Experimental Porphyric Neuropathy: A Preliminary Report

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SUMMARY: An experimental model for the study of porphyric neuropathy is presented. Injection of either tetraphenyl-porphinesulfonate (TPPS), hematoporphyrin derivative (HpD), or delta-aminolevulinic acid (ALA) into mice resulted in markedly decreased motor nerve conduction velocity (MNCV). The MNCV returned to normal within one week following the injection of large doses of ALA, and within three weeks following the injection of close to lethal doses of HpD, but there was no recovery of nerve function within 60 days following injection of substantially smaller doses of TPPS. Ultrastructural examination of motor nerves at various times following TPPS injection revealed the gradual development of structural abnormalities. Ultrastructural examination of the same nerves after a single dose of either ALA or HpD failed to demonstrate any abnormalities.

The present observations call for precaution as to the use of TPPS as photosensitizer in human cancer treatment.

INTRODUCTION

The acute intermittent (Swedish) form of hepatic porphyria is characterized by severe neurological abnormalities without obvious skin photosensitivity, while both skin photosensitivity and neurological abnormalities may be observed in patients with porphyria variegata, the South African Caucasian form of hepatic porphyria. It has long been known that the skin photosensitivity results from abnormally high concentrations of porphyrins in the tissues and that photosensitivity can be reproduced experimentally by the administration of different types of porphyrins (Meyer-Betz, 1913; Barker et al., 1970; Spikes, 1975). The mechanism underlying porphyric neuropathy, however, is less well understood. Acute intermittent porphyria is characterized by an increase in the excretion of the porphyrin precursors, delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) without a corresponding increase in excretion of the porphyrins. Many investigators have therefore tested ALA and PBG for possible neurotoxicity (Goldberg et al., 1954; Goldberg and Rimington, 1954; Berlin et al., 1954; Granick and Van den Schrieck, 1955; Jarrett et al., 1956; De Metteis, 1962; Biempica et al., 1967; Kosower and Rock, 1968; Feldman et al., 1968 and 1971; Marcus et al., 1970; Becker et al., 1971 and 1976; Isaacs et al., 1971; Shanley et al., 1972, 1975 and 1976; McGillion et al., 1973; Loots et al., 1975; Moore et al., 1976; Nicoll, 1976; Müller and Snyder, 1977; Dichter et al., 1977; Becker and Kramer, 1977; Bornstein et al., 1978; Cutler et al., 1978). Although there is good evidence that high concentrations of ALA may lead to changes in behavior, in cell membrane function, in neuromuscular and spinal transmission, there is no general agreement...
that ALA as such is directly respon-
sible for porphyric neuropathy.

In this study mice were injected with
single large doses of either tetrathing-
hydrorhodopsinate (TPPS), hemato-
porphyrin derivative (HpD) or
delta-aminolevulinic acid (ALA). We
demonstrate in the present report for
the first time decreased motor nerve
conduction velocities after the injec-
tion of TPPS, HpD and ALA, as well
as the development of structural
changes in peripheral motor nerves
after the injection of TPPS. Further-
more, the effects of these substances
were investigated as to direct toxicity,
persistence of photosensitivity and
extrahepatic synthesis of porphyrins
after ALA injection.

MATERIALS AND METHODS

CHEMICALS

TPPS. Tetraphenylporphyrin sodium te-
trasodium salt was prepared according
to the method of Busby et al (1975). The
material was dialyzed until alkaline barium
chloride solution failed to cause a precipi-
tate.

HpD. Hematoporphyrin derivative was
produced by a modification of the method
reported by Dougherty et al (1970). Since
HpD preparations may vary greatly in
purity and are relatively unstable, we have
provided a detailed description of our
method of preparation. Future reports will
refer to this information.

