 11(N_{11}), 49.83 units; deleted 11(D_{11}), 37.87 units. $(N_8 + N_{11}) - (T_8 + D_{11}) = 7.94$ units difference. Measurements represent 20 scans for each chromosome pair (40 separate chromosomes).

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14. R.L. was supported by a special fellowship (IFO 3 EY54569-01) from the National Eye Institute.

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10 May 1974

Toxic Polyneuropathy Produced by Methyl N-Butyl Ketone

Abstract. *A polyneuropathy affecting a large number of workers was recently observed at a plant producing plastic-coated and color-printed fabrics. Epidemiological data suggested strongly that methyl N-butyl ketone (MBK) was responsible for the outbreak. This hypothesis is now supported by the development of a peripheral neuropathy in chickens, rats, and cats exposed to MBK at atmospheric concentrations of 200 to 600 parts per million, 24 hours per day, 7 days per week. Although the animals were exposed continuously and the affected workers were exposed intermittently, the averages of the total number of hours of exposure for development of the peripheral neuropathy in the animals and workers were remarkably close.*

In June 1973, one worker from a plant producing plastic-coated and color-printed fabrics was examined at Ohio State University Hospital and found to have a polyneuropathy. Extensive screening of 1161 employees from that plant confirmed that there were 79 persons with clinical evidence of polyneuropathy and 182 persons with abnormal electrodiagnostic examinations (*1*). In all cases the onset of the disease was insidious. In severely affected individuals, symmetrical distal muscle weakness and loss of deep tendon reflexes were present in upper and lower extremities. Sensory impairment consisted of loss of sensitivity to superficial pain, temperature change, and light touch and vibration stimuli in toes, feet, and fingers. Electromyographic (EMG) findings consisted regularly of prolonged insertional activity, numerous positive waves, and fibrillations. Moderate slowing of nerve conduction velocities (NCV) were frequently observed. Workers with clinical peripheral neuropathy were found to have been regularly exposed to methyl *N*-butyl ketone (MBK) used as a dye solvent and cleaning agent. This solvent was introduced into the plant in August 1972, and reached full use by December 1972. No cases of polyneuropathy appeared prior to December 1972. Attack rates were highest in the work area with greatest usage of this solvent and were also correlated with increasing hours of exposure. These data suggested that MBK was related to the development of this polyneuropathy. On this basis laboratory studies were designed to simulate the environmental conditions of the affected workers by exposing animals to an atmosphere containing MBK at a concentration of 200 to 600 ppm, 24 hours per day, for 7 days per week. The results of these studies have demonstrated that several species of animals exposed to MBK under these conditions consistently developed a peripheral neuropathy as judged by clinical, physiological, and morphological criteria.

Animals used in our study included five domestic chickens, four Sprague-Dawley rats, and four domestic cats. They were held in environmental chambers with a volume close to 1000 liters. Delivery of room air at approximately 200 liter/min through the chamber maintained a normal environment with respect to O_2 (21 percent), CO_2 (less than 0.2 percent), relative humidity (less than 60 percent), and temperature (within 1° and 2°C of the normal 21° to 25°C). Solvent vapor was added to the chamber by diverting a metered portion of the airflow through the headspace of a container of MBK before it entered the chamber. Concentrations of MBK monitored by gas chromatography were initially at 200 ppm for chickens and 600 ppm for rats and cats. Food and water intake and body weights were measured regularly, and the MBK concentration was adjusted to 100 and 400 ppm, respectively, to minimize complications from inanition and weight loss. Pair-fed controls were killed when the exposed animals were killed.

To date we have observed the development of a peripheral neuropathy in all rats, cats, and chickens tested. The earliest development of overt clinical signs occurred at 4 to 5 weeks in chickens that showed an inability to stand on their legs. The cats were next to develop clinical weakness, as manifested at 5 to 8 weeks by dragging of their hind limbs and later by forelimb

