RETARDATION OF PERIPHERAL NERVE MYELINATION IN MICE TREATED WITH INHIBITORS OF CHOLESTEROL BIOSYNTHESIS

A Quantitative Electron Microscopic Study

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ABSTRACT

The effect of two inhibitors of cholesterol biosynthesis, triparanol and AY 9944, on peripheral nerve myelination, was studied. Suckling mice were intraperitoneally injected with both drugs on 3 consecutive days and were sacrificed 6 hr after the last injection; others were suckled by an injected mother and sacrificed at 2½ days of age. A single mouse which had been injected with both drugs at 1, 2, and 3 days of age was sacrificed 2 wk after the last injection.

Membranous and crystalline intracytoplasmic inclusions were observed in the Schwann cells of the sciatic nerves of all the experimental animals. Both the number of unmyelinated single axons and the number of myelin lamellae around each myelinating axon in the sciatic nerves were recorded for treated mice and of mice suckled by treated mothers.

The sciatic nerve of the experimental mice contained a larger proportion of unmyelinated single axons and smaller numbers of myelin lamellae around the myelinating axons, when compared with age-matched controls. The results suggest that a decrease of endogenous cholesterol in suckling mice may affect peripheral nerve myelination in two ways: by retarding the "triggering" of myelination in unmyelinated axons and by decreasing the rate of myelination already in progress.

INTRODUCTION

Cholesterol[1] is an essential component of all cytomembranes (8) and the most abundant lipid in myelin (23). Biochemical and histochemical studies have suggested that administration of certain drugs[2] (triparanol, AY 9944 and 20, 25-diazacholesterol) blocking cholesterol synthesis induces

[1] Names of sterols used in this work: cholesterol (Δ^5-cholesterol-3β-ol), desmosterol ($\Delta^{5,24}$-cholestadien-3β-ol), and 7-dehydrocholesterol ($\Delta^{5,7}$-cholestadien-3β-ol).

[2] Names of drugs used in this work: triparanol (MER 29): {1-[p(β-diethylaminoethoxy)phenyl]-1-(p-tolyl)-2-(p-chlorophenyl) ethanol}. A. Wassermann, Milan. AY 9944: trans-1,4-bis(2-chlorobenzylaminomethyl) cyclohexane dihydrochloride, was kindly supplied by Dr. D. Dvornik from Ayerst Laboratories, Montreal.

changes in the molecular composition of the myelin fraction in addition to retarding the myelination process of the central nervous system (3, 12, 22). AY 9944 is an inhibitor of Δ^7-reductase resulting in an accumulation of 7-dehydrocholesterol (6). 20, 25-diazacholesterol (10), and triparanol (MER 29) (2) are Δ^{24}-reductase inhibitors resulting in an accumulation of desmosterol.

Scott and Barber, using conventional histological enzyme reactions and lipid stains, concluded that triparanol delays myelination in mouse brain (22). Banik and Davison have reported that the myelin fraction isolated from 14-day old rats treated with triparanol contained large amounts of desmosterol, which, however, slowly decreased after treatment (3). Fumagalli et al. recently reported chemical analysis of myelin fractions from the brain and spinal cord of suckling rats treated with three different hypocholesteremic agents (AY 9944, 20, 25-diazacholesterol, and clofibrate). The myelin fraction isolated after AY 9944 or 20, 25-diazacholesterol treatment was decreased in amount and contained a higher concentration of 7-dehydrocholesterol or desmosterol, respectively, compared with the myelin fraction of the controls. The authors proposed that myelin formation is limited by the amounts of sterols available (12).

From the morphological point of view, the only study of the nervous system following the administration of cholesterol biosynthesis inhibitors to young and adult rats has been reported by Schutta and Neville (21). These authors reported the presence of crystalloid and membranous intracytoplasmic inclusions in neurons and in glial and Schwann cells of triparanol-injected rats, but not in rats injected with clofibrate or with 20, 25-diazacholesterol, the latter, like triparanol, being a Δ^{24}-reductase inhibitor. Schutta and Neville reported "no abnormality of the myelin structure". They concluded that the intracytoplasmic inclusions observed in the triparanol-treated rats may be due to a specific effect of triparanol, unrelated to its action on cholesterol metabolism. This conclusion is at variance with that of Dietert and Scallen. In their morphologic and histochemical studies of adrenal glands, they observed intracytoplasmic inclusions in animals treated with triparanol, AY 9944, and 20,25-diazacholesterol. The authors concluded that such inclusions were a common cytological response related directly to the inhibition of cholesterol biosynthesis (5).

The present study was undertaken to investigate the effect of two cholesterol biosynthesis inhibitors—AY 9944 and triparanol—on peripheral nerve myelination. To our knowledge, this communication constitutes the first morphologic evidence that cholesterol biosynthesis inhibition induces a retardation of the myelination process, demonstrated here in the sciatic nerves of suckling mice treated with AY 9944 and triparanol. In contrast to control animals, the proportion of unmyelinated single axons does not decrease with age; and the thickness of the myelin sheaths (i.e., number of myelin lamellae) is always less than observed in sciatic nerves of nontreated controls.

These results suggest that both the initiation of the myelination process and the growth (increase in thickness) of the myelin sheaths are retarded by drugs known to inhibit cholesterol biosynthesis. In addition, membranous and crystalline intracytoplasmic inclusions were observed in all the experimental sciatic nerves.

MATERIALS AND METHODS

Drugs Used

Swiss albino mice were treated daily with two drugs: triparanol, 300 mg/kg/day as a suspension in olive oil, and AY 9944, 30 mg/kg/day as a solution in glass distilled water. The drugs were administered in rapid succession by intraperitoneal injection.

Experiments

In an initial experiment, a litter of 10 mice was divided into two groups: five experimental and five control. The experimental mice were injected with both drugs at 1, 2, and 3 days of age. Two experimental mice were sacrificed 6 hr after the last injection, together with two controls. Three animals died during this experiment.