Twelve grams of hematoporphyrin hy-
drochloride (Laboratoire Rousel, Paris,
France) were added to 240 ml of a mixture
containing 19 volumes of glacial acetic acid
and 1 volume of concentrated sulfuric acid.
The reaction was allowed to proceed in the
dark for 5 days at room temperature, with
occasional stirring. The resulting solution
was passed through a 0.2 polycarbonate
filter and then divided equally among six 1-
liter polyethylene centrifuge bottles. The
bottles were immediately filled to the 1 liter
mark with 3% sodium acetate in water, and
the resulting red-brown precipitate pelleted
by centrifugation (4°C, 15 min at 2,500 g).
The lightly colored supernatant was
the resulting red-brown precipitate pelleted
for 5 days at room temperature, with
the method of Busby et al (1975). The
trasodium salt was prepared according to
purity and are relatively unstable, we have
produced by a modification of the method
isotonic saline. Solutions were neutralized
with 0.15N NaOH before injection.

Beta-carotene was Eastman Kodak
#3702. Since beta-carotene is insoluble in
isotonic saline, it was injected as a suspension
in isotonic saline.

ANIMALS

Mice. DBA/2J mice from the Jackson
Laboratory, Bar Harbor, Maine, and
Swiss Albino mice from either Bio-
Breeding Laboratories, Ottawa, Ontario,
or Canadian Breeding Farms and Labo-
ratories, Laprairie, Quebec, were matched
for strain, sex and age in each experiment.
All animals were injected at 10 weeks of
age.

MOTOR NERVE CONDUCTION
VELOCITY (MNCV)

MNCV was measured at various inter-
vals following injection of either TPPS,
HpD or ALA. Mice injected with saline
alone served as controls.

TPPS was injected intraperitoneally
(i.p.) in various doses (Fig. 2) in groups of
5 to 6 DBA/2J mice. Close to lethal doses of
HpD (100 mg/kg) were injected i.p. into
two groups of 6 DBA/2J mice (Fig. 4). HpD
in doses of 80 mg/kg were injected i.p. in a
group of 9 Swiss albino mice (Fig. 3).
Finally a group of 10 Swiss albino mice
were injected i.p. with 1000 mg/kg ALA
(Fig. 3).

The animals were anesthetized with
methoxyflurane and the MNCV was mea-
sured immediately. After measurements
were made, new MNCV measurements
were taken at various times post-
jection by exposure to Pyrex-filtered
sunlight at an intensity of approximately
95 mW/cm². Scratching and rubbing of the
ears, eyes, nose and tail during the
immediately following exposure was con-
sidered to be evidence of a mild phototoxic
reaction. More severe reactions resulted in
obvious edema of the same sites. The most
severe acute reaction was sudden death.
Late effects including thickening and
shortening of the ears and the development
of hyperkeratotic lesions on the ears and
tail. The mice were also examined for skin

ankle. Recordings were obtained from the
muscle of the first interosseous space.
Supramaximal stimuli (6V) were generated
at a frequency of 8/sec by a Grass SD9
stimulator. The action potentials of mus-
cles were displayed on an oscilloscope and
the latencies measured. Conduction velo-
city was calculated by dividing the distance
between the two stimulation points by the
difference between the two latencies.

MORPHOLOGICAL STUDIES

Groups of 5 control and 5 experimental
DBA/2J mice injected with 150 mg
TPPS/kg body weight were sacrificed 5,
30, 60 and 120 days later. Groups of 5
control and 5 experimental Swiss albino
mice were sacrificed 10 days after the
injection of 80 mg HpD/kg or 1000 mg
ALA/kg body weight. The mice were
anesthetized with methoxyflurane and
then perfused with cacodylate-buffered
paraformaldehyde-glutaraldehyde fixative
through the left heart (Karnovsky, 1965).
Specimens were taken from the right side
(opposite to that on which nerve conduc-
tion velocity was measured) from the
ventral root of the fifth lumbar segment,
the sciatic and pereoneal nerves. All nerves
were postfixed in 1 per cent caccylate-
buffered osmium tetroxide, dehydrated
and embedded in Epon. Grids were stained
with uranyl acetate and lead citrate and
examined under a Philips 400 electron
microscope.
DEMONSTRATION OF EXTRAHEPATIC SYNTHESIS OF PORPHYRINS

Swiss albino mice were injected intravenously, intraperitoneally or subcutaneously with 30 mg ALA/kg of body weight, following which they were examined at regular intervals under longwave ultraviolet light at an intensity of 25 mW/cm². The development of the typical red fluorescence of porphyrins was taken as an indication that substantial amounts of porphyrin had been synthesized as a result of the injection of ALA.

