Progressive Neuropathologic Lesions in Vitamin E-Deficient Rhesus Monkeys

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Abstract. A consistent group of progressive central and peripheral nervous system lesions developed in seven rhesus monkeys maintained on a vitamin E-deficient diet for 30 to 33 months. These lesions were absent from vitamin E-supplemented monkeys. The principal neuropathologic alteration was loss of sensory axons in the posterior columns, sensory roots, and peripheral nerves. Morphologic and morphometric studies indicated that the distal segments of the axons were affected most severely and large-caliber myelinated fibers are selectively involved. Swollen, dystrophic axons (spheroids) occurred infrequently. Degeneration and phagocytosis of small numbers of neuronal perikarya were observed in the dorsal root ganglia and the anterior horns. The number of affected neurons was not proportional to the number of affected axons. Accumulation of lipopigment was evident in neuronal perikarya and CNS endothelial cells. The nervous system lesions were usually accompanied by a chronic necrotizing myopathy. The neuropathologic lesions in vitamin E-deficient monkeys are compared with those in vitamin E-deficient rats and in humans with low serum vitamin E concentrations. A similar type of sensory axonopathy is associated with chronic deficiency of vitamin E in these three species.

INTRODUCTION

Nervous system lesions in mammals with chronic vitamin E deficiency have been delineated most extensively in rats (5, 14—16, 20). Swollen, dystrophic axons (spheroids) are found chiefly in the posterior columns, posterior horns, Clarke's column, the gracile and cuneate nuclei, the nucleus of the spinal trigeminal tract, and the nucleus of the tractus solitarius. The axonal dystrophy may be associated with loss of axons and myelin sheaths from the posterior columns. Accumulation of lipopigment in neuronal perikarya and CNS microcirculatory endothelium has also been observed (18, 20). A preliminary report by Towfighi (29) indicates that the distal segments of peripheral sensory axons may also degenerate.

Little is known, however, about the neuropathologic effects of chronic vitamin E deficiency in primates. Einarson and Telford (6) have described ac-

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cumulation of neuronal lipopigment and other degenerative nerve cell changes in four vitamin E-deficient rhesus monkeys. Axonal dystrophy and loss of posterior column axons were not observed. Recently, Hayes (11) reported that dystrophic axons were absent from the gracile and cuneate nuclei of vitamin E-deficient cynomolgus and cebus monkeys.

It should also be noted that both vitamin E-deficient rats and monkeys may develop a skeletal muscle disorder which is not directly related to nervous system lesions (8, 15, 20). The muscle changes are those of a chronic necrotizing myopathy similar to the muscle disorder induced in several other mammalian species by experimental vitamin E deficiency (1). Group atrophy of muscle fibers and other characteristics of neurogenic muscular atrophy do not develop as a consequence of mammalian vitamin E deficiency.

In this paper we report our observations on the neuropathologic lesions in rhesus monkeys with experimental vitamin E deficiency. Our findings demonstrate that prolonged vitamin E deficiency in the monkey is consistently associated with central and peripheral nervous system lesions as well as a chronic primary necrotizing myopathy. The neuropathologic lesions in mammals, including primates with experimentally induced vitamin E deficiency and in humans with known or suspected vitamin E deficiency, are compared.

MATERIALS AND METHODS

Induction of Vitamin E Deficiency

Ten sexually immature male and female rhesus monkeys (Macaca mulatta) weighing approximately 3 kg each were fed a purified, vitamin E-deficient diet consisting of 18% “vitamin free” casein, 22% sucrose, 47% corn starch, 8% lard stripped of vitamin E, and all of the essential vitamins and minerals except vitamin E (9). Seven of the ten monkeys were used to investigate the neuropathologic effects of experimentally induced vitamin E deficiency. The three remaining monkeys (two males and one female) served as controls and were supplemented with 100 mg of vitamin E (d-alpha tocopheryl acetate) three times per week by mouth.

The ten monkeys used in this study were derived from a group of twelve monkeys used in an investigation of the anemia of vitamin E deficiency (9). For identification purposes, the twelve monkeys were assigned consecutive numbers (M-1 through M-12) when they were initially received at our animal care facility. The same identification numbers were used in both the neuropathologic and the hematologic studies. Two of the twelve monkeys, M-2 (control) and M-12 (deficient), were not included in the present study.