In a second experiment, six mice from a litter of 10 mice were injected at 4, 5, and 6 days of age. Two injected animals were sacrificed 6 hr after the last injection, together with two litter mate controls. Four animals died during this experiment.

In a third experiment, the mother was fed a cholesterol-free diet[3] and given both drugs by daily intraperitoneal injection for three days starting on the day of delivery. The newborn mice were not injected but were suckled by their treated mother for 3 days, two such litter mates being sacrificed 6 hr after the mother's third injection. Two animals of the same age suckled by a nontreated mother were used as controls. No toxic effects were noted in other litter mates observed until 6 days of age.

Finally, a fourth experiment was done to study

[3] Nutritional Biochemicals Corp., Cleveland, Ohio.

recovery from any observed drug effects. Two mice injected intraperitoneally at 1, 2, and 3 days of age were transferred to another mother after the third injection, to assure removal from any possible drug source. Mother mice ingest urine from their offsprings, and in this experiment, the original mother had cannibalized injected siblings. Both potential sources of drug could have led to drug secretion in the milk. 1 wk later, one of the animals died, but was not studied; the remaining mouse was sacrificed 2 wk after the third injection. During these 2 wk, daily observations were made and, although the drug-treated mouse was always smaller than its foster litter mates, it was observed to retain its suckling capabilities in spite of the marked differential in size, weight, and coordinated motor activity.

Processing the Tissue for Electron Microscopy

The sciatic nerves of experimental and control animals were exposed under anesthesia with a lethal dose of Diabutal, fixed *in situ* with 5% cacodylate-buffered glutaraldehyde for 5 min, removed, and immersed in glutaraldehyde for 1 hr. They were then washed overnight in Sabatini's washing solution (19), and postfixed for 1 hr each in Dalton's chrome-osmium and in 1% uranyl acetate in 10% formalin. The nerves were dehydrated in graded acetones (15 min each), treated with propylene oxide, and finally infiltrated and embedded in an Epon-Araldite mixture (17).

Thin sections were cut on a Porter-Blum MT-2 ultramicrotome and examined with a Philips-200 electron microscope after staining with lead citrate.

Analysis of the Nerves

For each experimental and control animal, adjacent electron micrographs were taken of a complete transverse section through a sciatic nerve, care being taken to cut a block well above the level at which the sciatic divided into its three major branches.

The electron micrographs of the experimental nerves were carefully scrutinized for any morphological differences between them and the control nerves. The myelin periodicity was measured and both the number of unmyelinated single axons and the number of myelin lamellae around each myelinating axon were counted. Experimental and control nerves from the first three experiments were so examined. The number of myelin lamellae was measured by counting the major dense lines in "compact" myelin.

The electron micrographs of the nerves from the fourth (or drug recovery) experiment were analyzed only with respect to qualitative morphologic abnormalities.

RESULTS

Controls

No morphological abnormalities were observed in any of the control nerves from animals injected with olive oil and distilled water (Fig. 1). Membrane-limited dense bodies (probably lysosomes) were only rarely observed in control nerves. No crystalline intracytoplasmic inclusions were found.

Drug-Induced Intracytoplasmic Inclusions

Membranous and crystalline intracytoplasmic inclusions have been described by Yates and coworkers (1, 4, 26), Hruban and Swift (14, 24), Dietert and Scallen (5), and Schutta and Neville (21) in different tissues (including nerve) of animals treated with any one of several cholesterol biosynthesis inhibitors. Similar inclusions were observed in the present investigation in 44% of the Schwann cells (Figs. 2, 3, 4, 6, 7, 8, 9, 11, 12, 13), in fibroblasts, in macrophages (Fig. 5), and in endothelial cells (Fig. 10) of the sciatic nerves from all the drug-injected animals (first, second, and fourth experiments), as well as from the mice suckled by treated mothers (third experiment). Because a very precise description of the ultrastructure of these inclusions has been published by Chen and Yates (4), we will not describe them here but will use the descriptive categories adopted by these authors: type I: whorls of membrane (Figs. 2, 5, 10); type II: labyrinthine aggregates of smooth membranes (Fig. 5); type III: inclusions with a reticular internal structure (Figs. 3, 4, 5); and type IV: crystalline bodies showing a regular lattice pattern (Figs. 6, 7, 8, 12). Intermediate types were also observed (Figs. 5, 9).

Analysis of the Myelin Sheaths

No difference was observed in the periodicity (140A) and ultrastructure of the myelin sheath between experimental and control nerves. Measurements of the extent of myelination in both experimental and control nerves have been expressed as the per cent of single axons plotted against the number of myelin lamellae (Figs. 14, 15, 16). C-fibers were excluded from this analysis.

First Experiment(Fig. 14)

When the animals were injected at 1, 2, and 3 days of age and sacrificed 6 hr after the last injection, the per cent of unmyelinated (tabulated as "zero" myelin lamellae) single axons in the ex-