RESULTS

MOTOR NERVE CONDUCTION VELOCITY

TPPS. Figure 2 summarizes the results. Mice injected with saline alone showed a relatively constant mean MNCV ranging from 51 to 55 m/sec throughout the observation period. The muscle action potentials recorded from the interosseous muscle in TPPS injected mice showed increased latencies and decreased amplitudes (Fig. 1). Distal motor latencies were increased in proportion to the slowing of the MNCV (Fig. 1). The general shape of the action potentials did not differ between TPPS and control mice (Fig. 1). The motor nerve conduction velocity in TPPS injected mice showed a rapid decrease. Twenty four hours after the injection of 150 mg TPPS/kg body weight the MNCV was 32.2 ± 1.1 m/sec as compared with 53.1 ± 2.1 in mice injected with saline. The MNCV in all TPPS injected mice was significantly decreased (p < 0.001) 24 hours post injection (Fig. 2). Larger doses of TPPS resulted in a more severe decrease in MNCV than did lower doses (Fig. 2).

HpD. The injection of 100 mg HpD/kg body weight in DBA/2J mice resulted in a rapid decrease in MNCV. The observed decrease to 36.7 ± 3.2 m/sec within 20 hours of the injection was highly significant (p < 0.001). At day 2 the MNCV was reduced to 60% of normal and remained so for approximately one week. Thereafter the MNCV showed a gradual recovery with values not significantly different from normal, 17 days after the injection (Fig. 4).

The injection of 80 mg HpD/kg body weight in Swiss albino mice showed the same rapid decrease in MNCV. The recovery to normal values appeared faster. Hence, 7 days after the injection of MNCV-values were not significantly different from those of control animals (Fig. 3).

TABLE I

<table>
<thead>
<tr>
<th>Dose injected intravenously (mg/kg of body weight)</th>
<th>(a) Non-phototoxic deaths</th>
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(a) Mice held in subdued lighting for 7 days post-injection. All but two deaths occurred within 48 hours of injection, and all deaths occurred within 5 days.
Motor Nerve Conduction Velocity (MNCV) in TPPS Treated Mice

**Figure 2** — Mice were injected intraperitoneally with either saline or various doses of TPPS, and then maintained for 60 days in subdued lighting. MNCVs (tibial nerve) were measured at intervals. Each point represents the mean MNCV ± S.D. of the 5 or 6 mice in each group.

**ALA.** The intraperitoneal injection of 1000 mg ALA/kg body weight into Swiss albino mice caused the same rapid decrease in MNCV. Thus 20 hours after the injection the MNCV was 37.2 ± 1.4 m/sec and highly significant from normal (p < 0.001) (Fig. 3). The lowest values appeared on day 2 after which there was a relative rapid recovery to normal MNCV values 10 days after the injection (Fig. 3).

**Monitored body temperature.** The subcutaneous temperatures fluctuated as the mice recovered from anesthesia. The intraperitoneal temperatures remained stable. They did not differ between control animals and the various experimental groups. Accordingly TPPS injected mice (150 mg/kg) showed a temperature of 32.5 ± 1.2°C; HpD (80 mg/kg) 31.3 ± 1.7°C; ALA 34.4 ± 1.4°C and saline (control) injected mice showed a temperature of 33.4 ± 1.1. It is therefore highly unlikely that the observed decreases in MNCV in experimental animals are due to temperature variations.

**Morphological Studies**

The peroneal and sciatic nerves as well as the ventral root were examined ultrastructurally, at various intervals after the injection of 150 mg TPPS/kg body weight. No pathological changes were detectable 5 days post-injection. The first demonstrable changes were observed 30 days after injection. They consisted of membranous profiles in the axons and the accumulation of floccular substance in the matrix of

**Figure 3** — Mice were injected intravenously with either saline or HpD (80 mg/kg of body weight), or intraperitoneally with ALA (1000 mg/kg of body weight), and then maintained for 10 days in subdued lighting. MNCVs were measured at intervals. Each point represents the mean ± 95% confidence limits of the 9 or 10 mice in each group.