Each monkey in the neuropathologic study was maintained on the deficient or control regimen for 30 to 33 months. At that time, the three control monkeys and five of the vitamin E-deficient monkeys were sacrificed. The two remaining vitamin E-deficient monkeys were repleted with vitamin E for two months by feeding 100 mg of d-alpha tocopheryl acetate three times per week and then sacrificed. At monthly intervals throughout the study, each monkey was weighed, examined clinically, and its serum vitamin E concentration (21) and hematocrit (packed cell volume) determined.

Biopsy and Autopsy Procedures

Immediately prior to sacrifice, the monkeys were anesthetized with ketamine and acepromazine and biopsies of the sural nerves at the ankle and of the quadriceps muscle
were obtained for light and/or electron microscopy. Subsequently, the animals were killed by an overdose of pentobarbital or by intracardiac perfusion with 3% glutaraldehyde in 0.1 M phosphate buffer. The brain, spinal cord, three to four dorsal root ganglia, the brachial and lumbar sacral plexuses, the ulnar and sciatic nerves, and the deltoid, sternomastoid, intercostal, diaphragm, psoas, quadriceps, and gastrocnemius muscles were fixed in 10% neutral buffered formalin. Segments of ulnar nerve from one control and two experimental animals were excised after glutaraldehyde perfusion and immersed in 3% buffered glutaraldehyde. Blocks of all major viscera, the vertebral bone marrow, segments of rib, the gonads, and the eyes from all monkeys were fixed in 10% neutral buffered formalin.

Conventional Light Microscopy

Paraffin sections of frontal, temporal, parietal, occipital, and hippocampal cortex, basal ganglia, brainstem, cerebellum, spinal cord, dorsal root ganglia, the brachial and lumbar sacral plexuses, the ulnar and sciatic nerves, and the skeletal muscles from all monkeys were examined by light microscopy using one or more of the following stains: hematoxylin and eosin (H & E), luxol fast blue-PAS (LFB-PAS), Loyez, Sevier-Munger (axons), Masson trichrome, oil red "O," and phosphotungstic acid and hematoxylin (PTAH). Unstained paraffin sections were examined by fluorescence microscopy, using a mercury bulb with BG12 excitation and 510 nm emission filters. Paraffin sections of the heart, lung, gastrointestinal tract, liver, kidneys, spleen, adrenal, gonads, bladder, decalcified bone, and bone marrow from all monkeys were stained with H & E and PAS and examined by light microscopy. Unstained paraffin sections of selected viscera were examined by fluorescence microscopy, following the techniques used for the brain sections. The eyes were not included as part of this study.

Ultrastructural Methods

Segments of sural nerve from six experimental and three control monkeys and segments of ulnar nerve from two deficient and one control monkeys were fixed immediately after excision in 3% phosphate-buffered glutaraldehyde, pH 7.4, at room temperature. After initial fixation for one hour, the nerves were divided transversely into 1 mm³ blocks and kept in glutaraldehyde overnight at room temperature. Subsequently, the blocks were washed in buffer, postfixed in OsO₄, dehydrated in graded alcohols, transferred to propylene oxide and embedded in epon-araldite or Spurr’s medium (25). During embedding, the blocks were oriented so that uniform cross sections of the nerve could be obtained. Semi-thick (1.5 μ) and thin sections were prepared from these blocks. The thick sections were stained with alkaline toluidine blue, mounted in immersion oil, and coverslipped for light microscopic and morphometric studies. The thin sections were stained on copper grids with lead citrate and uranyl acetate and examined with a Philips 200 electron microscope.

