American Journal of Pathology, Vol. 132, No. 3, September 1988

Brain and Optic System Pathology in Hypocholesterolemic Dogs Treated with a Competitive Inhibitor of 3-hydroxy-3-methylglutaryl Coenzyme A Reductase

P. H. BERRY, BVSc, MPhil, J. S. MACDONALD, PhD, A. W. ALBERTS, BS, S. MOLON-NOBLOT, PhD, J. S. CHEN, BS, C.-Y. L. LO, BS, MS, M. D. GREENSPAN, BS, PhD, H. ALLEN, VMD, G. DURAND-CAVAGNA, PhD, R. JENSEN, DVM, PhD, Y. BAILLY, VM, P. DELORT, DVM, and P. DUPRAT, DVM, PhD

From the Department of Safety Assessment, Merck Sharp & Dohme-Chibret, Riom, France, the Department of Safety Assessment, Merck Sharp & Dohme, West Point, Pennsylvania, and the Department of Biochemical Regulation, Merck Sharp & Dohme Research Laboratory, Rahway, New Jersey

The cholesterol lowering compound lovastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34 HMG CoA reductase), was given in nine separate experiments to normocholesterolemic dogs at rates up to 180 times the maximum therapeutic dose in man (1 mg/kg/day). Mean serum total cholesterol concentrations were reduced as much as 88% below normal. Clinical evidence of neurotoxicity occurred in up to 37% of animals given 180 mg/kg/day lovastatin for 11 or more days, especially in one laboratory where the dosing regime resulted in higher concentrations of plasma drug levels. Dogs receiving 60 mg/kg/day or less never exhibited neurologic signs. The central nervous system (CNS) of affected dogs exhibited endothelial degeneration and hemorrhagic encephalopathy. Focal extravasation of horseradish peroxidase occurred frequently (6/8) in the retrolaminar optic nerve of asymptomatic or clinically affected dogs given 180 mg/kg/day lovastatin, with endothelial degeneration and discrete optic nerve degenerative lesions interpreted as ischemic. The association between the degree of hypocholesterolemia and occurrence of clinical signs was not exact. Total brain cholesterol was similar in treated and control dogs. Hypocholesterolemic dogs had proportionally lowered serum concentrations of alpha-tocopherol, but oral supplementation of this vitamin did not prevent the neurologic syndrome. Endothelial degeneration in the CNS and optic nerve may have reflected *in vitro* morphologic effects of HMG CoA reductase inhibitors due to extreme inhibition of nonsterol isoprene synthesis. Retinogeniculate axonal (Wallerian-like) degeneration occurred in ≥12% of dogs given 60 mg/kg/day or more lovastatin, with central chromatolysis of occasional retinal ganglion cells. These neuroaxonal changes may have been secondary to vascular effects, but superimposed direct neurotoxic action at the high dosage levels of lovastatin could not be excluded. There was no evidence of drug induced adverse effects in the CNS of dogs given up to 30 mg/kg/day lovastatin for 2 years. (Am J Pathol 1988, 132:427–443)

THE FUNGAL METABOLITE LOVASTATIN (formerly mevinolin) is a competitive inhibitor of the rate limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (EC 1.1.1.34).[1] Lovastatin or related compounds[2] can lower serum cholesterol concentrations by as much as 46% in several species including humans,[3–7] cynomologous monkeys,[8,9] rabbits,[9–11] and dogs[1,9,12,13] without significant toxicologic side effects. In adult rats and mice, the lack of a permanent cholesterol-lowering effect of these compounds may be due to their induction and stabilization of hepatic HMG CoA reductase.[14–16] Despite the importance of cholesterol as a constituent of the developing and mature nervous system,[17,18] there are no previously reported neuropathologic effects associated with the use of HMG CoA reductase inhibitors in experimental

Accepted for publication April 5, 1988.

Address reprint requests to P. H. Berry, Department of Safety Assessment, Merck Sharp & Dohme-Chibret, 63203 Riom Cedex, France.

animals. High doses of lovastatin in pregnant rats may cause fetal skeletal malformations, however. The incidence of these changes is suppressed by coadministration of the enzyme product mevalonate,[19] suggesting a mechanism-based effect due to extreme inhibition of HMG CoA reductase.