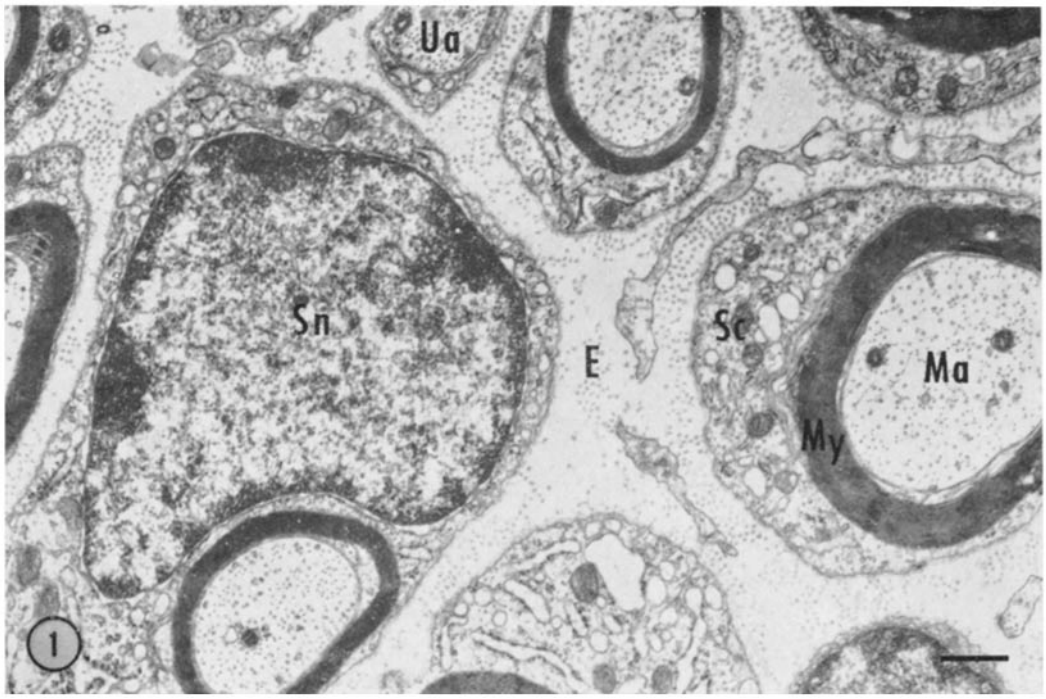


FIGURE 1 Sciatic nerves of a 3 day old control mouse. Animal injected three times with olive oil and water. No morphological abnormality was observed. 63% of the single axons are in the process of myelination (*Ma*) and 37% are classified as unmyelinated single axons (*Ua*). *E*, endoneurium; *My*, myelin sheath; *Sc*, Schwann cell cytoplasm; *Sn*, Schwann cell nucleus. Scale line, 0.5 μ. × 17,850.

perimental nerve was 1.8 times that of the control. An overall diminution in the rate of myelination was also observed as indicated by a smaller proportion of axons with 11–15, 16–20, or 21–25 myelin lamellae in the experimental nerve relative to the control nerve. Furthermore, the maximum sheath thickness attained by any fiber in the experimental nerve was 21 myelin lamellae, while that in the control nerve was 32.

Second Experiment (Fig. 15)

In an experimental animal injected at 4, 5, and 6 days of age and sacrificed 6 hr after the last injection, the percentage of unmyelinated single axons was 3.1 times that in the age-matched, litter mate control.

While the maximum sheath thickness attained by the experimental nerve (37 myelin lamellae) was similar to that of the control animal(40 myelin lamellae), an overall retardation of the myelination process was still observed. The percent of single axons attaining maturer thicknesses in the experimental nerve was smaller than the per cent in the control. It should be noted that the 6 ¼ day old control has only 10.7% of its single axons unmyelinated, while the experimental (with 33.2%) is very similar to the control in the first experiment (see Fig. 14) which showed 36.6% unmyelinated single axons at 3 ¼ days of age (approximately the age at which the second experiment was initiated).

Third Experiment (Fig. 16)

In this experiment, in which the animals were not injected, but suckled by their treated mother, an effect was found similar to that in the animals injected with the drugs, i.e., a greater percentage of unmyelinated single axons, and a delay in myelin lamellar deposition in myelinating axons of the experimental nerve compared with the control. The maximum thickness attained in the experimental myelin sheaths was 12 myelin lamellae while that of the control was 20. Again an overall diminution of myelination in the experimental nerve was observed. The difference in the percentage of unmyelinated axons between experi-