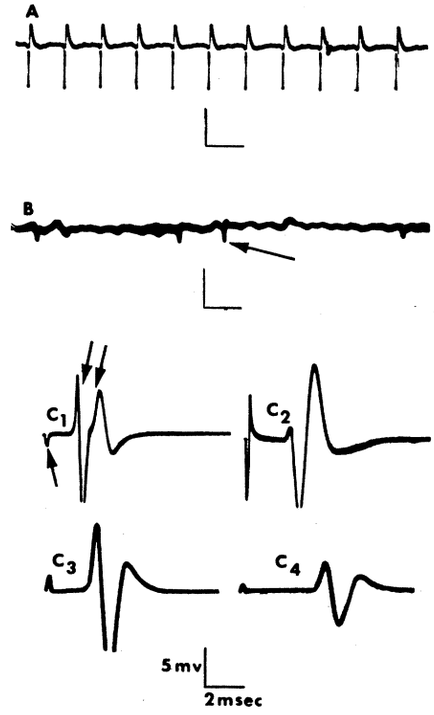

Fig 1. (A) Train of positive waves in anterior tibialis after 5 weeks of MBK exposure. Vertical scale, 200 μv; horizontal scale, 10 msec. (B) Fibrillation potentials (arrow) in gastrocnemius after 9 weeks of MBK exposure. Vertical scale, 200 μv; horizontal scale, 5 msec. (C_1) Control recording showing stimulus artifact (arrow) and recorded footpad muscle action potential (arrows) from stimulation of ulnar nerve (wrist to footpad); (C_2) control recording from elbow joint to footpad; (C_3) prolonged latency between stimulus and recorded muscle action potential from ulnar nerve stimulation (wrist to footpad); and (C_4) prolonged latency of ulnar nerve conduction from elbow joint to footpad.

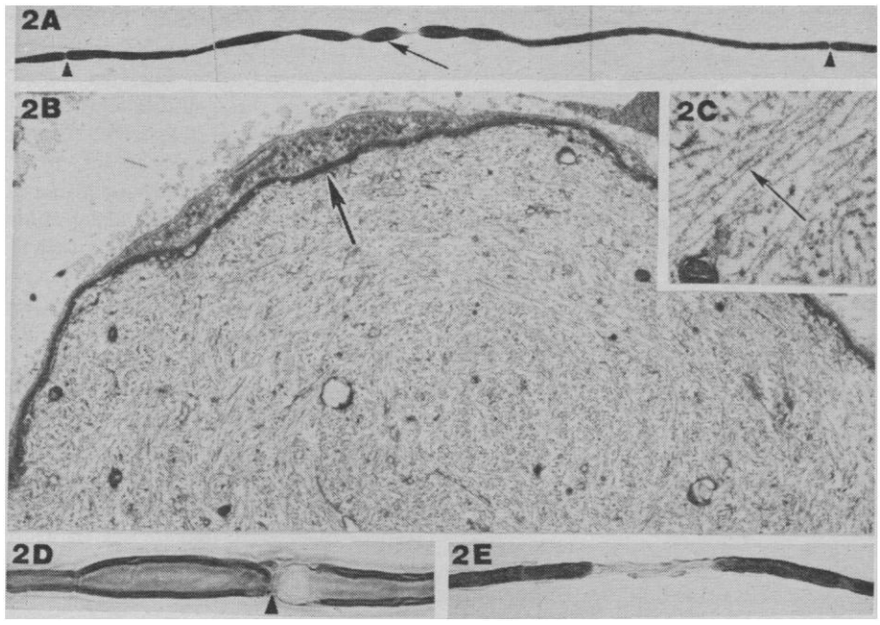

Fig. 2. (A) One internodal segment of a nerve fiber with the nodes of Ranvier at the arrowheads. Focal axonal enlargements (arrow) are seen (× 140). (B) Cross section of nerve fiber through area of axonal enlargement. There is marked thinning of myelin sheath (arrow). The axonal cytoplasm shows accumulations of bundles of neurofilaments (× 8,700). (C) Enlarged area of swollen axon showing neurofilaments (arrow) (× 34,000). (D) Axonal swelling on both sides of the node of Ranvier (arrowhead). Thinning of myelin sheath accompanies swelling close to the node of Ranvier at right (× 350). (E) Focal loss of myelin without axonal swelling (× 280).

weakness. Rats showed clinical weakness after 11 to 12 weeks, as manifested by dragging of their hind limbs. The water intake of the rats was greatly increased.

Electrodiagnostic studies were performed weekly in four cats exposed to MBK. For EMG, monopolar, Teflon-coated recording electrodes with a 0.1-mm bare tip were used. The reference electrode was located in the subcutaneous tissue. The interference pattern was obtained in the limb muscle by inducing a flexor reflex. Recording of the electrical activity of the muscle at rest and during insertion of the electrode was accomplished by general anesthesia with intravenous sodium pentobarbital (30 mg/kg). The following muscles were routinely tested: supraspinatus, triceps, extensor carpi, deep digital flexors, paraspinals, quadriceps, hamstring, gastrocnemius, and anterior tibialis. For determination of NCV, the ulnar nerve was stimulated at the elbow and carpal joints under general anesthesia, with a monopolar needle electrode with a Grass stimulator and accompanying Grass stimulus isolation unit.

