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Pathology of local anesthetic-induced nerve injury*

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Summary. Nerve fiber injury and endoneurial edema were induced by the injection of the local anesthetic 2-chloroprocaine, tetracaine, procaine, etidocaine or mepivacaine into the soft tissue and fascia surrounding the sciatic nerve of Sprague-Dawley rats. Light microscopy demonstrated that the perineurial barrier was not mechanically damaged by the surgical procedure but, at 48 h post-injection, perineurial permeability was increased. Previous observations of leakage of horseradish peroxidase and the present report of neutrophils and eosinophils in the endoneurium indicate a disruption of blood-nerve barrier systems. Endoneurial edema was observed in the subperineurial, interstitial and perivascular regions. Axonal degeneration and demyelination occurred; the latter associated with accumulation of large lipid droplets in Schwann cells. Degranulation of mast cells, proliferation of fibroblasts and macrophage activity were noteworthy in affected areas. The findings are remarkable in that this is the first model of endoneurial edema by a neurotoxin which penetrates the perineurium, disrupting barrier system and inducing nerve fiber injury.

Key words: Anesthetics, local – Peripheral nerves – Nerve degeneration – Histology – Pathology

The clinical risk of local anesthetic neurotoxicity is generally assumed to be minimal; thus, the majority of regional anesthesia morbidity has been attributed to needle trauma or inadvertent vascular injection. Several histological studies have supported this impression [5, 6, 13, 22]. For example, Gentili et al. [5, 6] and Selander et al [22] have demonstrated the absence of damage to peripheral nerves examined at least 10 days after extraneural exposure to clinical concentrations of local anesthetics. However several clinical reports [11, 16, 19] of long-lasting neurological deficits secondary to epidural Nesacaine-CE (2-chloroprocaine) in the past few years have prompted a reevaluation of the neurotoxic potential of local anesthetic solutions.

Different experimental studies of spinal cord and nerve, have variously led to conclusions that nerve injury was produced by all local anesthetics [5, 6, 10, 20], some local anesthetics [1, 13, 17, 22], or some other property of local anesthetic solutions [17, 21, 23]. Recently, Gissen et al. [7] provided data in support of this last hypothesis that much of the reported toxicity could be attributed not to the local anesthetics, but to the low pH and antioxidant sodium bisulfite which are found in Nesacaine-CE.

Our laboratory has conducted additional studies on the role of pH and sodium bisulfite in injury to rat sciatic nerves following exposure to various solutions with and without the local anesthetics [8, 12]. Using a clinically relevant in vivo experimental design, we found that 48 h after the extraneural application of several different local anesthetics including 3% 2-chloroprocaine, 1% tetracaine, and 2% lidocaine, both axons and Schwann cells were damaged and the accumulation of endoneurial fluid was of sufficient severity to increase endoneurial fluid pressure. Since no nerve injury was produced by control solutions consisting of pH-adjusted vehicles for local anesthetics, these comparisons clearly implicated the local anesthetics. It is particularly noteworthy that both tetracaine and lidocaine solutions produced edema greater than control solutions, although these two solutions are not as acidic as Nesacaine-CE. The apparent inconsistency with the results of Gissen et al.

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may reflect different aspects of neurotoxicity and its temporal manifestations.

The present studies were designed to provide a more extensive evaluation of the pathological consequences of extraneural application of various clinical local anesthetic preparations. In this report, we describe the morphology of nerve fiber and Schwann cell lesions occurring after several local anesthetics were applied to the surface of rat sciatic nerves.

Materials and methods

Eighty-three female Sprague-Dawley rats (250–400 g) were used in these experiments. The animals were anesthetized by intraperitoneal injection of a mixture of Nembutal (sodium pentobarbital, 50 mg/ml), Valium (diazepam, 5 mg/ml) and 0.9% saline, in volume proportions of 1:1:2. Both sciatic nerves of each rat were exposed by lateral incision of the thigh and reflection of the fascia and underlying muscle. Using a 30-gauge needle, each solution to be tested was injected in a volume of 1 ml beneath the clear fascia surrounding the nerve but external to the epineurium with extreme care taken to avoid nerve trauma.

