reflected fluorescent microscope of Olympus, Tokyo (ext. 405 nm, em. 475 nm over). Adding to this, the specimens were stained with hematoxyline-eosine (HE) for a general view, periodic acid Schiff reaction and sudan black B, respectively, after a short exposure of formaldehyde gas for 5 min.

Figure 1, a and b, are examples of capillary nets revealed by the authors' method. Capillary lumina were filled with faint green fluorescence, while slender areas along the capillary wall were stained with a strong green fluorescence. These areas were identified as endothelial cells referring to the specimens stained with HE. Further, oval black shadows in capillary lumina were blood cells. With these observations, running courses of capillaries could be traced up to their periphery. Often, along the outer side of capillary wall, cells containing yellow fluorescent granules were observed. They appeared only in the perivascular areas and especially in bifurcating regions of capillaries, arterioles or venules. Occasionally, from capillaries, finer branches sprouted out (figures 3 and 4). The average diameter of capillaries in specimens was estimated as 2–8 μm (figures 1 and 2), but that of smallest branches as shown in figures 3 and 4 was only 1 μm. Here questions arose in mind; whether the blood cells could pass through those narrow portions or not, and whether the capillaries in the brain cortex were susceptible to the administration of L-DOPA or not.

Concerning a deformability of red cells, several reports had already been published from the stand point of rheology[4–6]. Further, in capillary nets a tangle of capillaries sometimes occurred. That is, in figures 2–4, 2 capillaries ran spirally and in figures 3 and 4, the finest capillaries were twined around each other.

At the first step of this investigation, the authors considered these findings as artifacts during a preparation of specimens. However, a twist or a tangle of capillaries could not be produced by the authors' procedure, because the stretching force for brain tissue was applied only from left to right as described afore. The fluorescent cells along the brain vessels appeared to embrace capillaries as depicted in figure 5. They contained a lot of autofluorescent granules of about similar size and often made a cluster of 2 to 3 cells close to bifurcating regions. The histochemical properties of intracellular granules were similar to type II of mast cells reported by Ibrahim[7]. That is, the granules were acidophile and stained with PAS reaction as shown in figure 6, a and b. They did not show a metachromasia with toluidine blue, but partially stained with sudan black B. Different from the report of Ibrahim, alcohol or acetone treatment could not induce a decrease of fluorescency. According to the authors' observation, the fluorescency of the granules was first detected at second week after birth (unpublished). They are assumed as one type of macrophage from the granular size and quality, and their function is a store house of some substances transported by a brain circulation. This speculation is being confirmed by the experimental studies in the author's laboratory.

1 B. Romeis, Mikroskopische Technik. Leibniz Verlag, München 1948.
2 J.C. de la Torr, Adv. Neurol. *2*, 59 (1973).
3 B. Falck, N.-Å. Hillarp, G. Thieme and A. Torp, J. Histochem. Cytochem. *10*, 348 (1962).
4 P.-I. Brånemark, Biorheology *1*, 139 (1963).
5 Y.C. Fung, J. Biomech. *2*, 353 (1964).
6 E.H. Bloch, Trans. Soc. Rheol. *8*, 26 (1962).
7 M.Z.M. Ibrahim, J. neurol. Sci. *21*, 431 (1974).

## Experimental data on the neurotoxicity of methyl-ethyl-ketone (MEK)

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*Summary.* A severe potentiating effect of methyl-ethyl-ketone (MEK) on the peripheral and central neurotoxicity of n-hexane could be demonstrated in a chronic inhalation study in rats.

The hexacarbon compounds n-hexane, methyl-n-butyl-ketone (MBK) and 2,5-hexanedione have been identified as neurotoxins in several outbreaks of occupational neuropathies. There have been various reports of polyneuropathies in factory workers following exposure to n-hexane or MBK[2–4] and in 'sniffers'[5], i.e. juveniles who had used cements or solvents for their euphoric properties. In the latter cases, n-hexane was thought to be the major neurotoxic agent. N-hexane and MBK are metabolically related with 2,5-hexanedione and 2,5-hexanediol. Spencer and Schaumburg have demonstrated in extensive neurobiological studies that these compounds cause neuropathies of the 'dying-back' type in the form of central-peripheral distal axonopathies in rats and cats[6–8].