**Figure 4** — Mice were injected intravenously with 100 mg HpD/kg of body weight, and then maintained for 25 days in subdued lighting. MNCVs (tibial nerve) were measured at intervals. Each point represents the mean MNCV ± S.D. of the 6 mice.

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**Experimental Porphyric Neuropathy**

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axonal mitochondria. These structural alterations were found in localized areas within large axons and were more frequent in the peroneal and sciatic nerves than in the ventral root. Large myelinated fibers displayed extensive adaxonal Schwann cell ingrowths into the axoplasm partly enclosing organelle debris (Fig. 5).

Sixty days post injection, large myelinated fibers particularly in the peroneal nerve showed extensive accumulations of membranous profiles, degenerated mitochondria and clear vesicles in the axon. The axons were often sequestered by the adaxonal Schwann cell cytoplasm (Fig. 6). Longitudinal sections showed accumulations of excessive neurofilaments in the paranodal regions. These neurofilaments were arranged in a swirling array and formed a juxtanodal cuff, leaving a central area containing neurotubules and mitochondria (Fig. 7). These axonal swellings appeared strictly localized to the juxtanodal areas and were sometimes large enough to override the adjacent paranodal area of the subsequent internode (Fig. 7). The myelin sheath surrounding the swellings showed attenuation and splitting. In this age-group myelin vacuoles and so called bubbling of myelin were found along the entire internode (Fig. 8). Sequestration of axoplasm was frequently observed and consisted of adaxonal ridges engulfing and invaginating parts of the axoplasm giving rise to islets of axoplasm within the inner lip of the Schwann cell cytoplasm (Fig. 9). This mechanism of axonal sequestra-

Figure 5 — Peroneal nerve 30 days after injection of 150 mg TPPS/kg body weight. Large myelinated fiber shows a complex array of adaxonal processes extending into the axoplasm (arrows). The axon contains organelle debris. 5325 x.

Figure 6 — Sciatic nerve 60 days after injection of 150 mg TPPS/kg body weight. The axon is interrupted and contains an abundance of organelle debris. The myelin sheath shows disruption and splitting of lamellae. 8280 x.

Figure 7 — Peroneal nerve 60 days after injection of 150 mg TPPS/kg body weight. Paranodal area showing juxtanodal axonal swelling due to the accumulation of neurofilaments. The axons show centrally located channel containing neurotubules and mitochondria (arrow-heads). Note distortion of the nodal gap due to the over-riding cuff of the swollen axon (arrows). 9200 x.

Figure 8 — Myelinated internode of the sciatic nerve 60 days after injection of 150 mg TPPS/kg body weight. Note atrophied axon containing altered mitochondria. The myelin sheath shows splitting and intramyelinic vacuoles. 5,980 x.

Figure 9 — Peroneal nerve 60 days after injection of 150 mg TPPS/kg body weight. Longitudinal section shows sequestered organelles in the inner lip of the Schwann cell. 16,000 x.
tion was very occasionally found in control animals but was not nearly as frequent as in experimental animals. In the mice injected with TPPS 120 days previously the most striking lesion was marked shrinkage of axons surrounded by delaminated and desquamated myelin sheaths (Fig. 10). Regeneration and remyelination were not observed.

The described changes were most frequently found in the peroneal nerve (Fig. 11) and least frequently in the ventral root (Fig. 12), although they were qualitatively similar.

Examination of the same nerves ten days after a single dose of either HpD or ALA revealed no structural changes.