The solutions of local anesthetics and their commercial pH ranges included (a) Nesacaine-CE (3% 2-chloroprocaine hydrochloride, 0.2% $NaHSO_3$, and pH 2.7–4.0) (number of nerves = 22), (b) Novocain (10% procaine hydrochloride, 0.4% acetone $NaHSO_3$, and pH 3.0–5.0) ($N = 6$), (c) Pontocaine (1% tetracaine hydrochloride, 0.2% acetone $NaHSO_3$, and pH 3.2–6.0). ($N = 12$), (d) Carbocaine (2% mepivacaine hydrochloride, 0.1% methylparaben, and pH 4.5–6.8) ($N = 14$), and (e) Duranest (1.5% etidocaine hydrochloride, 0.05% sodium metabisulfite, and pH 3–4.5) ($N = 13$). In addition to 2-chloroprocaine, the selected agents are examples, respectively, of low and high potency ester and amide local anesthetics. Controls consisted of nerves exposed to distilled water ($N = 16$), 0.9% bacteriostatic saline ($N = 26$), and the vehicle used for Nesacaine-CE consisting of 0.2% sodium chloride, 0.2% sodium bisulfite and adjusted to pH less than 4.0 ($N = 27$). For tests of concentration dependence of nerve injury, nerves ($N = 30$) were challenged with procaine HCl solutions of 0, 2, 3.5, 6 or 10% freshly-prepared with distilled water. Drug injections were made by an investigator who was "blinded" to the solution being administered.

Following drug injection, the wounds were closed with metal clips and the rats allowed to recover for 48 h. Nerves were excised under the anesthetic described above and fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer at a pH of 7.4. After glutaraldehyde fixation for 24 h, nerves were rinsed in buffer and postfixed for 3 h in 1% osmium tetroxide. The tissue was subsequently dehydrated in serial alcohol solutions and later in propylene oxide prior to infiltration with araldite. Blocks were sectioned for light microscopy and stained with either paraphenylenediamine or methylene blue-azure II. Electron microscopy was performed on selected blocks after preparation of ultrathin sections stained with uranyl acetate and bismuth subnitrate. Sections were examined in a Siemens 101 ultramicroscope operating at 80 kV.

Concentration dependence of nerve injury was tested using a subjective scoring system for each of three measures of local anesthetic effects. Using a light microscope, an investigator, blinded to the identity of the test solution, assigned a score of 0, 1, or 2 to the largest fascicle of each nerve. Maximum severity was assigned a score of 2, a score of 0 indicated no injury, and moderate or equivocal injury was indicated by a score of 1. The 3 measures of interest were edema ("structureless space"), nerve fiber injury (degeneration and demyelination), and lipid droplets (osmiophilic inclusions in epineurial, perineurial, and endoneurial cells).

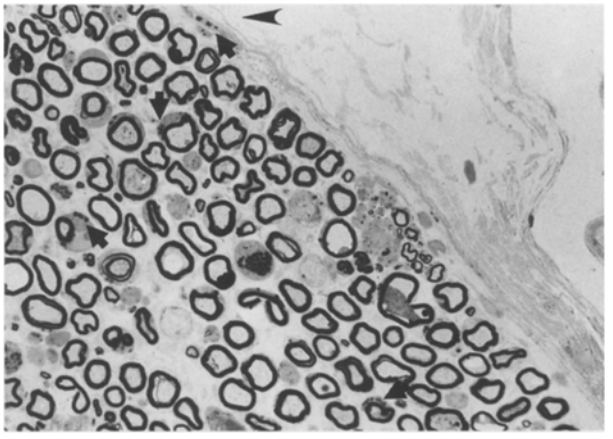

Fig. 1. Light micrograph of araldite section from rat sciatic nerve. Nerve pathology observed at 48 h after the extraneural administration of 10% Novocain included a thinning and disorganization of the perineurium (*arrowhead*). Darkly staining intracytoplasmic inclusions (*arrows*), consisting of lipid droplets can be seen both in Schwann cells and in the perineurium. Several degenerating axons are also present in this filed. Stained with paraphenylenediamine, ×457