The first EMG findings consisted of pathological insertional activity with positive waves which appeared between 4 to 6 weeks of exposure in all cats. Subsequently trains of positive sharp waves were observed (Fig. 1A). Between 9 and 10 weeks, fibrillation potentials appeared in muscles at rest and with insertional activity (Fig. 1B). Slowing of the ulnar nerve conduction velocity from an average of 115 to 50 m/sec developed in all cats from 7 to 9 weeks (Fig. 1C). The amplitude of motor unit action potentials did not significantly change from controls of 1000 μv; however, a few polyphasic potentials were occasionally seen at 8 to 9 weeks. The EMG findings occurred in all muscles tested, with a predilection for more marked changes distally.

For histologic examination, the sciatic nerves were either fixed in situ with 3 percent glutaraldehyde in 0.1*M* phosphate buffer, *p*H 7.4, or the animals were perfused through the abdominal aorta with Karnovsky's fixative (*2*). The nerves were postfixed with 1 percent osmium tetroxide and embedded in Spurr low-viscosity embedding media. Sections (1 to 2 μm) were studied by light microscopy. Thin sections of portions of the nerve were viewed with a Hitachi Hu12 electron microscope. Other portions of the nerve were prepared for nerve fiber teasing (*3*).

The pathological alterations were similar in all three species. The changes consisted of focal, often abrupt, swelling of the axon along the nerve fiber (Fig. 2A) or close to the nodes of Ranvier. The myelin sheath surrounding the swollen axon was often thinner than normal (Fig. 2B); at areas close to the node of Ranvier there was denudation of myelin for varying lengths depending on the severity of the process (Fig. 2D). Axonal changes in the form of marked accumulation of neurofilaments and loss of neurotubules were observed at the sites of axonal swelling (Fig. 2, B and C). Focal areas of denudation of myelin from the axon unaccompanied by axonal swelling (Fig. 2E) were frequently observed. At times this reached one-third to one-half of an internodal segment and did not necessarily begin at the node of Ranvier. Less frequently observed were nerve fibers containing myelin ovoids. These changes were associated with little or no macrophage infiltration, but myelin breakdown products were frequently observed in the cytoplasm of Schwann cells. No abnormalities were identified in the controls.

Our study demonstrates that a peripheral neuropathy can be induced in three species of animals exposed to MBK at an initial range of 200 to 600 ppm. Although these were continuous exposures compared to the intermittent exposures of the workers, there was a good correlation on the basis of the total number of hours of exposure between the development of clinical neuropathy in the animals and humans. MBK had been used in the plant for 9 months prior to detection of the polyneuropathy, leading to an approximate total exposure of 1584 hours (22 days per month for 8 hours per day for 9 months) compared to the average number of hours of exposure for development of a peripheral neuropathy in the animals of 1440 hours (24 hours per day for 2 months).

By clinical criteria the neuropathy observed in our experimental animals compares favorably with that of the workers. The involvement was predominantly motor in the most severely affected humans, and muscle weakness was the principal clinical manifestation in the experimental animals. Further, the major electrodiagnostic abnormalities of positive waves, fibrillation potentials, and slowed NCV's in the patients were reproduced in the exposed animals.

At present no adequate samples of human nerve biopsy material have been examined from afflicted workers to allow for histological comparison with the experimental observations.

The findings in the experimental neuropathy consisted of axonal swelling with thinning of the myelin sheath or denudation of myelin near the node of Ranvier associated with accumulated masses of neurofilaments. This type of abnormality is very similar to the giant axonal change described by Asbury *et al.* (*4*) in a sporadic case of a slowly progressive mixed polyneuropathy without known exposure to toxins. It is also of interest that the peripheral nerve can show neurofilamentous alterations with other agents including acrylamide (*5*), β,β'-iminodipropionitrile (*6*), and vincristine (*7*) but none are identical to those described here with MBK or in the case of the giant axonal neuropathy. It is also important that in our experimental neuropathy focal areas of denudation of myelin were present without swelling of the axon. This change could be secondary to axonal damage, although we cannot rule out the possibility of direct toxicity of MBK for the myelin sheath.