DIRECT TOXICITY STUDIES

Table 1 summarizes data from an experiment in which DBA/2J mice were injected intravenously with various doses of either TPPS or HpD. It is apparent that HpD is more toxic than TPPS, on a per mg basis. However, since the dodecahydrated sodium salt of TPPS has a molecular weight almost double that of HpD, the toxicities of the two porphyrins on a molar basis were not strikingly different. It was noted that the mice began to die only when the doses of porphyrin became large enough to overload the porphyrin-binding capacity of the plasma proteins. As might be expected if serum-bound porphyrin is relatively non-toxic, we found that the toxicity of intraperitoneal injections of HpD was greater than that of intravenous injections, and that this toxicity could be reduced significantly by adding serum albumin to HpD prior to injection.

No deaths resulted from intraperitoneal injections of ALA at doses up to 1000 mg/kg of body weight.

PERSISTENCE OF PORPHYRIN FOLLOWING INJECTION

Intraperitoneal injections of 1000 mg ALA/kg of body weight into Swiss albino mice caused skin fluorescence and a mild skin photosensitivity which began immediately post-injection and could be detected for approximately 5 weeks. After smaller doses the fluorescence was weaker and lasted for an appropriately shorter time. Swiss albino mice injected intraperitoneally with 150 mg TPPS/kg of body weight 51 days prior to testing were killed within 20 minutes by whole-body exposure to Pyrex-filtered sunlight. After 86 days, exposure to sunlight for only 5 minutes was sufficient to cause non-lethal phototoxic reactions. Even after 107 days the mice still developed swollen ears, tails and faces after a 30 minute exposure, and the serum still showed fluorescence. The intraperitoneal injection of 500 mg of beta-carotene per kg of body weight 5 hours prior to exposure to the filtered sunlight resulted in a substantial degree of protection against the phototoxic reactions.

In DBA/2J mice, definite serum fluorescence persisted for at least 96 days in mice given as little as 50 mg of TPPS/kg of body weight. Phototoxic damage in the DBA/2J mice was limited to the ears, the periorbital tissues, the nose, and the tail, since the dark fur protected the rest of the body from exposure. The severity of phototoxic damage was directly proportional to the amount of TPPS injected.

Microscopic examination of the ears showed extensive fibroblast proliferation in the dermis with a diffuse mononuclear inflammatory reaction and prominent vascular proliferation. The

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Figure 10 — Ventral root 120 days after the injection of 150 mg TPPS/kg body weight. Myelinated fiber showing marked axonal atrophy. The redundant myelin sheath shows delamination. 21,450 x.

Figure 11a — Peroneal nerve 60 days after the injection of 150 mg TPPS/kg body weight. Large myelinated fibers show axonal swellings with attenuated myelin sheath (arrows). 1408 x.

Figure 11b — Peroneal nerve of age-matched control mouse. 1408 x.
epidermis showed focal separation from dermis at the basement membrane, so called bullae.

**EXTRAHEPATIC SYNTHESIS OF PORPHYRINS**

None of the mice injected either intravenously or intraperitoneally with 30 mg ALA/kg of body weight developed any detectable skin fluorescence. However, those mice which had been given a subcutaneous injection localized to either the anterior third or posterior half of the tail showed the typical red fluorescence of porphyrin at the injection site, but nowhere else. The fluorescence was well-developed within 2 hours of the injection, and lasted for slightly less than 24 hours. Control mice given comparable amounts of citric acid as localized subcutaneous injections did not develop detectable fluorescence at the injection sites.

**DISCUSSION**

The injection into mice of a single large dose of ALA (1000 mg/kg) resulted in photosensitivity and skin fluorescence which lasted approximately 1 day, and a rapid decrease in MNCV lasting approximately one week before it recovered to normal values. No structural changes could be demonstrated in the peripheral nerves after this injection. The injection of a single large dose of HpD (100 mg/kg) resulted in photosensitivity and skin fluorescence which lasted approximately 5 weeks, and a rapid decrease in MNCV which lasted less than 3 weeks before recovering to normal values. Structural changes of peripheral nerves failed to develop after a single injection of HpD. The injection of a single large dose of TTPS (150 mg/kg) resulted in photosensitivity and skin fluorescence which lasted more than 3 months, serum fluorescence that persisted for even longer, a rapid decrease in MNCV which appeared to be irreversible. First one month after the injection the gradual development of morphological abnormalities in the peripheral nerves could be demonstrated. These results strongly suggest that ALA, HpD and TTPS are capable of inducing a metabolic disturbance in peripheral motor nerves resulting in impaired propagation of the electrical impulse. Since the decreased MNCV recovered to normal after the injection of single doses of ALA or HpD, and since no structural abnormalities developed after these injections, it can be assumed that the metabolic derangement was reversible as the substances cleared from the circulation and tissues. TTPS on the other hand persisted in the circulation for a considerably longer time allowing it not only to cause an irreversibly decreased MNCV but also later occurring structural changes.