Teased Fibers

Preparations of 70 to 80 teased, myelinated fibers from the sural nerves of each of two deficient monkeys were examined. Samples of whole sural nerve were fixed in 3% phosphate-buffered glutaraldehyde for 30 to 45 minutes at room temperature, washed in buffer, postfixed in 1% OsO₄, washed in buffer, infiltrated with graded solutions of glycerin at 45°C, and stored in 100% glycerin at 4°C (4). Individual myelinated fibers consisting of at least 4 to 5 internodes were teased from fascicles with the aid of a dissecting microscope and fine forceps, mounted in a water-soluble mounting medium, coverslipped, and examined by light microscopy (4).
NEUROPATHOLOGY OF VITAMIN E-DEFICIENT MONKEYS

Morphometry

The frequency distribution of myelinated fiber diameters per mm² of endoneurium and the number of myelinated fibers per mm² of endoneurium (fiber density) in the sural nerves of five deficient and two control monkeys were determined from photographic enlargements of transverse, semi-thick sections of the nerves stained with alkaline toluidine blue (4). The frequency distribution of fiber diameters and the fiber density are subsequently referred to as the fiber spectrum. An area equivalent to 0.3 mm² of endoneurium was determined from the photographs by planimetry. With the exception of one deficient monkey with profound axon loss, a minimum of 3,000 myelinated fibers per monkey were counted and their diameters measured with a Zeiss TGZ 3 particle size analyzer set in the exponential mode. The frequency distribution of fiber diameters and fiber density were calculated from these data with a programmable calculator, and histograms were prepared (4).

RESULTS

Clinical and Laboratory Observations

Serum vitamin E concentrations in each of the seven deficient monkeys fell to 0.4 mg/dl within 8 to 13 months after initiation of the dietary regimen, and subsequently reached undetectable levels after 20 months on the regimen. Thus, during the course of the study, each deficient monkey had been fed the vitamin E-deficient diet for 30 to 33 months and had exhibited markedly reduced serum vitamin E levels for 17 to 24 months. Serum vitamin E concentrations in the three control monkeys varied slightly between 1.2 and 1.6 mg/dl throughout the course of the study.

During the first 24 months of the study, the vitamin E-deficient monkeys gained weight and appeared healthy. During the third year of the study, however, each of the seven deficient monkeys developed one or more of the following clinical and laboratory abnormalities which are regularly associated with vitamin E deficiency in the monkey (9): weight loss, ranging from 2 to 30% of maximum weight, generalized muscle weakness, and anemia (hematocrit less than 35%). The rate of onset and severity of these abnormalities varied.

The three control monkeys continued to gain or maintain their weight during the study. Their hematocrit consistently remained above 40%.

The two vitamin E-deficient monkeys repleted for two months with vitamin E had been fed the deficient diet for 30 months at the time repletion was begun. Each monkey had exhibited markedly reduced serum vitamin E levels for 21 months. The clinical and laboratory findings at the time repletion was begun were as follows: weight loss of 2% and 8% of maximum body weight, generalized, moderate muscle weakness, and anemia with hematocrits of 31% and 27%. At the time of sacrifice following two months of repletion, one of the monkeys had regained all of the weight previously lost. The other monkey not only regained all of the lost weight but increased its weight by 12% over its previous maximum level. In both monkeys, the generalized muscle weakness disappeared and the anemia resolved. Each monkey’s hematocrit was 40%. Serum vitamin E concentrations were 1.0 mg/dl and 1.3 mg/dl.
Fig. 1. Quadriceps, vitamin E-deficient monkey. Changes characteristic of the chronic necrotizing myopathy found in four vitamin E-deficient monkeys. Variation in fiber diameter, vacuolated fibers, and fibers undergoing necrosis (N) and phagocytosis (P) are evident. Hematoxylin and eosin stain. ×250.

General Autopsy

Accumulation of PAS-positive, xylol-insoluble lipopigment with yellow autofluorescence was evident in smooth muscle, cardiac muscle, and macrophages in sections of thoracic and abdominal viscera from each deficient monkey. Mild to severe atrophy of testicular germinal epithelium was present in all deficient males. Both testicular atrophy and lipopigment accumulation appeared to be less extensive in the two repleted animals than in most of the other deficient monkeys. Pigmentary changes and testicular atrophy were entirely absent from controls. Osteoblasts were focally present around bony trabeculae in sections of rib and vertebral body from all monkeys. Osteoid seams were not observed.