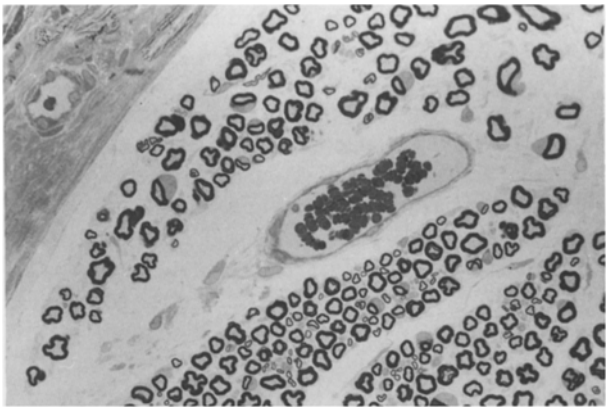

Fig. 2. Rat sciatic nerve 48 h after extraneural injection of 3% Nesacaine. Note marked edema, visualized as "structureless space", in perivascular, subperineurial and interstitial regions. The 1-μm-araldite section was stained with paraphenylenediamine, ×294

Results

Pathological changes were produced by all of the local anesthetics studied, and the nerve injury was observed to be dose-dependent in a study of various concentration of procaine HCl. Light microscopy at 48 h after exposure to local anesthetics demonstrated

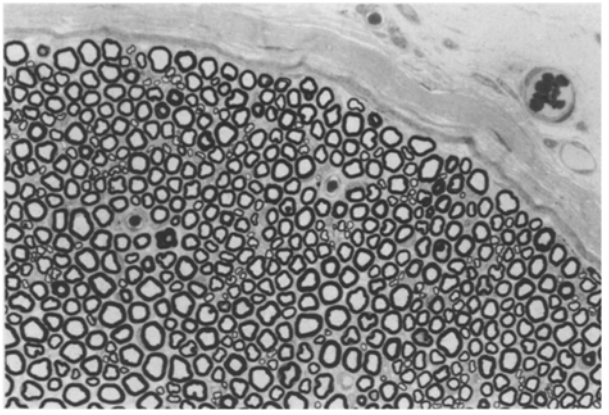

Fig. 3. Cross-section of rat sciatic nerve 48 h after exposure to control solution consisting of 0.9% saline, × 294

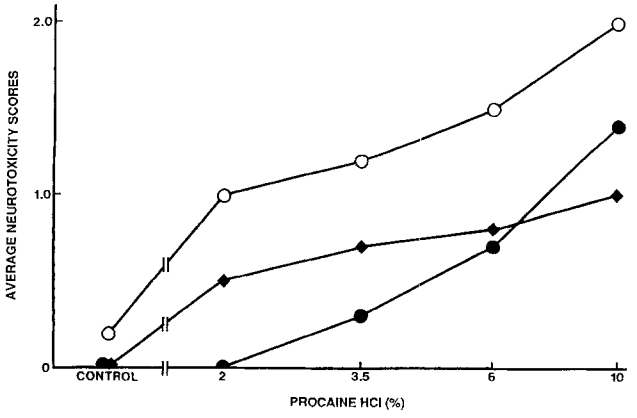

Fig. 4. Concentration-dependent increase in endoneurial edema (*open circles*), nerve fiber injury (*diamonds*), and lipid droplets (*closed circles*) at 48 h after extraneural exposure to procaine hydrochloride

moderate to severe evidence of nerve fiber injury, as well as endoneurial edema (Figs. 1, 2). Control nerves treated with saline or drug vehicles did not show any edema (Fig. 3). Edema fluid, visible as structureless space, accumulated principally in the subperineurial region but was found in all parts of the endoneurial compartment including perivascular spaces within 48 h of application (Fig. 2). Although nerve injury was found with all local anesthetics, the degree and extent of nerve injury varied. Both edema and nerve injury were typically greatest subjacent to the site of local anesthetic injection, but the most severe edema was not always found with the most severe nerve injury.