In contrast to the effects of HMG CoA reductase inhibitors in rats, when these animals are given agents such as triparanol or AY-9944, which restrict cholesterol synthesis after squalene cyclization, there is accumulation of cholesterol-precursor sterols in several tissues including the brain.[20,21] Cholesterol-suppressed, fetal holoprosencephalic malformations may also occur,[22] and in rats dosed neonatally with either agent, there is disturbed myelinogenesis with accumulations of numerous intracytoplasmic inclusions predominantly in oligodendroglia throughout the central nervous system (CNS).[23-25]

The HMG CoA reductase inhibitor lovastatin is used in human hypercholesterolemia at 1 mg/kg/day or less to reduce abnormally high serum cholesterol concentrations and the associated risk of coronary heart disease.[4,5,26,27] In the studies reported here, lovastatin was given to normocholesterolemic mature beagle dogs at rates up to 180 times the maximum human therapeutic dose, causing rapid reduction in mean serum cholesterol concentrations by as much as 88%. There was associated abetalipoproteinemia, and serum alpha-tocopherol was proportionally reduced with cholesterol (Alberts AW, unpublished observation) to levels previously associated with canine hypovitaminosis E.[28,29] Sudden tremors and convulsions occurred in some treated dogs. The purpose of this study is to describe the clinical signs, changes in serum cholesterol concentrations, and morphologic findings in brain and optic system of the affected dogs, to examine the effect of alpha-tocopherol prophylaxis on the development of the neurologic syndrome, and to consider possible pathogenetic mechanisms that may be related to extreme inhibition of the pivotal enzyme HMG CoA reductase.

Materials and Methods

Animals and Methods

At Merck Sharp & Dohme (MSD)-Chibret, Riom, France (Laboratory A), or MSD Research Laboratories (MSDRL), West Point, PA (Laboratory B), nine experiments were completed between 1980 and 1986 with 303 individually-caged beagle dogs 27 to 53 weeks old initially, weighing 6.6 to 13.6 kg. Treatment groups usually were comprised of 4 to 8 dogs per sex, used as controls or given 5 to 180 mg/kg/day lovastatin orally in a capsule each morning for up to 105 weeks. In laboratory A, American beagles (Marshall Farms, North Rose, NY) or French beagles (Centre d'Elevage du Domaines des Souches, Toucy, France) were each offered 300 g per day of certified canine chow (Ralston Purina, St Louis, MO; or Usine d'Alimentation Rationnelle, Villemoisson, France) within 40 minutes of treatment. In laboratory B, each beagle (Marshall, or Hazelton Research Animals, Denver, PA) received 350 or 400 g per day of chow (Purina) up to six hours after treatment.

A study conducted at laboratory A examined the effect of alpha-tocopherol prophylaxis on the development of neurologic changes. In each of four groups there were one female and two male dogs serving as controls, or dosed orally for up to 12 weeks with either 0.5 g b.i.d. alpha-tocopherol acetate (in Rovimix E-50, provided by Hoffman-La-Roche, Basel, Switzerland), 180 mg/kg/day lovastatin, or both 0.5 g b.i.d. alpha-tocopherol acetate and 180 mg/kg/day lovastatin.

Biochemical Analyses

Following absorption, lovastatin, an inactive lactone, is hydrolyzed to its active, open dihydroxy acid form. Inhibitory activity in plasma was measured 1, 2, 4, and 24 hours after treatment of dogs with 5 to 180 mg/kg/day lovastatin continuously for at least 25 weeks. In laboratory A, on the day of collection of plasma for these analyses, dogs were fed within 5 minutes of receiving lovastatin. In laboratory B, food was offered between the 2- and 4-hour blood sampling.

In all dogs, serum total cholesterol concentrations were estimated[30] at intervals of 1 or more weeks. Analyses of serum high-density lipoprotein (HDL) cholesterol, as well as lipoproteins by gel electrophoresis, were done[31] less frequently. Plasma alpha-tocopherol levels were determined[32] at weekly intervals in all dogs in the alpha-tocopherol supplementation study, and in brain homogenate of the frontal cortex of dogs given 180 mg/kg/day lovastatin for 40 weeks in laboratory A. Frontal cortex levels of mevalonate products cholesterol, dolichol, and ubiquinone were also determined by HPLC analysis.[33] Extracts of brain lipids were chromatographed on a normal phase system using a cyanopropyl column and 0.1% isopropanol in heptane as the solvent system. The lipids were detected with a CRB 2140 diode array detector and quantitated by comparison with known amounts of standards.