Skeletal Muscle

Chronic necrotizing myopathy was evident in each muscle examined from four of the deficient monkeys (Fig. 1). Changes in the muscle fibers included increased numbers of internal nuclei, random variation in diameter, atrophy, vacuolation, segmental necrosis, phagocytosis, and regeneration. Connective
tissue was increased focally around areas of muscle fiber loss. Occasional collections of mononuclear cells were present in the endomysium and perimysium. There was no evidence of neurogenic atrophy. Random variation of fiber diameter and accumulation of lipopigment within muscle fibers were the only alterations apparent in muscle sections from the two repleted monkeys. Muscles from one deficient monkey and the three control monkeys were free of lesions. The deficient monkey without myopathy is the animal noted in the neuropathology section as having the least extensive central and peripheral nervous system lesions. Ultrastructural and histochemical studies of the quadriiceps biopsies will be described in detail in a subsequent report.

Neuropathology

Significant histologic and ultrastructural lesions were found only in the deficient monkeys, including the two monkeys repleted with vitamin E for two months. The local severity of the lesions and the extent of their anatomic distribution varied from monkey to monkey. The severity and extent of the neuropathologic changes were not correlated with the duration of markedly reduced serum vitamin E levels, the severity of the anemia, the degree of muscle weakness, or with the extent of the weight loss.

The neuropathologic alterations in the two vitamin E-repleted monkeys paralleled those in the five unrepleted deficient monkeys. The extent and severity

Fig. 2. Cervical spinal cord, vitamin E-deficient monkey. Marked degeneration of fasciculus cuneatus with lesser involvement of the fasciculus gracilis. Loyez stain. ×10.
of the lesions were marked in one of the repleted monkeys and moderate in the other. Prominent regenerative changes such as the extensive proliferation of Schwann cells and abundant axonal sprouts were absent in the peripheral nerves, posterior nerve roots, and posterior horns. With the exception of certain ultrastructural changes in the peripheral nerves, the neuropathologic lesions in the two repleted monkeys were indistinguishable from those observed in the five unrepleted monkeys. Ultrastructural changes suggestive of early axonal injury (intraaxonal accumulation of membrane-bound vesicles and vac-

Fig. 3. Small numbers of swollen, dystrophic axons (spheroids) (arrows) in posterior horn of vitamin E-deficient monkey. This type of axonal change occurred infrequently, most often in the posterior horns, and rarely in the gracile or cuneate nuclei. Sevier-Munger stain. ×250.
Fig. 4. Posterior cervical roots, vitamin E-deficient monkey. Moderate loss of axons and myelin sheaths from two large adjacent roots. Two small roots are spared. Luxol fast blue-periodic acid-Schiff stain. ×100.

ules) were absent in the peripheral nerves of the two repleted monkeys, while these changes were evident in the unrepleted monkeys. Advanced degenerative changes such as dissolution and phagocytosis of axons and myelin sheaths were evident in both the repleted and unrepleted monkeys.

A composite description of the neuropathologic lesions in the deficient monkeys is presented in the following paragraphs. Excluding the previously-noted exception regarding certain ultrastructural changes in the peripheral nerves, the description is applicable to both the repleted and the unrepleted monkeys.

Central Nervous System (CNS)

The most striking CNS alteration was concomitant loss of axons and myelin sheaths in the posterior columns accompanied by mild, fibrillary astrocytosis (Fig. 2). All deficient monkeys and none of the control animals were affected. The lesions were more severe in the fasciculus cuneatus than in the fasciculus gracilis; in one monkey only the cuneatus was affected. Axonal loss occurred throughout the course of each tract, but appeared more severe in rostral segments of the cord. Small numbers of swollen, dystrophic axons (spheroids) occurred in most but not all of the deficient monkeys (Fig. 3). They were present in greatest numbers in the posterior horns and were difficult to find in the gracile and cuneate nuclei. There was slight to moderate axonal loss within
the posterior roots (Fig. 4). The extent of the loss was proportional to, but not as severe as, the loss in the adjacent posterior columns. The anterior roots were intact. Sections of mid-pons with attached trigeminal roots were available for examination from five deficient monkeys and two controls. Symmetrical loss of small to moderate numbers of axons from the trigeminal roots was evident in four of the deficient monkeys. The deficient monkey without trigeminal lesions also had the least extensive posterior column changes. Trigeminal roots were unaffected in the two control monkeys examined.