Nerve fiber injury, endoneurial edema, and lipid droplet formation were all produced in a dose-dependent fashion. Examination of nerves exposed to various concentrations of procaine HCl indicated a coincident increase in edema, lipid, and nerve injury (Fig. 4).

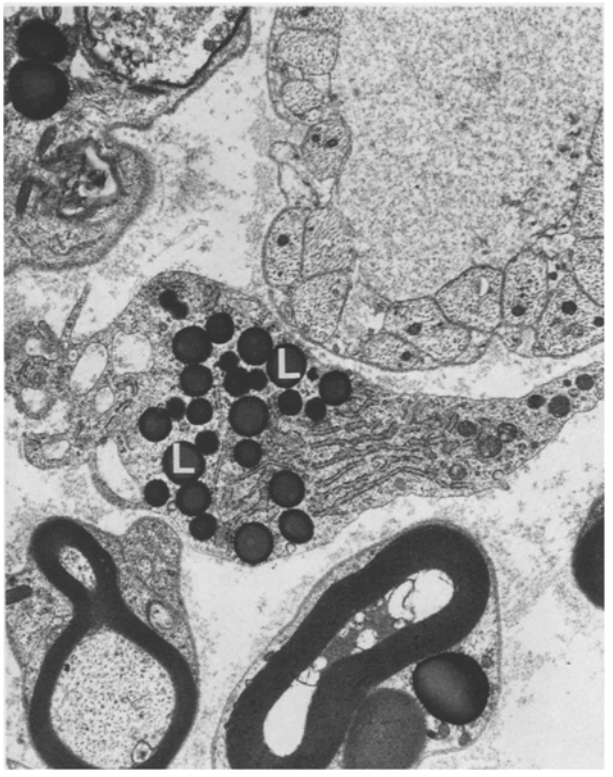

Fig. 5. Electron microscopy showed many changes in fibroblasts including elaboration of many cytoplasmic processes and lipid droplets (*L*). Tissue was obtained from rat sciatic nerve 48 h after initial exposure to extraneural etidocaine, × 8,099

Diffuse axonal swelling consistent with acute axonal degeneration was apparent as well as cytoplasmic changes in swollen Schwann cells, which were often packed with osmiophilic inclusions (Fig. 1). These spheroid inclusions were also frequently noted in perineurial cells and in the epineurial region surrounding the nerve. In control nerves, these droplets were not normally seen in perineurial or endoneurial cells, although epineurial cells often were affected. Occasionally, focal thinning and disaggregation of the perineurium and subperineurial fibrosis were seen subjacent to the injection site (Fig. 1).

Electron microscopy confirmed the presence of edema which appeared as structureless space in the endoneurial interstitium. In affected regions, nerve injury was manifested by both mast cell degranulation and reactive fibroblasts, which often characteristically included arrays of thin cytoplasmic processes as well as increased endoplasmic reticulum and abnormal cellular configurations (Fig. 5). Additionally, a peculiar abnormality noted in some nerves exposed to local anesthetic was the appearance of eosinophils (Fig. 6) in the endoneurium. These cells were particularly conspicuous in the subperineurial space and appeared in areas where edema was most pronounced. Eosinophils