Clinical Assessment

All dogs were examined daily and selected animals were subjected to detailed neurologic examination.[34]

The optic fundus of all dogs was examined at regular intervals using an indirect ophthalmoscope. Tropicamide, 0.5% (Mydriaticum, MSD-Chibret), was used as a mydriatic to facilitate visualization of the fundus. Cerebrospinal fluid (CSF) pressure was measured[35] before necropsy of five overtly normal dogs given 180 mg/kg/day lovastatin for 40 weeks and four untreated control dogs.

Necropsy and Morphology

In routine studies, dogs were killed by exsanguination during barbiturate anesthesia. Brains were weighed (wet) and fixed by immersion in 10% neutral-buffered formalin with cervical spinal cord, optic, and sciatic nerve (midfemoral). Portions of thoracic and lumbar spinal cord from selected dogs with neurologic signs were similarly fixed. Eyes were preserved in Zenker's fixative. Paraffin-embedded, hematoxylin and eosin-stained (H&E) sections of these tissues were examined and selected special stains also were used. Vertical, semi-serial H&E sections of eye and optic nerve head (ONH, defined here as papilla with approximately 4 mm of retrolaminar optic nerve) were cut across the entire thickness of the optic nerve of 24 of the dogs given 180 mg/kg/day lovastatin.

Dogs were also selected for perfusion fixation and transmission electron microscopy (EM) of the brain and optic system after intravenous injection of horseradish peroxidase (HRP). In one study, each dog was given 20 ml of 60 to 65 mg/ml HRP (Sigma, type II) by slow (2 to 3 minutes) intravenous injection (Table 1). After 30 to 105 minutes dogs were heparinized, anesthetized, and perfused via an intra-aortic cannula with 8 l of phosphate-buffered, pH 7.3 fixative (0.5% paraformaldehyde, 2, 2.5, or 3% glutaraldehyde), delivered at 120 to 140 mm mercury using a peristaltic pump. Eyes with orbital optic nerve and coronally-sliced (3 to 5 mm) brain were post-fixed in 2%, phosphate-buffered glutaraldehyde for 2 to 48 hours at 4 C. Brain slices were rinsed subsequently in phosphate buffer, incubated at 20 C for 40 minutes in 0.1% diaminobenzidine substrate solution,[36] rinsed, and photographed. Longitudinal, 50 μ serial sections of each left ONH were cut on a vibratome (Lancer Company, St Louis, MO). These and similar sections of amygdala and thalamic optic tract were incubated as described above to localize HRP. Vibratome sections were mounted unstained, or post-fixed in osmium. Osmium-fixed tissues were dehydrated in serially-concentrated alcohols and embedded in Epon resin. One micron, toluidine blue-stained sections were examined from the amygdala, thalamic optic tract, and the prelaminar, laminar and retrolaminar ONH. Ultrathin sections from selected sites were cut, stained with uranyl acetate and lead citrate, and examined in a JEOL 1200EX electron microscope. Paraffin and H&E-stained sections of brain and semi-serial sections of the contralateral (right) eye and ONH from each perfusion-fixed dog were examined also.

Table 1—Dogs Selected for Morphologic Study Using Perfusion Fixation After Intravascular Horseradish Peroxidase (HRP)

	No. of days treatment*	Neurologic signs	Final total serum cholesterol (mg/100 ml)	HRP circulation time (min)†
Control				
84M‡	24	–	148	95
86M	30	–	123	65
91F	87	–	135	105
79F	88	–	200	65
Treated				
94M	11	+	19	30
96M	12	+	54	55
78M	15	–	22	55
04M	15	–	26	90
98M	24	–	8	65
93F	28	+	16	30
81F	87	–	2	58
92M	88	–	2	100

* 180 mg/kg/day lovastatin.