Neuronal perikarya were affected only in deficient monkeys. These changes, however, were much less extensive than those involving axons. Degeneration and phagocytosis of small numbers of nerve cell bodies were evident in the dorsal root ganglia and in the anterior horns (Figs. 5 and 6). The number of affected dorsal root ganglion cells did not vary appreciably from monkey to monkey and was not proportional to the axon loss in the adjacent posterior roots or columns. Neurons in the anterior horns were affected much less frequently than those in the dorsal root ganglia. In some monkeys, no neuronal degeneration or phagocytosis was found in the anterior horns.

In each deficient monkey, accumulation of PAS-positive, xylol-insoluble
lipopigment with yellow autofluorescence was present in most neurons in the dorsal root ganglia, anterior horns, and motor nuclei of the brainstem (Fig. 5). Lipopigment granules were not seen in cortical neurons or in small neurons in other parts of the nervous system. Lipopigment granules were present, however, in the CNS endothelium of six deficient monkeys. The deficient monkey without endothelial changes was also the animal with least extensive posterior column lesions and intact trigeminal roots. The neurons and CNS endothelium of controls were free of pigmentary changes.

Peripheral Nervous System (PNS)

Moderate to marked loss of axons was evident by light microscopy in sections of ulnar nerve from three of the seven deficient monkeys. Individual fascicles were selectively involved (Fig. 7). In two of these three monkeys, the sural nerves were also sites of marked axonal loss that appeared to increase in severity in a proximal to distal direction (Fig. 8). These two monkeys were also the animals with most extensive posterior column lesions. Light microscopic examination of sural nerves from the five other deficient monkeys disclosed a small number of degenerating axons. Sections of the sciatic nerves and lumbarosacral and brachial plexuses from six of the deficient monkeys were free of abnormalities. In the seventh monkey, these nerves contained occasional perivascular endoneurial collections of mononuclear cells with adjacent focal axonal loss. In the three control monkeys, examination by light microscopy of the brachial and lumbarosacral plexuses and ulnar and sciatic nerves was normal and, rarely, a degenerating axon was noted in the sural nerves.

Sural nerves from six deficient and three control monkeys and ulnar nerves from two deficient and one control monkey were examined by electron micros-
copy. Structurally similar degenerative changes involving myelinated and unmyelinated axons were present in each of the deficient nerves (Figs. 9 and 10). The number of affected axons, however, varied from monkey to monkey. The least extensive alteration consisted of accumulations of individual or coalescent membrane-bound vacuoles and membranous profiles within the axoplasm (Figs. 9 and 10). Frequently, there was loss of microtubules and filaments adjacent to these accumulations (Figs. 9 and 10). The basal lamina was focally reduplicated or elevated around Schwann cells with unmyelinated axons (Figs. 9 and 10). A few Schwann cells surrounding myelinated and unmyelinated fibers contained one or two cytoplasmic vacuoles without limiting membranes. More advanced lesions comprised dissolution of axonal contents and, eventually, disappearance of the axon itself with the formation of bands of Büngner (Figs. 9 and 10). The Schwann cell processes within the bands of Büngner contained cytoplasmic filaments. In some processes, cytoplasmic dense bodies and round or irregularly shaped vacuoles without limiting membranes were seen. Phagocytes containing myelin figures, irregularly shaped dense bodies, and vacuoles were centrally present within some of the bands of Büngner. In each instance, the phagocyte was entirely surrounded by the basal lamina enveloping the band. Phagocytes or cells identifiable as monocytes lying within

Fig. 7. Ulnar nerve, vitamin E-deficient monkey. Marked loss of axons and myelin sheaths selectively involving entire fascicles or well-defined portions of fascicles. Plastic section, 1.5 μ. Alkaline toluidine blue stain. ×250.
Fig. 8. Sural nerve, vitamin E-deficient monkey. Moderate loss of axons and myelin sheaths. A few degenerating axons are evident (arrows). The fiber spectrum of this nerve is shown in Figure 13. Plastic section, 1.5 μ. Alkaline toluidine blue stain. ×250.

the endoneurium but outside the bands of Büngner were not observed. Spheroids were absent. In addition to the axonal and Schwann cell alterations, a small number of vacuolated endoneurial fibroblasts were seen (Fig. 11). In contrast to the findings in the deficient monkeys, degenerating axons were rarely encountered in ulnar and sural nerves from control animals; axoplastic accumulations, bands of Büngner, reduplication of basal lamina, and vacuolated endoneurial fibroblasts were entirely absent.