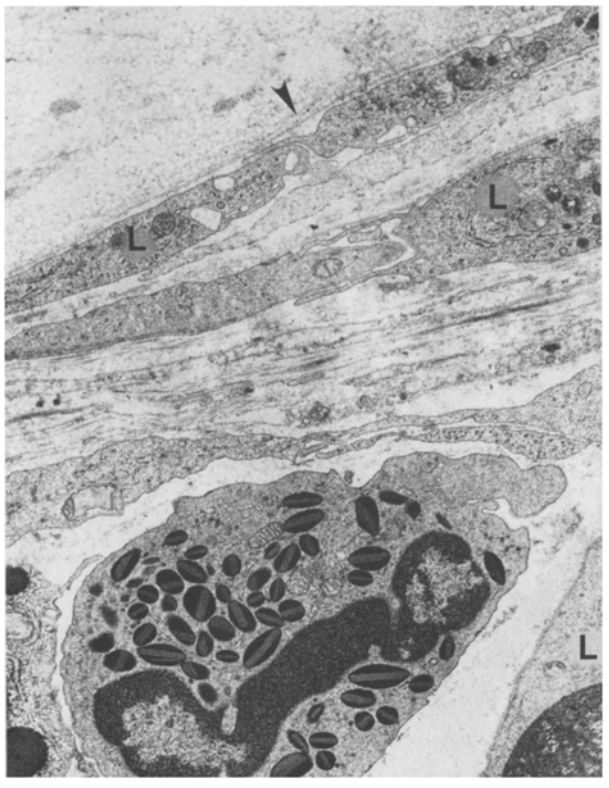

Fig. 6. Thin section from rat sciatic nerve at 48 h after the extraneural administration of 3% Nesacaine. Injury to the perineurial barrier is indicated by a loosening and disaggregation of the perineurial sheath, and separation (*arrowhead*) of the basal lamina from perineurial cells. Further evidence of altered permeability of endoneurial barrier systems is the subperineurial infiltration of an eosinophil, identified by its characteristic bar granules. Lipid inclusions (*L*) are seen both in perineurial and endoneurial cells. Stained with uranyl acetate and bismuth subnitrate, ×10,800

were distinguished from neutrophils by their electron-dense granules which contained central bars. Other inflammatory cells, including neutrophils, were also seen.

Both axonal dystrophic changes and some demyelination were seen by electron microscopy. Axonal degeneration was manifested by swelling and accumulations of masses of darkly staining organelles, vesicles and filaments (Fig. 7). Reactive changes were also seen in Schwann cells consisting of increased amounts of endoplasmic reticulum. Disintegrating cells contained swollen degenerating organelles, membranous debris, and lipid droplets (Fig. 8).

Schwann cells often included pale-staining intracytoplasmic inclusions typical of lipid deposits or Elzholz bodies (Fig. 8). These deposits were characteristically homogenous rather than laminated as seen with Elzholz bodies. The lipid inclusions seen in both normal appearing Schwann cells and in the disintegrating Schwann cells were also observed in endoneurial macrophages and fibroblasts and in perineurial cells. They were not seen in axons. In addition to the presence of lipid droplets in perineurial cells, other abnormalities were noted, including disaggregation of the perineurium and separation of the basement membrane (Fig. 6). Tight junctions between perineurial cells could not be visualized in affected regions.

Discussion

Fine structural analysis of local anesthetic neurotoxicity reveals that both injury to nerve fibers and disturbances of the endoneurial microenvironment occur when local anesthetics are applied to the exterior of peripheral nerves. Although previous research [5, 6] indicated that axonal injury occurred as well as altered vascular permeability, it was possible that traumatic injury due to intrafascicular or intraneural injection may have produced these changes as artifacts [3]. The methods employed in the present series of experiments avoided needle trauma and confirm that extraneural injection results in nerve fiber injury and indicate that these agents can penetrate the perineurial sheath and disturb the barrier system creating endoneurial edema of sufficient severity to increase endoneurial fluid pressure [12]. This opening of the perineurial barrier not only represents a direct neurotoxicity of the local anesthetic agents, but also would provide direct exposure of endoneurial blood vessels to the local anesthetics in relatively high concentrations. Having already shown altered perineurial permeability to intravenous horseradish peroxidase (HRP) that had leaked from the epineurial circulation [12], we present additional evidence of a permeability change with the observation that eosinophils and polymorphonuclear neutrophils – not normal constituents of the endoneurium – infiltrate the endoneurial interstitium, subjacent to the site of local anesthetic administration.