† Time in minutes between intravenous injection of HRP and commencement of perfusion.

‡ Individual dog numbers; M, male; F, female.

Results

Biochemical Analyses

Mean plasma concentrations (measured as inhibitory activity) of lovastatin increased with dose, but individual values varied widely within treatment groups (Table 2). In laboratory A, where chow was offered immediately after treatment, the highest plasma levels of active drug (2249.5 ± 1694.9 ng/ml) were measured 4 hours after treatment of dogs with 180 mg/kg/day lovastatin. In laboratory B, where feed was offered between 2 to 4 hours after treatment, peak plasma levels of active drug (438.7 ± 312.5 ng/ml) were found 2 hours after treatment of dogs with 180 mg/kg/day lovastatin.

Reductions in average total serum cholesterol concentrations of treated dogs of both sexes were closely proportional to dose, and varied to 88 or 66% below pretest values in dogs given 180 mg/kg/day lovastatin in either laboratory A or B, respectively. When male dogs alone in laboratory A were considered, reductions to 93% occurred (Figure 1). Serum HDL- and LDL-cholesterol were both reduced, the latter in particular. Gel electrophoresis often showed lowering of the serum betalipoprotein fraction to below measur-

Table 2—Plasma Levels of Inhibitory Equivalents of Lovastatin in Dogs Treated Continuously for 25 or more Weeks.

Lovastatin treatment	No. of dogs	Lovastatin inhibitory equivalents (ng/ml)			
		24 hours*	1 hour	2 hours	4 hours
Laboratory A†					
20 mg/kg/day	5	21.9 ± 20.5	51.5 ± 53.3	-	178.8 ± 95.2
60 mg/kg/day	4	33.2 ± 18.2	75.1 ± 43.3	-	2082.0 ± 1731.8
180 mg/kg/day	6	74.1 ± 51.3	169.1 ± 96.0	-	2249.5 ± 1694.9
Laboratory B‡					
Control	10	0	14.1 ± 32.8	0	1.9 ± 6.0
5 mg/kg/day	10	4.3 ± 9.1	42.0 ± 36.2	65.4 ± 35.0	38.3 ± 37.8
30 mg/kg/day	10	7.8 ± 12.2	95.2 ± 98.4	175.9 ± 140.3	94.6 ± 69.1
180 mg/kg/day	8	31.4 ± 23.6	231.6 ± 348.0	438.7 ± 312.5	317.1 ± 336.4

* Hours after last dose of lovastatin, or empty capsule in case of controls, values expressed as mean ± SD.
† Laboratory A (MSD-Chibret, Riom, France)—dogs offered 300 g of certified chow within 5 minutes of drug treatment.
‡ Laboratory B (MSDRL, West Point, PA)—dogs offered 350 g of certified chow between 2 and 4 hours after treatment.

able limits in dogs treated with 60 or 180 mg/kg/day lovastatin. Only dogs treated with 180 mg/kg/day showed neurologic signs. These subjects always had substantially lowered serum total cholesterol concentrations (Table 1) but the relation between serum cholesterol concentrations and the appearance of neurologic signs was not direct; not all dogs with very low serum cholesterol values showed clinical signs (Table 1, Figure 1).

In the 12-week alpha-tocopherol acetate supplementation study, mean serum alpha-tocopherol concentrations of dogs given 180 mg/kg/day lovastatin alone decreased from 6.72 ± 0.82 to 2.68 ± 1.03 μg/ml at termination (Figure 2). With concurrent lovastatin, alpha-tocopherol treatment, serum alpha-tocopherol levels increased more than threefold in each dog by the end of the second week, from 7.20 ± 0.89 to 25.24 ± 1.57 μg/ml. Different individual response patterns then were observed, but in each dog, including one that showed neurologic signs (Figure 2), serum alpha-tocopherol concentrations were almost always higher than in the unsupplemented treated dogs.