The slowing of the nerve conduction velocity in these experiments was accompanied by decreased amplitude of the action potential. The decreased MNCV is most likely the result of a conduction block of large myelinated fibers.

In TTPS-mice the earliest pathological changes were found in axonal mitochondria one month post-injection. These changes were followed by axonal sequestration and paranodal accumulation of deranged neurofilaments, an indication of impaired axonal transport. Changes also occurred in the myelin sheath, such as vacuolization and myelin bubbling, which appeared to be independent of the axonal pathology. Later changes were characterized by axonal atrophy accompanied by secondary myelin breakdown. These changes involved mainly large myelinated fibers and showed a clear gradient of severity with the most severe changes in the peroneal nerve and least severe in the ventral root. Both the qualitative and the quantitative changes were consistent with a dying back phenomenon. A similar sequence of events has been demonstrated in hexacarbon neuropathies (Spencer and Schaumburg, 1976; Sabri et al., 1979), and in different experimental models of diabetes mellitus (Sima and Robertson, 1979, Sima, 1980). Certain other changes such as vacuolization of the myelin sheath are similar to changes characteristic of lead neuropathy (Lampert and Schochet, 1968).

Comparable electrophysiological and morphological changes have been reported in humans with acute intermittent porphyria. Flügel and Druschky (1977) measured various electrophysiological parameters in such patients and concluded that the severity of the decrease in MNCV in the tibial nerve was directly related to the severity of the disease, and that it showed the characteristics of a primary axonopathy rather than that of primary demyelination. Recent morphological studies support this con-
clusion (Cavanagh and Mellick, 1965; Kaeser, 1965; Cavanagh and Ridley, 1967), although there certainly have been studies which support a mechanism of primary segmental demyelina-
tion (Denny-Brown and Sciarrà, 1945; Gibson and Goldberg, 1956) and others which indicate that both mechanisms may be operative (Anzil and Dozic, 1978). Wochnik-Dyjase et al (1978) presented data indicating that a decrease in MNCV was present in some patients following acute attacks associated with clinical evidence of motor polyneuropathy, and that both the electrophysiological and the clinical abnormalities were sometimes transient, the measurements returning to normal some time after the termi-
nation of the acute attack. They concluded that the neurological ab-
normalities caused by porphyria are strictly functional at first, but that prolonged or repeated acute attacks can lead to permanent damage in the form of axonal degeneration. This is in keeping with the results of the present experimental model.

Although we can reproduce in mice both functional and morphological changes similar to those observed in humans who develop porphyric neu-
ropathy, this by itself does not prove that our animal model accurately reproduces the pathogenesis of por-
phyric neuropathy at the biochemical level. The biochemical basis for por-
phyric neuropathy is not well understood at present. However, since porphyric neuropathy is commonly found in association with acute inter-
mittent porphyria, in which the concentration of porphyrins in the circulation is not usually elevated, it has generally been assumed that porphyrins as such could not be responsible for the neuropathy. Con-
sequently, suspicion has been directed primarily at the porphyrin precursors ALA and PBG, abnormally large amounts of which are synthesized by the liver and released into the circulation.

It has been demonstrated in orga-
notypic tissue culture (Whetsell et al, 1978) that porphyrins can be synthe-
sized from ALA by both Schwann cells and neurons. This is not surprising since all cells that contain the bio-
chemical machinery necessary for synthe-
izing heme-containing enzymes might be expected to produce porphyrins from ALA. Extrahepatic synthesis of porphyrins was also demonstrated in the present study.