The present observations of neurotoxicity are not consistent with the view that nerve fiber injury is a consequence of only some local anesthetics [1, 13, 17, 22] or only a particular local anesthetic vehicle [7]. Specifically, five different local anesthetics were observed to produce a comparable spectrum of nerve injury. The anesthetics included both low- and high-potency ester and amide linkage local anesthetics; pH ranged from 2.7 (Nesacaine) to 6.8 (Carbocaine); and the antioxidant sodium bisulfite was an ingredient of some, but not all, preparations.

The time of nerve injury with local anesthetics in this model provides a possible basis for the difference between the present results and those of others [1, 5,

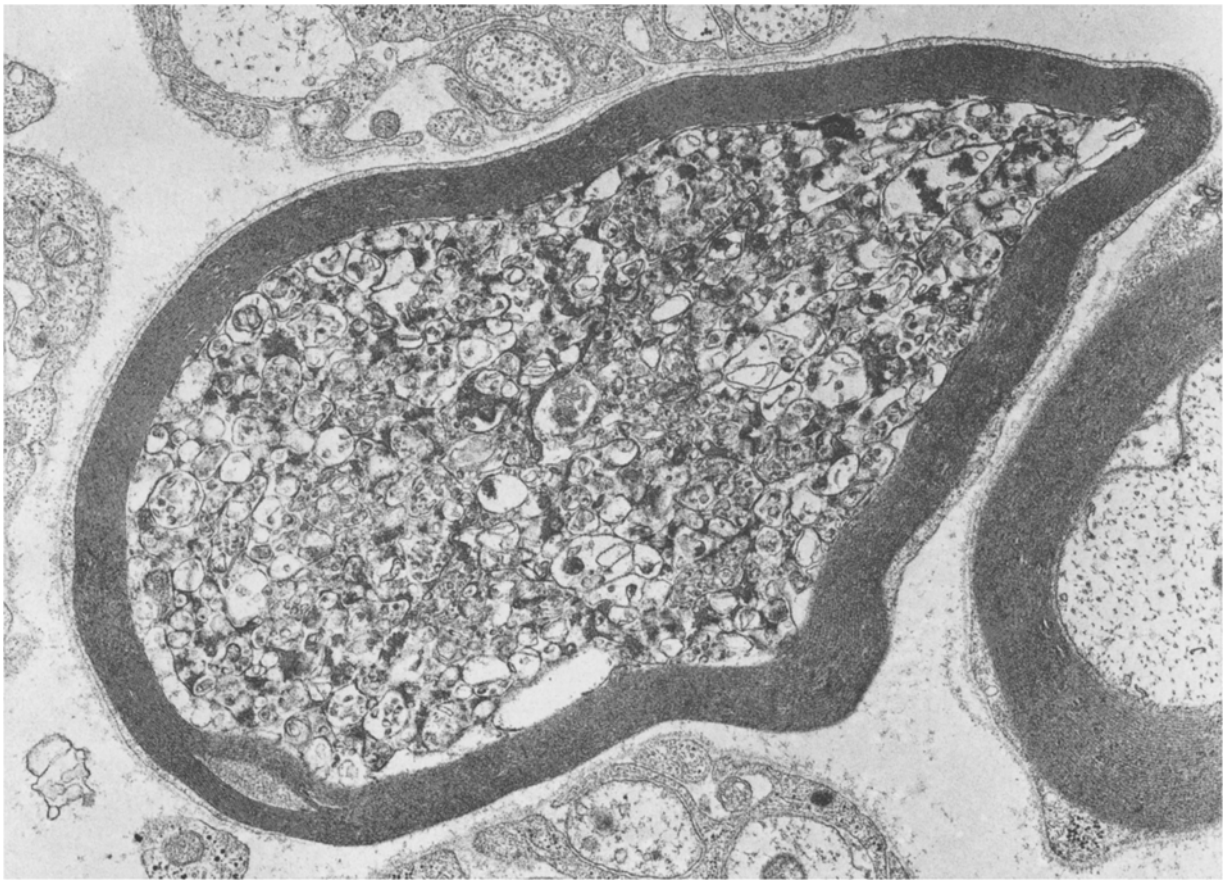

Fig. 7. Dystrophic axon from a rat sciatic nerve 48 h after extraneural exposure to 3% Nesacaine. The axoplasm is packed with abnormal organelles and the myelin sheath is inappropriately thin for the axonal caliber. Thin section was stained with uranyl acetate and bismuth subnitrate, × 19,000

6, 7]. Barsa et al. [1] and Gentili et al. [5, 6] conducted their experiments at least 10 days after exposure, while Gissen et al. [7] actually produced only an acute exposure of 15 min followed by thorough washing of the tissue, thus avoiding the possibile development of neuropathology such as described here. If Gissen's approach is viewed as a "minimal" local anesthetic exposure, then the toxic effect of low pH and sodium bisulfite may be valid only in the in vitro situation in which the normal body fluids are not present to provide a timely dilution or buffering of the acidic fluid.