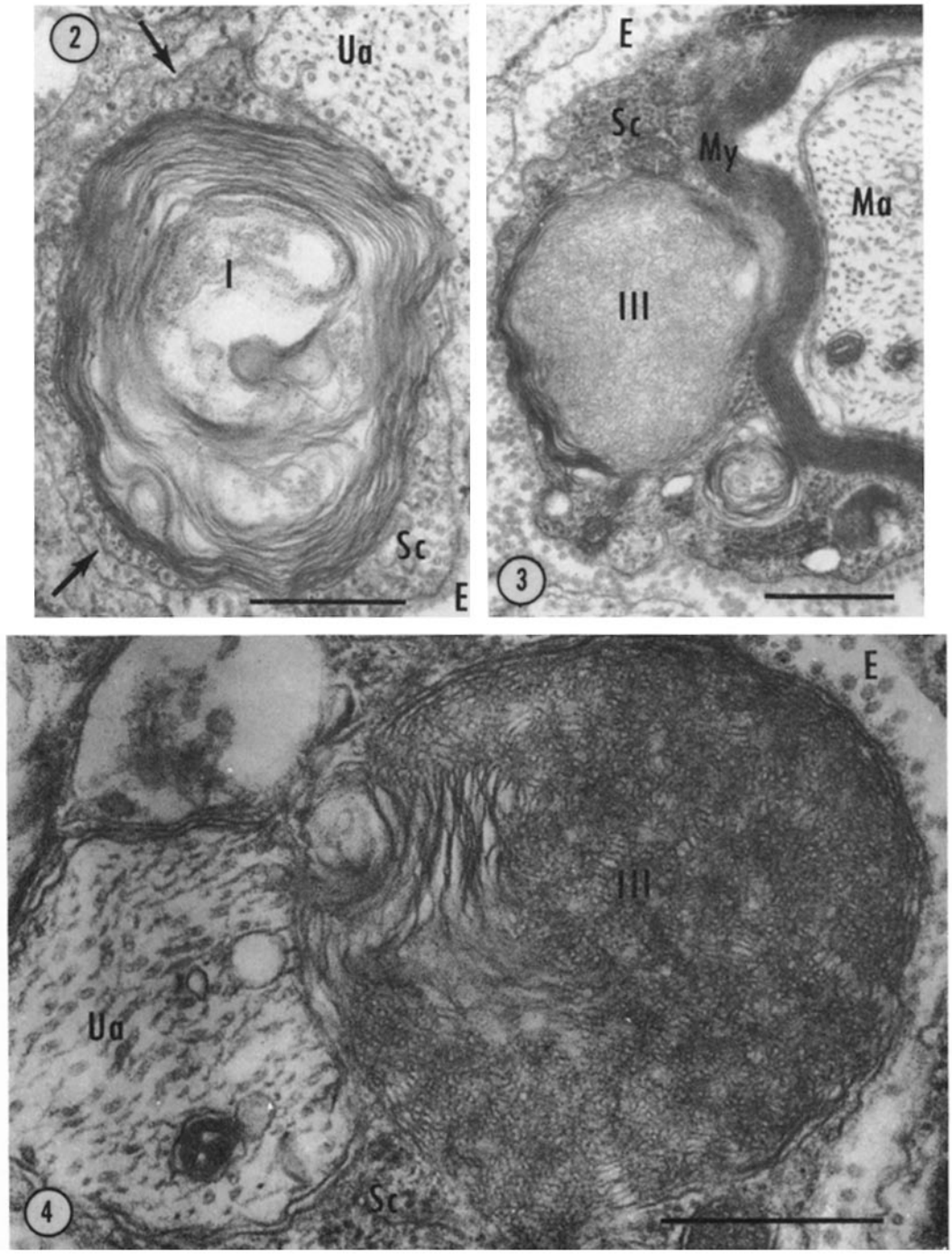


FIGURE 2 Sciatic nerve of mouse injected at 1, 2, and 3 days of age and sacrificed 6 hr after the last injection. A drug-induced inclusion (*I*) formed by a "whorl of membranes" (Chen and Yates, type I inclusion) occupies almost all the Schwann cell cytoplasm (*Sc*) of an unmyelinated axon (*Ua*). Arrows indicate the Schwann cell limiting membrane. Scale line, 0.5 μ. × 46,295.

FIGURES 3 and 4 Drug-induced inclusions formed by ≃70A smooth membranes arranged in a reticular fashion (Chen and Yates, type III inclusion). In Fig. 3 this inclusion (*III*) occupies a great portion of the Schwann cell cytoplasm (*Sc*) surrounding a myelinating axon (*Ma*) of a 6¼-day old mouse (second experiment). In Fig. 4 the inclusion (*III*) totally occupies the Schwann cell cytoplasm (*Sc*) surrounding an unmyelinated axon (*Ua*) of a 3¼-day old mouse (first experiment). *E*, endoneurium; *My*, myelin sheath. Scale lines, 0.5 μ. Fig. 3, × 38,250; Fig. 4, × 65,920.

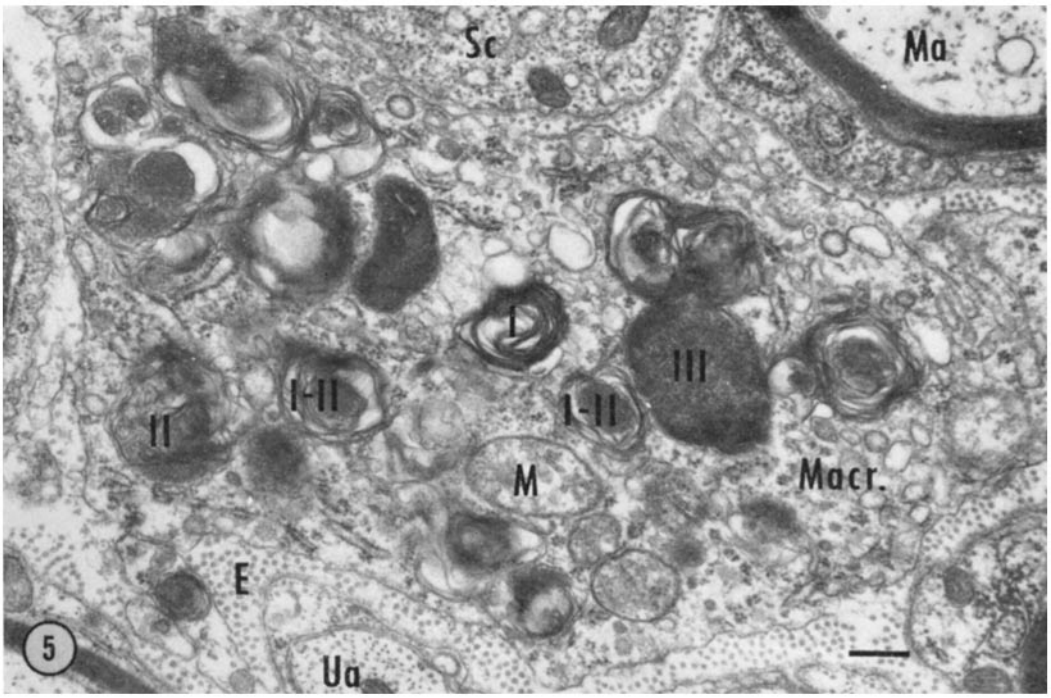


FIGURE 5 Animal injected at 1, 2, and 3 days of age and sacrificed 6 hr after the last injection. Numerous drug-induced inclusions are present in the cytoplasm of a macrophage (*Macr.*). Chen and Yates, type I, II, and III inclusions are observed as well as an intermediate type exhibiting characteristics of type I and type II (*I–II*). *M*, mitochondria; *Sc*, Schwann cell cytoplasm; *Ma*, myelinating axon; *Ua*, unmyelinated axon; *E*, endoneurium. Scale line, 0.5 μ. × 16,200.

mental (65%) and control (51.4%) nerves is not as great, the ratio being 1.3:1 compared to the injected animals of the first two experiments where the ratios were 1.8:1 and 3:1, respectively.