Analyses of brain alpha-tocopherol in clinically normal dogs given 180 mg/kg/day lovastatin for 40 weeks showed no difference between control and treated dogs (Table 3). Analyses of brain cholesterol

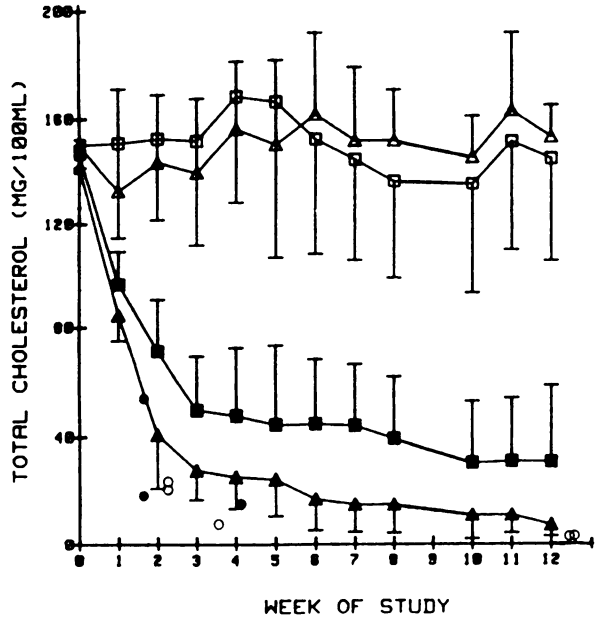

Figure 1—Mean and standard error of concentrations of serum total cholesterol from dogs used in study of brain and optic system pathology. Male (△) and female (□) control. Male (▲) and female (■) treated, 180 mg/kg/day lovastatin. Final cholesterol values for individual treated dogs studied with (●) or without (○) overt neurologic signs.

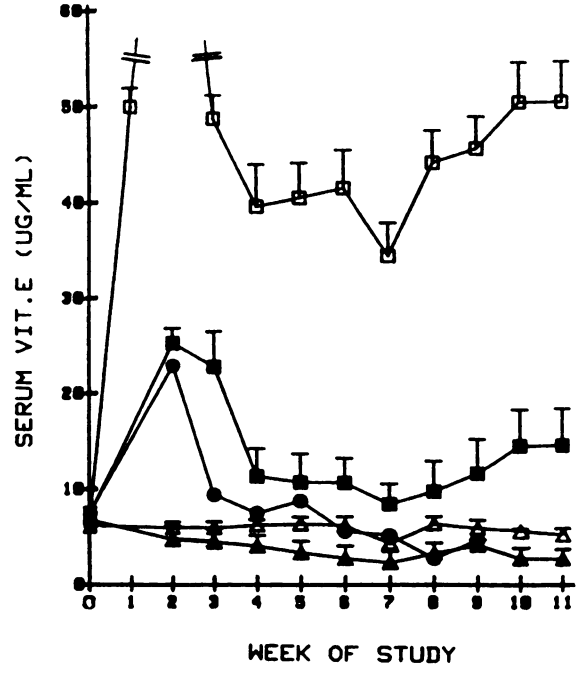

Figure 2—Mean and standard error of serum concentrations of alpha-tocopherol (Vitamin E) from dogs used in study of effect of alpha-tocopherol treatment on occurrence of neurologic signs after treatment with 180 mg/kg/day lovastatin. Control (△), oral alpha-tocopherol (□), lovastatin (▲), lovastatin and alpha-tocopherol (■), individual dog that showed neurologic signs (●).

Table 3—Levels of alpha-Tocopherol and the Mevalonate Metabolites Cholesterol, Dolichol, and Ubiquinone in Brain Homogenates of Dogs given 180 mg/kg/day Lovastatin for 40 Weeks

Treatment	No. of dogs	alpha-Tocopherol (μg/g)	Cholesterol (μg/g)	Dolichol* (μg/g)	Ubiquinone (μg/g)
Control	4	9.97 ± 2.2	12044 ± 1573	48.2 ± 10.2	21.1 ± 2.9
Lovastatin	6	9.78 ± 1.3	12431 ± 1356	41.8 ± 11.5	15.9 ± 2.5†

* Unesterified.
† $P < 0.05$.

content similarly showed no differences (Table 3). There was no treatment-related effect on wet brain weight. Dolichol and ubiquinone content were slightly reduced in the treated dogs (Table 3); the difference in ubiquinone content was statistically significant ($P < 0.05$).