Endoneurial edema as a result of altered perineurial permeability has only recently been described [12]. Four pathophysiological mechanisms of nerve edema have previously been recognized [14] but none of them involved primarily the perineurium. Electron microscopy of local anesthetic-treated nerves in this study also showed subtle morphological changes of perineurial cells (Fig. 6), including lipid inclusions consistent with a toxic effect, attenuation of the perineurial sheath, separation of individual perineurial cells and lack of tight perineurial junctions. The significance of the perineurial breakdown and edema is supported by the parallel increase in degeneration associated with increasing doses of local anesthetic.

Lipid deposits in Schwann cells were conspicuous in the present study and represent a distinctive morphological finding. Schwann cell cytoplasm was characteristically packed with lipid droplets and they were often associated with disintegrating cells. Lipid was also noted in fibroblasts, macrophages, and perineurial cells, but did not appear in axons. Elzholz bodies, which are a type of lipid droplet found in normal Schwann cells, are infrequent, usually solitary, and laminated. In normal cells, particularly hepatocytes, lipid inclusions are presumed to be storage depots for short carbon chains, which might be used for the later synthesis of lipophilic cellular components [4]. Although such oil droplets are only rarely seen in the peripheral nervous system [9], they have been observed in nerve injury produced by crush, lysophosphatidyl choline, and compound 48/80 [2], and are