An outbreak of severe toxic neuropathies among Berlin solvent sniffers in 1975 was closely related to an alteration of the abused solvent mixture by denaturation with 11% methyl-ethyl-ketone (MEK), a substance previously considered as safe concerning neurotoxic properties[9]. The solvent was composed of 16% n-hexane, 26% benzine fraction, 29% toluene and 18% ethylacetate. The production of the MEK-containing thinner was subsequently officially stopped; 6 more cases occurred, however, in 1976 and 1977, after old, MEK-containing batches had been sold[10].

*Material and methods.* The following experiments were devised to investigate the effects of inhalation of MEK and n-hexane in rats. In plastic chambers with smooth floors 5 rats were exposed to 10,000 ppm n-hexane (99% purity; Merck Nr. 4367) for 15 weeks, 7 days per week, 8 h per day. 5 other rats were exposed to a mixture of 1100 ppm MEK and 8900 ppm n-hexane, another group of 5 animals to 6000 ppm MEK (99% purity; Merck Nr. 6014). The initial concentration of 10,000 ppm had to be decreased within a few days in the latter group because of severe irritation of the upper respiratory tract. 5 rats served as controls. In another set of experiments under the same conditions, 2 rats were sacrificed each week in each group in order to determine the date of earliest morphological alterations. At the time of sacrifice, animals were perfused in a slightly modified method described by Pease[11]. The peripheral nerves of the brachial plexus, sciatic nerve and its branches and several levels of the spinal cord and medulla were studied by light and electron microscopy. All representative specimens were embedded in araldite, hardened and ex-

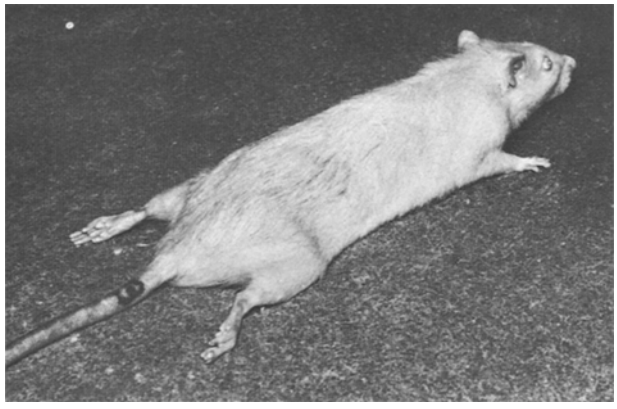

Fig. 1. Severe distal and proximal hindlimb weakness in a rat after 7 weeks of MEK/n-hexane exposure.

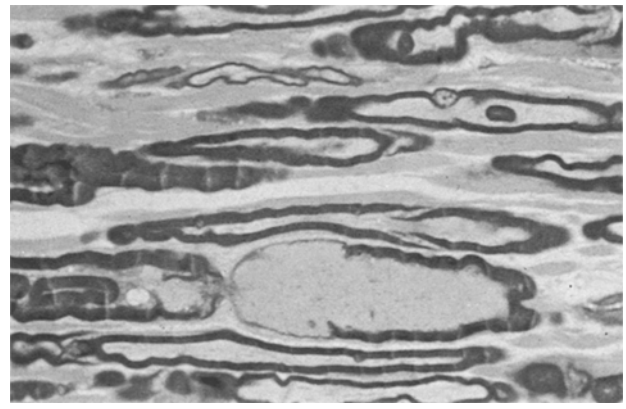

Fig. 3. Giant paranodal axonal swelling and myelin thinning in the tibial nerve of a rat. MEK/n-hexane, 4th week. Light micrograph. × 1500.