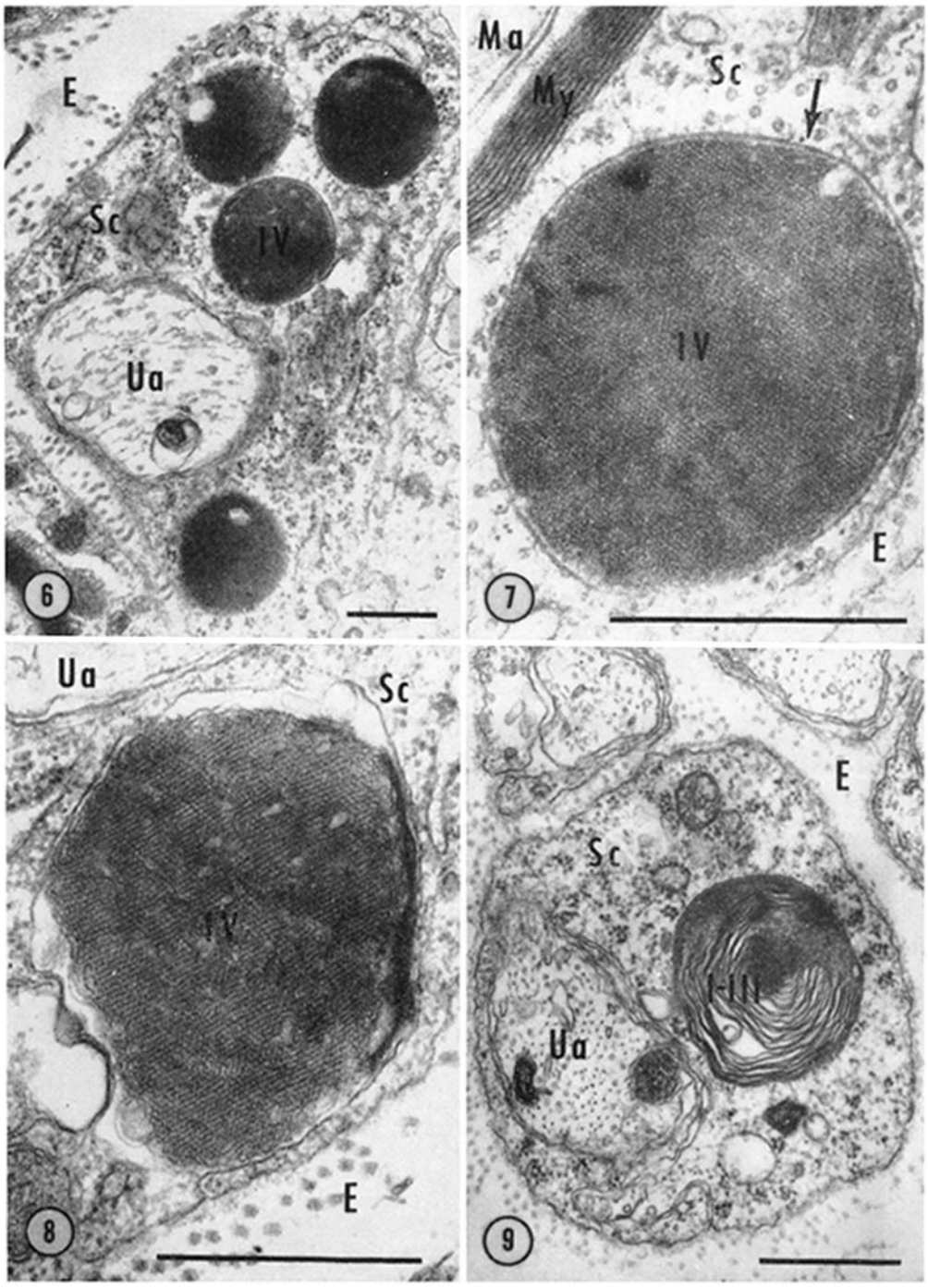
Fourth Experiment

In this experimental animal sacrificed 2 wk after the last injection at age 3 days, inclusions were still present in the Schwann cells (Figs. 12, 13). The

FIGURE 6 Sciatic nerve of a mouse injected at 1, 2, and 3 days of age and sacrificed 6 hr after the last injection. Four crystalline inclusions (*IV*) (Chen and Yates, type IV inclusions) are observed in the cytoplasm of a Schwann cell (*Sc*) surrounding an unmyelinated axon (*Ua*). *E*, endoneurium. Scale line, 0.5 μ. × 26, 400.

FIGURES 7 and 8 High magnification of a crystalline drug-induced inclusion (Chen and Yates, type IV inclusion), showing an internal regular lattice pattern with a periodicity of approximately 120A. The limiting membrane is clearly distinguished (arrow). Sciatic nerve of a mouse injected at 4, 5, and 6 days of age and sacrificed 6 hr after the last injection. *Ma*, myelinating axon; *My*, myelin sheath; *Ua*, unmyelinated axon; *Sc*, Schwann cell cytoplasm; *E*, endoneurium. Scale lines, 0.5 μ. Fig. 7, × 86,520; Fig. 8, × 76,220.

FIGURE 9 A drug-induced inclusion (*I–III*) showing intermediate structure or transitional stage between Chen and Yates type I and type III inclusions. Sciatic nerve of a 2-day old noninjected mouse, suckled by an injected mother. *Ua*, unmyelinated axon; *Sc*, Schwann cell cytoplasm; *E*, endoneurium. Scale line, 0.5 μ. × 40,000.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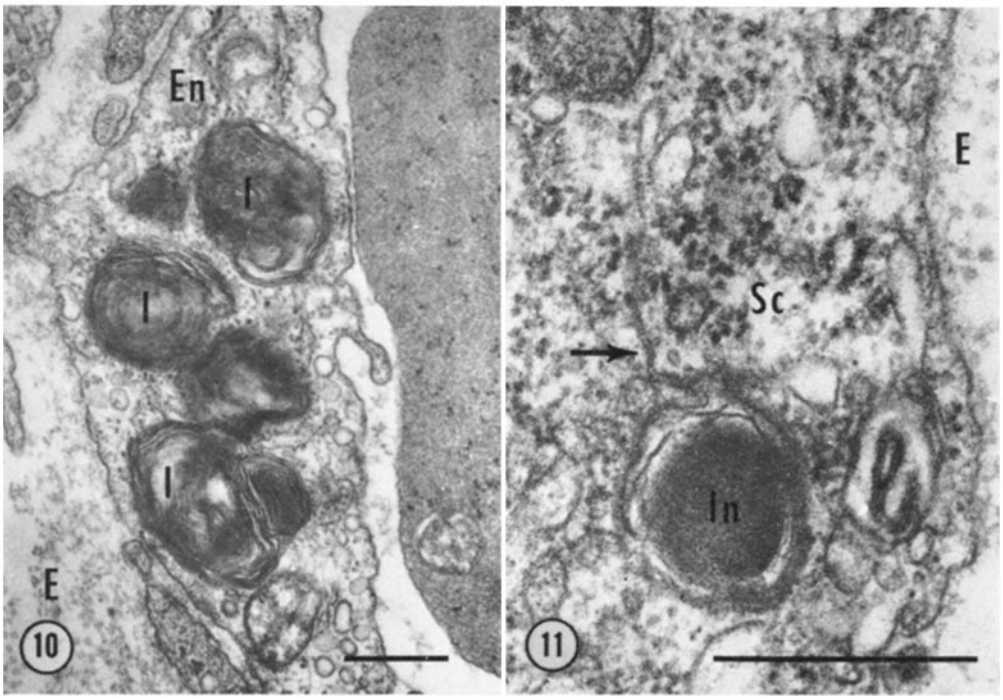