Safe atmospheric levels of MBK should be established; it is also necessary to ascertain whether the recommended threshold limit value of 100 ppm for MBK provides an adequate margin of safety for workers exposed to this solvent.

Note added in proof: After this manuscript was submitted for publication, Spencer and Schaumberg (*8*) reported similar findings in rats exposed to MBK at 1300 ppm, 6 hours per day, 5 days a week, for up to 4 months.

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5 May 1974; revised 21 June 1974

Complement-Dependent Stimulation of Prostaglandin Synthesis and Bone Resorption

Abstract. *Complement-sufficient heterologous serum induced prostaglandin synthesis and resultant resorption in cultures of fetal rat long bones. Bone resorption was enhanced with unheated normal rabbit serum as compared to heated serum or serum from rabbits lacking the sixth component of complement (C6). Addition of functionally purified C6 restored resorptive activity in C6-deficient serum. Concentrations of prostaglandin E were increased in the culture media of bones incubated with complement-sufficient serum. The resorptive effects of active serum as well as the appearance of prostaglandin E in the media were inhibited by indomethacin.*

The resorption of fetal bone in organ culture can be stimulated by the addition of serum to the medium (*1*). Histological evidence for the role of complement (C) in the breakdown of cartilage matrix, impaired growth, and increased bone resorption in cultures of mouse and chick bone rudiments containing serum was obtained by Fell, Lachmann, Coombs, Dingle, and Weiss (*2*). The destructive effects on cartilage probably required the presence of connective tissue cells (*3*). These investigators attributed the observed effects to immune activation of C, since antiserum to cell surface antigens or to bone tissue intensified the activity and depletion of C from serum or the use of C-deficient serum abrogated the response. Complement activation increased the release of lysosomal enzymes in their cultures.

We have assessed the role of C in stimulation of bone resorption by serum, using a quantitative assay based on the release of previously incorporated radiocalcium from fetal rat bone in organ culture (*4*). Nineteen-day, fetal rat long bone shafts (radius and ulna) previously labeled with ^{45}Ca, were cultured in modified BGJ medium; 50 percent rabbit serum served as the source of C. The bones were cultured for 6 days, with one medium change at 3 days. Paired bones were used for control and experimental cultures.

Serum from ten normal rabbits all showed heat-labile stimulation of bone resorption (Table 1) and only 1 of 16 individual assays failed to show a significant difference between experimental and control cultures after 6 days. The mean increase in release of ^{45}Ca was approximately 60 percent and most of this occurred during the second 3 days in culture. When an increase in release of ^{45}Ca was observed during the first 3 days, it was usually less than 30 percent. The experimental-to-control ratios achieved were not as high as those attained with other experimental models that employed parathyroid hormone or osteoclast-activating factor from human leukocytes (*4*, *5*), largely because (i) no preculture was used to remove exchangeable ^{45}Ca and (ii) a relatively high rate of control resorption was produced by the presence of 50 percent heated serum in the medium. At the end of 6 days, bones cultured in unheated serum showed marked histologic change; compared with bones cultured in heated serum, there was loss of matrix and proliferation of osteoclasts and

Table 1. Bone-resorbing activity of rabbit serum. Values are means ± standard errors for number of assays given in parentheses; each assay consisted of four pairs of bones cultured with the indicated serum. C6, the sixth component of complement.

Rabbit serum		6-day ^{45}Ca release (experimental/control ratio)
Experimental	Control	
Normal, unheated	Normal, heated*	1.59 ± 0.06† (16)
C6-deficient, unheated	C6-deficient, heated*	1.05 ± 0.05 (14)
C6-deficient plus human C6, unheated	C6-deficient plus human C6, heated*	1.41 ± 0.11† (4)
C6-deficient plus guinea pig C6, unheated	C6-deficient plus guinea pig C6, heated*	1.44 ± 0.15† (4)
Normal plus human C6	Normal	1.05 ± 0.02 (3)
Normal plus guinea pig C6	Normal	1.14 ± 0.02† (3)
Normal plus guinea pig C2	Normal	0.94 ± 0.13 (1)
C6-deficient plus guinea pig C2	C6-deficient	0.98 ± 0.23 (1)
Human C6	Vehicle	1.02 ± 0.05 (1)
Guinea pig C6	Vehicle	1.09 ± 0.01† (1)

* 56°C, 30 minutes. † Significantly greater than 1.0, $P < .05$.