Axonal degeneration was observed in the teased fiber preparations of sural nerve from both vitamin E-deficient monkeys studied with this technique (Fig. 12). Approximately 5% of the teased axons were affected in one monkey and 10 to 15% in the other.

In four of five deficient monkeys, fiber spectrum analysis demonstrated a selective loss of those myelinated sural nerve axons which were greater than 6 μ in diameter (Figs. 13 and 14). In the fifth deficient monkey, the evidence for selective axon loss was inconclusive. This monkey had also exhibited the least extensive central and peripheral nervous system lesions. In the four deficient monkeys with definitely abnormal fiber spectrum studies, myelinated sural nerve axons greater than 6 μ in diameter constituted 11 to 25% of the total myelinated axon population. The largest axons observed were between 12.5
Fig. 9. Ulnar nerve, vitamin E-deficient monkey. Focal accumulation of vacuoles and membranous profiles within a small caliber axon with an intact myelin sheath. There is a slight loss of filaments and tubules around the periphery of the accumulation. A band of Büngner (B) and focal elevations of the basal lamina of Schwann cells (arrows) are also seen. Lead citrate and uranyl acetate stain. ×18,000.

and 13.5 μ in diameter. In histograms, there was obvious flattening of the usual large fiber peak between 6 and 14 μ. Fiber density was reduced by 50% or more of control values in only the two deficient monkeys with unequivocal light microscopic sural nerve findings (Figs. 8 and 14). Fiber spectrum analysis of two control monkeys demonstrated a normal bimodal frequency distribution of myelinated sural nerve axons (Fig. 13). Myelinated axons greater than 6 μ in diameter constituted 40% of the total myelinated sural axon population in each of the controls. The largest-diameter myelinated sural axons observed were between 13 and 14 μ in one of the controls and 14 to 14.9 in the other. The fiber density was 9,772/mm² in one control and 9,996/mm² in the other.

DISCUSSION

Our studies demonstrate that prolonged vitamin E deficiency in young rhesus monkeys is associated with a homogeneous group of degenerative central and peripheral nervous system lesions. Sensory axons are chiefly affected. The axonopathy is progressive, as evidenced by the systematic increase in the severity of the lesions with regard to local axonal loss and to involvement of functionally related pathways, e.g., fasciculus gracilis. The involvement of sensory axons is most apparent in the CNS. Axonal loss was demonstrable by light microscopy in the posterior columns and posterior roots of each deficient
monkey. Less frequently, the trigeminal roots were sites of axonal depletion. Loss of axons from the posterior columns was greatest in the rostral cord segments. Possibly, the rostral accentuation of the axonal damage is indicative of a distal or dying-back type of axonopathy. A topographic study, however, including various levels of the posterior columns, the posterior roots, and the dorsal root ganglia during the evolution of the axonopathy, would be necessary to confirm this supposition.

In the peripheral nerves, the extent of the axonopathy ranges from light microscopic changes comparable in severity to the posterior column lesions to subtle alterations detectable with ultrastructural or morphometric techniques. Our view that, in the peripheral nervous system, sensory axons are also the primary site of the degenerative process is based upon the following observations on the deficient monkeys: (1) regular occurrence of axonal lesions in the sural nerves, (2) selective degeneration of fascicles in the ulnar nerve (a mixed motor and sensory nerve), and (3) absence of neurogenic muscular atrophy, especially in the monkeys with peripheral nerve lesions evident by light microscopy. Further, several of the peripheral nerve alterations (e.g., increased axonal loss in distal sural nerve, absence of axonal lesions in proximal nerves and plexuses, and selective involvement of large caliber myelinated sural nerve
Fig. 11. Ulnar nerve, vitamin E-deficient monkey. Vacuolated endoneurial fibroblasts. This change was seen most often in severely affected nerves. Lead citrate and uranyl acetate stain. ×18,000.

axons) suggest that a distal axonopathy affects peripheral as well as central sensory axons in the vitamin E-deficient monkey.