FIGURES 10 and 11 Portion of a sciatic nerve of a mouse injected at 1, 2, and 3 days of age and sacrificed 6 hr after the last injection. In Fig. 10, Chen and Yates type I inclusions (*I*) are seen in the cytoplasm of an endothelial cell (*En*). Fig. 11 shows a portion of Schwann cell cytoplasm (*Sc*). The membranes surrounding the inclusion (*In*) are continuous with the endoplasmic reticulum (arrow). *E*, endoneurium. Scale lines, 0.5 μ. Fig. 10, × 29,070; Fig. 11, × 70,000.

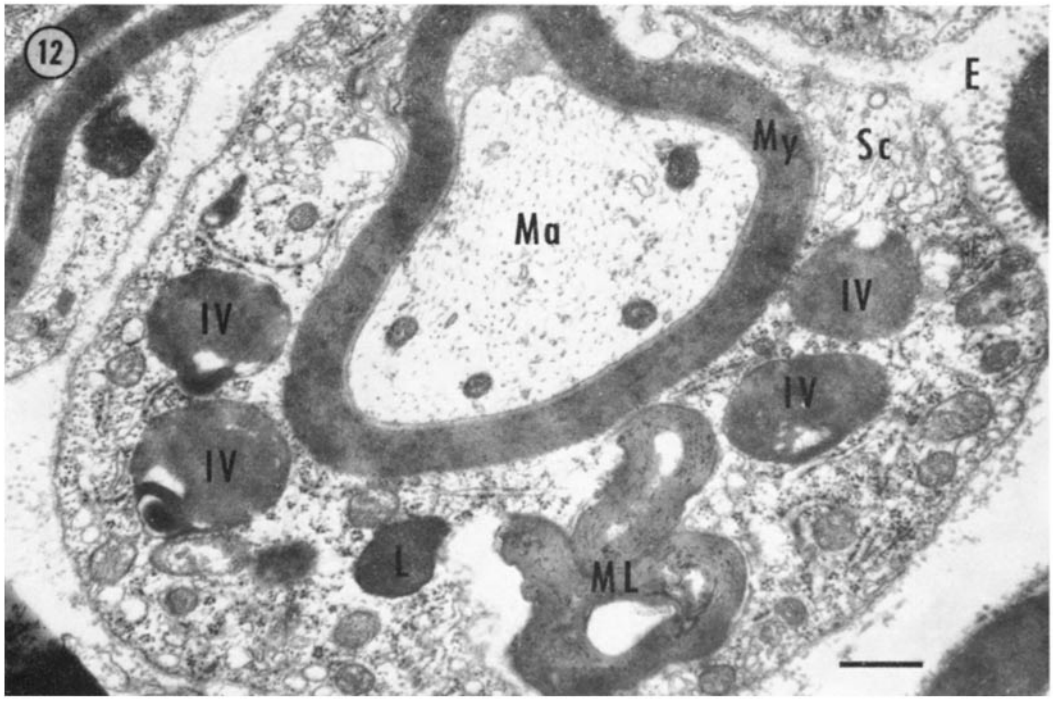
largest proportion of the inclusions observed in the Schwann cells had a crystalline structure (Chen and Yates, type IV inclusion). This is in contrast to both of the animals sacrificed 6 hr after their last injection (first and second experiments). In these instances, crystalline, lamellar, and reticular inclusions were almost equally represented. Membrane-limited "bags" invaginated toward the axon, but separated from axoplasm by the axolemma, were occasionally observed (Fig. 13). The plane of section in all such cases has so far prevented clear-cut delineation of the exact relation of the innermost myelin lamella or Schwann cell "tongue" to these bodies. The fact that they contain inclusions resembling the drug-induced inclusions seen in Schwann cell cytoplasm suggests that the bag may be an inner Schwann cell cytoplasmic tongue.

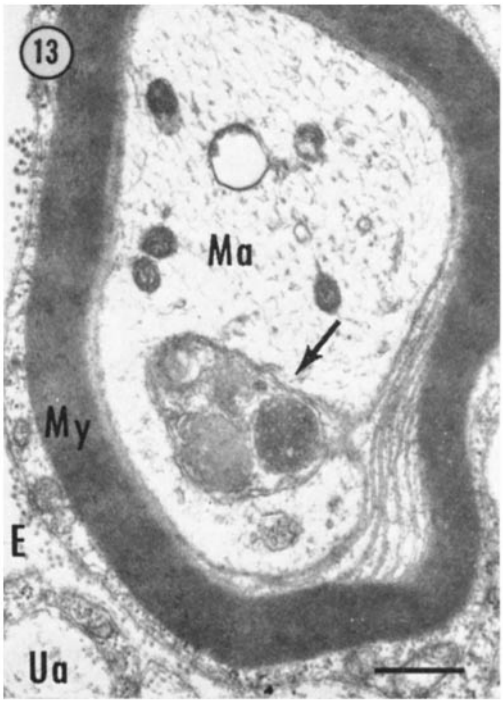
DISCUSSION

The present study indicates that the sciatic nerves of mice treated with AY 9944 and triparanol contain a larger proportion of unmyelinated single axons and a smaller number of myelin lamellae in the myelinating axons, when compared with the sciatic nerves of age-matched control animals.