In the present experimental design the question arises however, how ALA and porphyrins get access to the peripheral nerves. Two possibilities are plausible. It would be possible that the substances may pass the blood-
nerve-barrier and thereby get access to the endoneurium. However, it is known that ALA and probably porphyrins do not cross the blood-
brain-barrier (McGillion et al, 1973; Shanley et al, 1975). It may also be possible that the substances are taken up at unprotected sites of the periph-
eral nerves such as the motor end plates. They would then be transported in a retrograde fashion in the same way as certain viruses, toxins and other macromolecules gain access to the nervous system (Ryser, 1970; Haber-
mann, 1977). These possibilities are at the present time being investigated (Sima).

The effect of ALA, HpD and TPPS on the peripheral nerve metabolism is not known and can only be speculated upon. It has been suggested by Labbe (1967) that porphyrins may inhibit the incorporation of structural proteins into mitochondrial membrane and thus cause interference with mito-
chondrial function. On the other hand, the possibility exists that HpD and TPPS may exert a feedback inhibition of \( \delta \)-ALA-dehydrase (cf Bacchus, 1976) and thereby indirectly elevate the ALA concentration.

Delta-ALA is known as a potent (Na\(^+\))-ATP-ase inhibitor (Becker et al, 1971; Niklowitz, 1977). It may therefore very well impoverish the membranous ion handling and prevent the transmission of the electric impulse. In this context it is interesting to note that one of the underlying mechanisms of lead neuropathy is thought to be the same, since lead also inhibits \( \delta \)-ALA-dehydrase (Bacchus, 1976). In the present study structural changes of the myelin were demonstrated that are similar to those of lead neuropathy (Lampert and Schochet, 1968).

We therefore suggest as a working hypothesis, until further knowledge has been gathered, that both ALA and porphyrins should be considered as inducers of the abnormalities of nerve function and morphology in porphryc neuropathy.

In summary the present study has demonstrated for the first time func-
tional abnormalities of motor peripheral nerves in mice after the injection of ALA, HpD and TPPS. In addition TPPS caused later occurring struc-
tural peripheral nerve changes indica-
tive of an axonal neuropathy of dying back type, not dissimilar from human porphyric neuropathy.

The present models may therefore be useful experimental tools for the study of porphyric neuropathy and will permit detailed neurophysiologi-
ical, biochemical, structural and mor-
phometric studies to be carried out in a sequential fashion.

However, this work has significance in another area also. Photoradiation therapy for cancer is an experimental form of therapy which appears to have substantial advantages over conven-
tional radiation therapy in certain clinical situations. Most of the clinical trials which are in progress at present make use of HpD for semi-selective photosensitization of the malignant tissue (Kelly and Snell, 1976; Dougherty et al., 1978; Kennedy, 1979), but the replacement of HpD by TPPS has been strongly advocated (Tsutsui et al., 1975) since a substantial amount of work in experimental animals and in vitro indicates that TPPS is clearly superior to HpD in some important respects (Winkelman, 1962 and 1967; Winkelman and Hayes, 1963; Wink-
elman et al., 1967). However, our finding that TPPS can persist in the circulation of mice for months follow-
ing a single injection and can lead to a persistent decrease in MNCV and the gradual development of structural changes in peripheral nerves is a warn-
ing that TPPS could be a dangerous substitute for HpD. We therefore strongly urge that electrophysiological and morphological studies of peri-
nervous nerves be included in the pre-
clinical evaluation of any other natural or synthetic porphyrin which may be considered for use as a sensitizer in photoradiation therapy for cancer.
ACKNOWLEDGEMENTS

The assistance of Dr. L. Kerur with the MNCV measurements and the technical skills of Mrs. Kazy Hay are gratefully acknowledged. We wish also to thank Dr. G.S. Marks for his stimulating and informative discussions.

Supported by Grants MA-8587 and MA-7117 from the Medical Research Council of Canada, Grant No. 352 from the Ontario Cancer Treatment and Research Foundation, and a grant from the National Cancer Institute of Canada.

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