Although the pathogenesis of the axonopathy is similar in the CNS and PNS, the extent of axonal loss is greater in the CNS. The basis for the difference between central and peripheral axonal loss is unknown. Possibly, central axons are particularly vulnerable to injury induced by the vitamin E-deficient state. Alternatively, both central and peripheral sensory axons may be equally susceptible to this type of injury, but, in the PNS, regenerative activity compensates in part for the axonal loss.

In addition to the axonopathy, accumulation of lipopigment granules in large neuronal perikarya and CNS endothelial cells as well as neuronophagia in the dorsal root ganglia and anterior horns were also observed. In view of the widespread unsystematized distribution of the lipopigment, the very limited extent of the neuronophagia, which involved both motor and sensory neurons, and the absence of ischemic parenchymal damage, it is unlikely that either the pigmentary changes or the neuronophagia play a significant part in the development of the systematized sensory axonopathy.

The precise role of vitamin E deficiency in the pathogenesis of the nervous system lesions observed in the monkeys cannot be fully explained. Accumulation of lipopigment in neuronal perikarya and CNS endothelium has been reported in vitamin E-deficient rats and chicks (7, 18, 20). The excessive forma-
Fig. 12. Sural nerve, vitamin E-deficient monkey, teased fiber preparation. An intact myelinated fiber lies adjacent to a fiber with changes characteristic of axonal degeneration (arrows). Note the adjacent nodes of Ranvier (N). The myelin sheath and axon of the degenerating fiber can still be identified above this point. Osmium impregnation. ×100.

...tion of lipopigment at these sites is thought to result from peroxidative decomposition of cell membranes and other structures that is accelerated in the absence of effective antioxidants such as vitamin E (28). The relationship of the axonopathy, however, to the deficiency of vitamin E cannot be readily explained. The absence of regenerative nervous system changes in the two repleted monkeys, which could be directly attributed to treatment with vitamin E, does not preclude the possibility that deficiency of vitamin E was directly responsible for development of the axonopathy. The period of repletion was brief, whereas axonal regeneration proceeds slowly in peripheral nerves and develops only to a limited extent, if at all, in posterior roots and columns. Possibly, the absence of ultrastructural changes indicative of early axonal injury noted in the peripheral nerves of the two repleted monkeys signifies the cessation of axonal degeneration induced by the vitamin E supplements. Some observations in the literature and in this study point to a direct relationship between the axonal degeneration and vitamin E deficiency. Axonal degeneration in the posterior columns and/or gracile and cuneate nuclei has been de-
Fig. 13. Myelinated fiber spectrum in sural nerve of control monkey M-3. The histogram demonstrates a normal bimodal distribution of myelinated fibers from 2 to 14 μ in diameter, with peaks between 2 to 4 and 6 to 10 μ. The density of myelinated fibers per mm² of endoneurium is indicated above the histogram.

Fig. 14. Myelinated fiber spectrum in sural nerve of vitamin E-deficient monkey M-6. Selective loss of large caliber myelinated fibers is indicated by the flattened component of the histogram between 6 and 12 μ. The fiber density is approximately half of the control values. The light microscopic characteristics of this nerve are illustrated in Figure 7.
scribed in vitamin E-deficient rats and dogs (5, 12, 14–16, 20). Further, the accumulation of membranous profiles and vacuoles observed by us in peripheral sensory axons may reflect membrane damage occurring within axonal structures as a consequence of vitamin E deficiency. The resolution of this problem will require further studies.

The neuropathologic alterations observed in our study are more extensive than those previously noted in primates with chronic vitamin E deficiency. Einarsen and Telford (6) examined four vitamin E-deficient rhesus monkeys and noted relatively minor degenerative changes confined to nerve cell bodies. Some of these alterations, such as the accumulation of lipopigment and neuronophagia, were also evident in our monkeys. Axons in the CNS were not affected, however, in Einarsen’s monkeys, and the peripheral nerves were not commented upon. It should be kept in mind that three of Einarsen’s monkeys were fed the deficient diet from 6 to 17 months before sacrifice; the fourth animal received the diet for almost 3 years. In our study, the animals were maintained for 30 to 33 months on the deficient regimen. Thus, the absence of axonal lesions in Einarsen’s monkeys may reflect a slow and variable rate in the development of the axonopathy during vitamin E deficiency. This explanation is supported by our own observations concerning the variable extent of central and peripheral axonal degeneration in the seven monkeys uniformly maintained on a longer vitamin E-deficient regimen.