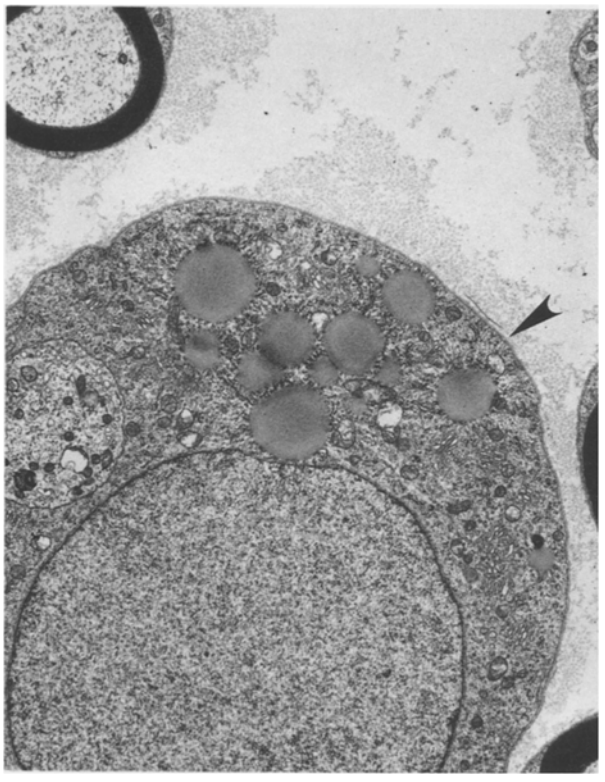

Fig. 8. Electron micrograph of swollen Schwann cell (note identifying basal lamina, *arrowhead*). This thin section was obtained from a rat sciatic nerve 48 h after the extraneural injection of local anesthetic. The numerous intracytoplasmic vacuoles consisting of non-membrane bounded, faintly osmiophilic material are characteristic of lipid (*L*). Stained with uranyl acetate and bismuth subnitrate, ×7,921

apparently characteristic of Wallerian degeneration of myelinated nerve fibers. The lipid inclusions reported with most models of nerve injury apparently differ from those reported here in that local anesthetic-induced nerve injury produces non-membrane-bounded droplets. The large numbers of such deposits in the present experiments suggest a direct toxic effect. Other toxins such as carbon tetrachloride are known to induce neutral fat accumulation in hepatic and renal cells. Increased lipid peroxidation has been cited as a possible mechanism [18]. The present results are consistent both with a local anesthetic-mediated alteration in lipid metabolism and with an accumulation of lipophilic metabolites of the anesthetic molecules, but the precise mechanism of the lipid droplet formation is not known.

Demyelination was frequently observed in association with lipid accumulation in Schwann cells but axonal injury also occurred [12]. The mechanism of axonal injury is likely to be directly related to administration of the toxin. Axonal degeneration may occur as a result of increased endoneurial fluid pressure, but this complication is slower in onset [12], whereas abnormal axons in the present study appeared earlier than 48 h after administration. Since edema formation and increased endoneurial fluid pressure may develop secondary to Wallerian degeneration, it is possible that local anesthetics exert a direct toxic effect on nerve fibers which precedes the subsequent development of edema at 48 h.

Within the endoneurial interstitium in these anesthetic-treated nerves, a variety of cellular abnormalities was seen. These included endoneurial macrophages and fibroblasts often packed with lipid droplets as well as other inflammatory cells. In addition there were occasional polymorphonuclear cells and eosinophils. Eosinophils are not normally found in peripheral nerve and are not associated with inflammatory neuropathies such as allergic neuritis. They have previously been described in peripheral nerves of the twitcher mouse, a genetic mutant model for Krabbe's leukodystrophy [15]. In the latter condition their presence is unexplained but occurs in the presence of a permeability disorder. Their presence in the endoneurium of nerves exposed to extraneural anesthetic can best be explained as the result of altered permeability associated with extraneural injection of a foreign material. This is consistent with the present observation of disaggregation of the perineurial sheath.

Altered perineurial permeability with local anesthesia is of further interest, because this model may be used to elucidate the mechanisms of local anesthetic action. Although there are pharmacological explanations for local anesthesia, less information is available about mechanisms through which anesthetics reach their targets and about untoward effects on target organs and supporting cells. The possibility that local anesthetics might provide an early or immediate breakdown of the perineurial barrier would imply that the entry of these agents into the nerve would be markedly enhanced by unimpeded diffusion rather than the alternative passage through perineurial cells.

Since the changes reported here appear to be maximal at 48 h, their relevance to the long-lasting clinical neurological deficits [11, 16, 19] may be based in individual differences which might potentiate mechanisms of nerve damage or in undetermined factors which produce an abnormally elevated local concentration of the anesthetic agent. Taken together, the present results have general clinical relevance in that it has been demonstrated that a wide-spectrum of local anesthetics are neurotoxic, that the nerve injury includes a breakdown of the perineurial barrier, and finally that the pattern of neuropathology is characterized in part by cytoplasmic lipid droplets,

which have not previously been described following local anesthetic-induced nerve injury.

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