Since it is well established that the drugs utilized in these experiments act as inhibitors of cholesterol synthesis in the nervous system (2, 6, 7, 11, 20) it is suggested that such inhibition in suckling mice affects peripheral nerve myelination in two ways: (*a*) by retarding the "triggering" of myelination in unmyelinated axons, and (*b*) by decreasing the rate of myelin lamellar deposition in the myelinating axon.

As a quantitative evaluation of the extent of myelin deposition at any one time, we counted the number of myelin lamellae around single axons in electron micrographs of experimental and control nerves. Friede and Samorajski, using a similar method, have shown that 5 hr are required for the deposition of a myelin turn during the first 10 days of peripheral nerve myelin formation in rats (9). Such a slow rate of myelin lamellar deposition indicates that longer periods of exposure of the ani-





FIGURES 12 and 13 Sciatic nerve of a mouse injected at 1, 2, and 3 days of age. Sacrificed 2 wk after the third injection. In Fig. 12, four crystalline inclusions (*IV*) (Chen and Yates type IV inclusion) are present in the Schwann cell cytoplasm (*Sc*) surrounding a myelinating axon (*Ma*). Myelin-like inclusions (*ML*) similar to the ones observed in this electron micrograph have also been observed in control nerves. In Fig. 13, the arrow is pointing to a membrane-limited "bag" containing drug-induced inclusions and invaginating the axoplasm of a myelinating axon (*Ma*). My, myelin sheath; *E*, endoneurium; *Ua*, unmyelinated axons; *L*, lysosome-like inclusion. Scale lines, 0.5μ. Fig. 12, × 21,600; Fig. 13, × 24, 395.

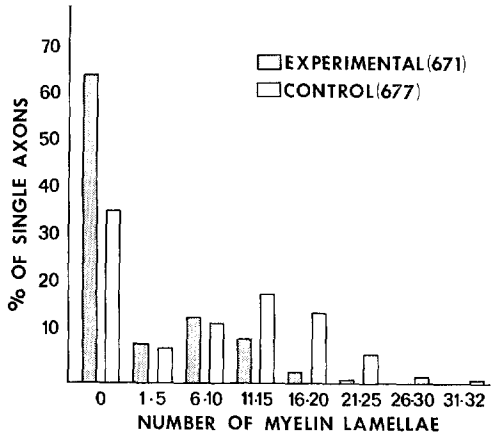


FIGURE 14 Animal injected at 1, 2, and 3 days of age with triparanol and AY 9944. Sacrificed 6 hr after the third injection. The figures in parenthesis indicate the total number of single axons counted.

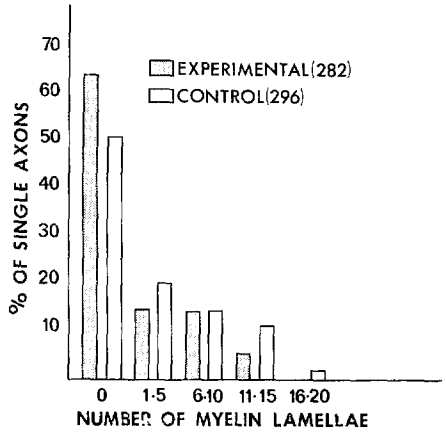


FIGURE 16 A 2½ day old noninjected animal suckled by a mother injected for 3 days (starting the day of delivery) with triparanol and AY 9944 and fed a cholesterol-free diet. The animals were sacrificed 6 hr after the mother's third injection.

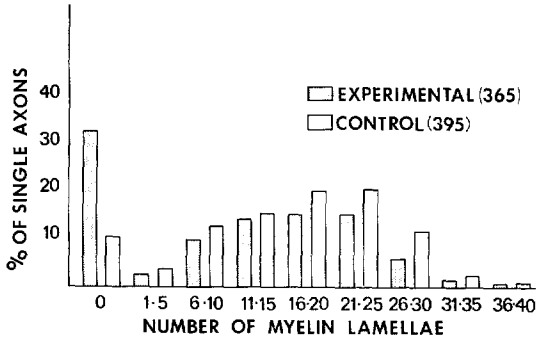


FIGURE 15 Animal injected with triparanol and AY 9944 at 4, 5, and 6 days of age. Sacrificed 6 hr after the third injection.

mals to the drugs than the one used in the present experiment (3 days) would be necessary to see a more accentuated difference in the number of myelin lamellae between experimental and control nerves.

The results of the present morphological study of changes in peripheral nerve myelin deposition support the findings in the brain and spinal cord reported by investigators who isolated "myelin fractions" (12) or estimated myelin formation by histochemical staining methods (22). Results of all three types of investigations can now be said to show a retardation of myelination in central or peripheral nervous systems of animals treated with cholesterol biosynthesis inhibitors.

Since triparanol and AY 9944 act on different sites in cholesterol biosynthesis (5, 11), both drugs were injected to minimize availability of endogenous cholesterol to Schwann cells during rapid myelination. Examination of the effect of AY 9944 alone has been assessed in similar experiments on the same strain of mice. The results are reported elsewhere (18) and indicate the ability of AY 9944 to inhibit myelination when administered in the first few days of life. The effect of triparanol alone is currently under study. Recent evidence has suggested that both endogenous and exogenous cholesterol are incorporated by the nervous system during myelination. In a preceding paper, we have reported that cholesterol-^{3}H injected intraperitoneally in suckling mice is rapidly incorporated by peripheral nerve during myelination and is detectable for long periods of time by radioautography in the myelin sheath and cellular components of the sciatic nerve (13). On the other hand, by the administration of labeled cholesterol precursors to myelinating animals, others have shown that biosynthesized cholesterol is also present in myelin (15, 23). One may question whether both sources are indispensable.