The absence of nervous system lesions in the large series of vitamin E-deficient new and old world monkeys studied by Hayes (11) is difficult to assess. The neuropathologic examination in these monkeys is summarized by a single statement noting that histologic examination of the brainstem disclosed no evidence of neuroaxonal dystrophy in the gracile or cuneate nuclei.

Comparison of our observations with neuropathologic findings reported in vitamin E-deficient rats (5, 14–16, 20) demonstrates both similarities and differences. Degeneration of axons in the posterior columns and sensory nerves and the accumulation of lipopigment in neurons and CNS endothelium occurs in both species. In rats, however, the fasciculus gracilis is more severely affected than the fasciculus cuneatus (20), while the opposite was true in our monkeys. Further, swollen, dystrophic axons or spheroids are prominent at a number of sites in the deficient rat, including the posterior columns, posterior horns, major sensory nuclei in the caudal medulla, and the distal peripheral nerves (20). In contrast, spheroid formation occurred only to a limited extent in our monkeys and rarely involved nuclei in the caudal medulla. The basis for the difference in the extent of spheroid formation is uncertain. Some studies suggest that development of spheroids in vitamin E deficiency may be related to the concentration of dietary fat (12).

Neuropathologic lesions resembling those seen in our monkeys and in vitamin E-deficient rats have been described in humans with known or suspected vitamin E deficiency. The human cases most often involve intestinal malabsorption of fat-soluble vitamins as a consequence of cystic fibrosis or biliary atresia (10, 26, 27). Posterior column degeneration and extensive spheroid formation have been observed in most of the human cases. We have recently
completed studies on two children dying at five and nine years of age with congenital biliary atresia, intestinal malabsorption, and documented, continuously low, serum vitamin E levels (19, 22). Some of the neuropathologic changes are remarkably similar to those in our deficient monkeys, e.g., greater involvement of cuneatus than of gracilis, selective loss of large caliber, myelinated sural nerve axons, and absence of proximal peripheral nerve degeneration. Spheroid formation in the caudal medulla and posterior horns was extensive, however, in both patients.

Very low serum vitamin E levels are also regularly observed in patients with abetalipoproteinemia (Bassen-Kornzweig syndrome) (13). These patients often develop clinical evidence of peripheral neuropathy and spinocerebellar degeneration (2, 3, 23, 24). Improvement of the neurological disorder following treatment with vitamin E has been reported (17). Neuropathologic data in these cases are limited to reports of two autopsies and a nerve biopsy (3, 23, 24). In the autopsied cases, demyelination of the posterior columns, spinocerebellar tracts, and focal areas in peripheral nerve were noted along with loss of neurons from the anterior horns and cerebellar nuclei in one case and accumulation of neuronal lipopigment in the other (3, 24). Patchy demyelination was also observed in sections of the nerve biopsy (23). No spheroids were described in any of the cases.

Conclusive statements cannot be made concerning the pathogenesis and interrelationships of the neuropathologic lesions in vitamin E-deficient rats, monkeys, and humans because necessary data are lacking. Considering our own observations and the studies reviewed in the preceding paragraphs, we suggest the following hypotheses. Chronic vitamin E deficiency in rats, monkeys, man, and possibly other mammals leads to degeneration and loss of sensory axons in the posterior columns, sensory roots, and peripheral nerves. The degeneration may result from axonal membrane injury and develop as a distal and dying-back type of axonopathy. The extent to which spheroid formation occurs is variable and may be influenced by the concentration of dietary fat. Pigmentary changes in neurons and CNS endothelium are characteristic of vitamin E deficiency and occur relatively early after the deficient state is established. In some cases, phagocytosis of a few motor or sensory neurons may be observed. Neither the pigmentary accumulation nor the neuronophagia plays a role in the development of the axonopathy. Should these formulations be confirmed, chronic mammalian vitamin E deficiency may prove to be a useful model for the investigation of systematized degeneration of sensory axons.

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Note added in proof. A recent study by Muller et al. (Neurology, 30: 1286−1291, 1980) demonstrates a selective loss of large-caliber myelinated sural nerve fibers in a patient with abetalipoproteinemia.
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