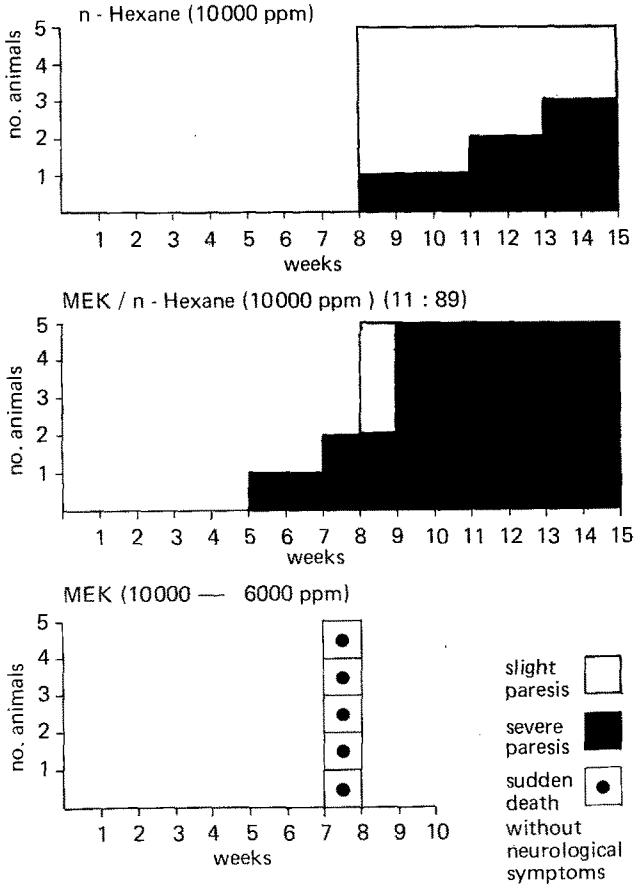


Fig. 2. Effects of chronic inhalation of pure n-hexane, MEK/n-hexane mixture and pure MEK in rats.

amined in 1-μm sections stained with methylen blue. Thin sections of selected areas were stained with uranyl acetate and lead citrate and examined by electron microscopy.

*Results and discussion.* Animals exposed to pure MEK did not develop clinical signs of a neuropathy; all animals in this group died with pathologically confirmed bronchopneumonia. Severe paresis of the hindlimbs (figure 1) occurred earliest in animals exposed to the MEK/n-hexane mixture (in the 5th week in one animal and in the 9th week in all other animals). In animals exposed to pure n-hexane, the first permanent motor deficits appeared in the 8th week and were less severe (figure 2).

The earliest morphological alterations were seen in the 4th week in animals exposed to the MEK/n-hexane mixture, but not before the 8th week in the n-hexane group. These changes appeared first in the tibial nerve supplying the calf muscles and consisted in an increase of number of the neurofilaments in the large myelinated fibres which were enlarged in paranodal regions (figure 3). Paranodal axonal swelling was regularly associated with abnormally attenuated myelin sheaths, these areas sometimes being remyelinated at later stages of exposure. In the severely affected animals, distal portions of the nerve were destroyed and appeared as a chain of homogeneous myelin ovoids. Similar morphological changes - swelling of axons, myelin retraction and fibre breakdown - were found at different levels of the long tracts of the spinal cord and medulla. No difference could be determined between the 2 groups concerning the type of morphological lesion, except that the extent of damage seemed to be more pronounced in animals exposed to MEK/n-hexane. Animals under exposure to pure MEK did not show any comparable morphological alterations after up to 7 weeks exposure.

The results of the present study indicate that the addition of a small amount of MEK to n-hexane (ratio 1 : 9) manifested markedly enhanced neurotoxicities with a shortened time for occurrence of morphological and clinical signs. The effects of low-grade concentrations on the nervous system and on different enzyme systems are presently under investigation.

1 Bundesgesundheitsamt Berlin, Unter den Eichen, 1000 Berlin (West) 45.
2 A. Herskowitz, N. Ishii and H. Schaumburg, New Engl. J. Med. *285*, 82 (1977).
3 J.S. Mallov, J. Am. med. Ass. *235*, 1455 (1976).
4 J.R. Mendell, K. Saida and M.F. Gansia, Science *185*, 787 (1974).
5 R. Korobkin and A.K. Asbury, Arch. Neurol. *32*, 158 (1975).
6 P.S. Spencer and H.H. Schaumburg, Progr. Neuropath. *3*, 253 (1977).
7 P.S. Spencer and H.H. Schaumburg, Neuropath. exp. Neurol. *36*, 276 (1977).
8 H.H. Schaumburg and P.S. Spencer, Brain *99*, 173 (1976).
9 H. Altenkirch, J. Mager, G. Stoltenburg and H. Helmbrecht, J. Neurol. *214*, 137 (1977).
10 H. Altenkirch, G. Stoltenburg and H.M. Wagner, Int. Symp. Periph. Neuropath. Univ. Studi Milano, suppl. 5, 17 (1978).
11 D.C. Pease, Histological techniques for electron microscopy, 2nd ed. Academic Press, New York 1964.