The morphologic observations reported here have been interpreted as suggesting that endogenous (biosynthesized) cholesterol is essential for myelin development and that exogenous (dietary) cholesterol from the maternal milk does not compensate for a lack of synthesized cholesterol. The possibility that the drugs used induced a cytotoxic

effect on the cells responsible for myelin formation cannot be excluded.

The third experiment (see Materials and Methods) attempted to test the indispensability of exogenous cholesterol by limiting the amount supplied in the diet. Noninjected newborn mice were suckled by a mother injected three times with AY 9944 and triparanol and fed a cholesterol-free diet, since we thought that in this way the cholesterol available in the milk would be decreased. Electron microscope analysis of the nerves from the suckling mice revealed not only the retardation of myelination but also the presence of cytoplasmic inclusions similar to those observed in drug-injected animals (Fig. 9). Since the formation of these inclusions has already been shown to be induced by each of these drugs (4, 5), the presence of such inclusions is interpreted as indicating that the drug was transmitted through the milk, and may directly inhibit cholesterol synthesis in the suckling mice. Attempts to measure the levels of cholesterol in the maternal milk have failed because of insufficient sample volume. However, preliminary attempts to supply suckling mice with an artificial cholesterol-free diet are underway, in order to allow a direct evaluation of the effect of exogenous cholesterol deprivation.

The morphology of the intracytoplasmic inclusions observed in the experimental nerves is similar to that of the intracytoplasmic inclusions described by several investigators in different tissues of animals injected with various inhibitors of cholesterol biosynthesis. Yates and coworkers (1, 4, 26) have given a very good description of the ultrastructure and chemical composition of the cytoplasmic inclusions induced by triparanol in Syrian hamster tissues—adrenal cortex, corpus luteum, testis, and small intestine. In the present study, we have used Chen and Yates' (4) classification of such inclusions. A cellular fraction from hamster adrenal gland rich in triparanol-induced inclusions has been analyzed and was shown to contain phospholipids, desmosterol, protein, and triparanol (1, 26), confirming an earlier interpretation (25). No such reports are available concerning the chemical composition of the inclusions induced by AY 9944. Dietert and Scallen reported studies of the effect of three different cholesterol biosynthesis inhibitors on murine adrenal gland and testis: triparanol, AY 9944, and 20,25-diazacholesterol. They found that the accumulation of intracytoplasmic inclusions is a general phenomenon produced by each of the three drugs that is related directly to the inhibition of cholesterol biosynthesis, suggesting that the accumulated cholesterol precursors are unable to substitute for cholesterol in the normal cellular metabolism (5). Preliminary observations in this laboratory have shown similar intracytoplasmic inclusions in the sciatic nerve of mice separately treated with AY 9944 and triparanol, supporting Dietert and Scallen's suggestion.

We have examined the sciatic and optic nerves of adult mice (>75 days of age) injected with both drugs. They contain the same types of inclusions seen in drug-treated suckling mice but in a smaller proportion. If the formation of the inclusions is a phenomenon related directly to the inhibition of cholesterol biosynthesis (5), their presence in Schwann cell cytoplasm of adult mice suggests the ability of such cells to synthesize cholesterol. This is consistent with observations indicating an active cholesterol metabolism in adult brain (7, 15, 23).

Some of the cytoplasmic inclusions are in direct continuity with membranes resembling those of the agranular endoplasmic reticulum (Fig. 11) and support Dietert and Scallen's suggestion that the drug-induced inclusions may be formed in the endoplasmic reticulum.

Catabolism of the drug-induced inclusions is suggested by their previously demonstrated acid-phosphatase content (5), and the possibility that such degradation is a slow process is indicated by the existence in Schwann cell cytoplasm of inclusions 2 wk after the last drug injection (Fig. 12). Such inclusions were occasionally observed in membrane-limited bags invaginating the axoplasm (Fig. 13). An attractive hypothesis would postulate their formation in the Schwann cell cytoplasm prior to myelination (as seen in Fig. 6) with invagination into the axon as a consequence of the cellular activity associated with myelination.

Banik and Davison have reported substantial amounts of desmosterol in the myelin fraction of brain from 14-day old rats injected with triparanol (3). In addition, Fumagalli et al. have found that triparanol and AY 9944 induce high levels of desmosterol or 7-dehydrocholesterol in the myelin fraction from the brain and spinal cord of treated rats. They suggested that both desmosterol and 7-dehydrocholesterol, which are known cholesterol precursors, may be able to substitute for cholesterol in the lipid-cholesterol complex of membranes and myelin, but are metabolized more rapidly than cholesterol (12).

As shown in this paper, approximately 44% of

the Schwann cells in the sciatic nerves of the drug-treated animals presented the membranous and crystalline intracytoplasmic inclusions. In some cases one inclusion occupied almost all the Schwann cell cytoplasm in a cross-section of unmyelinated (Figs. 2, 4) or myelinating (Fig. 3) fibers. In other fibers, several inclusions occupied a large portion of the Schwann cell cytoplasm (Figs. 6, 12). The inclusions have also been observed by Schutta and Neville in the central nervous system of triparanol-injected mice (21). The fact that a cell fraction rich in triparanol-induced inclusions contains desmosterol (1, 26) strongly suggests that desmosterol is forming part of such inclusions.

In conclusion, triparanol and AY 9944 affect peripheral nerve myelination by retarding the triggering of the process in unmyelinated axons and by decreasing the rate of myelin lamellar deposition in the myelinating axon. It is suggested that the observed retardation in myelination is due to a decrease in the available endogenous cholesterol, and that endogenous cholesterol is essential for myelination. Maternal milk does not compensate for a lack in endogenous cholesterol. The drug-induced membranous and crystalline inclusions are still present in the sciatic nerve 2 wk after the last injection. Because of the association of cholesterol precursors with the early stage of myelinogenesis (3, 16), isolation and analysis of the cholesterol precursor-containing inclusions at varying times after drug injection might be of significance in the broader study of myelin fractions at varying times during the development of the nervous system.

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