Clinical Signs

Neurologic signs occurred only in dogs given 180 mg/kg/day lovastatin. In laboratory B, 1 of 28 dogs treated at this rate for up to 105 weeks was affected, whereas in laboratory A the incidence was 16 of 63 in dogs treated for up to 40 weeks. Clinical signs occurred after 11 to 91 days, with sudden onset of overt disturbance 2 to 24 hours before death or when the dogs were killed. One mildly affected dog recovered. Signs consisted of hypoactivity; with ataxia evidenced by swaying, stumbling, and hypermetria. Affected animals walked into objects but there were no discernible changes in ocular reflexes or fundic morphology at ophthalmic examination. Recumbency, vomiting, ptyalism, transient tremors, as well as tetanic and clonic convulsions occurred in most affected dogs. Neurologic and fundic examination of overtly asymptomatic dogs given 180 mg/kg/day lovastatin showed no abnormalities. In five apparently normal dogs given 180 mg/kg/day lovastatin for 40 weeks, CSF pressures (mean, 87.5 mm; range, 79 to 92 mm) were similar to those of four control dogs (mean, 100.75; range, 92 to 110 mm).

Morphological Changes

Brain and Orbital Optic Nerve

Changes occurred mainly in dogs given 180 mg/kg/day lovastatin, particularly those with neurologic signs (Table 4), including one animal supplemented with alpha-tocopherol. The amygdala and frontal cortex were most affected, but the whole cerebral and cerebellar cortex, and the brainstem caudal to the medulla oblongata were also involved in some dogs. Edema was indicated by multifocal to diffuse zones of perivascular HRP in grey matter and to a much lesser extent in white matter (Figure 3). Perivascular "ring" hemorrhages (Figure 4A), foci of extravasated HRP, and homogeneous, eosinophilic material (Figure 4B) surrounded capillaries and other small vessels, which also showed fibrinoid degeneration and regenerative hyperplasia. Hemorrhages were present in areas of neuropil free of extravasated HRP, and edema and hemorrhages were sometimes found in overtly normal dogs treated with 180 mg/kg/day lovastatin (Table 4). Slight dilatation of lateral ventricles was present in occasional treated and control dogs.

There were changes on electron microscopy in vascular endothelium of treated dogs without apparent alteration of adjacent neuropil. Affected endothelial cells had distended cisternae of rough endoplasmic reticulum (RER), intracytoplasmic membrane-bound vacuoles, and were sometimes partially separated from adjacent cellular processes (Figure 5A). Horseradish peroxidase extended into interendothelial clefts and densely infiltrated basal laminae. Intra-endothelial vesiclelike profiles (<500 nm) containing HRP were seen occasionally. In some small vessels there was more than one layer of degenerative cells (Figure 5B). In more advanced vascular degeneration, flocculent or dense luminal deposits of HRP extended through interendothelial spaces to adjacent neuropil where there were swollen perivascular astrocytic processes, extravasated erythrocytes, and fibrin (Figure 6). These degenerated vessels were sometimes surrounded by two or more layers of directly apposed basal laminae, and had luminal thrombi including masses of necrotic cellular debris. Reactive endothelial cells had swollen nuclei, expanded cytoplasm with prominent RER, free ribosomes, and phagolysosomallike bodies, as well as numerous luminal microvillous processes.

Perivascular malacia was sometimes associated with vascular degeneration (Table 4). Very slight to moderate in degree, the multifocal to diffuse zones occurred most consistently in the amygdala and were usually accompanied by infiltrations of polymorphonuclear leukocytes with a few monocytes and Gitter cells.

Scattered axonal (Wallerianlike) degeneration (WD) of retinogeniculate fibers was evident in both

Table 4—Histologic Changes in Paraffin-HE Sections of Brains of Dogs given 5 to 180 mg/kg/day Lovastatin for up to 59 Weeks*

	Dogs clinically normal					Dogs with clinical signs
Lovastatin (mg/kg/day)	0	5	20	60	180	180
No. of dogs	62	16	16	16	48†	15
Brain histologic changes						
Microvascular hemorrhagic angiopathy	-‡	-	-	-	5	15
Focal perivascular malacia	-	-	-	-	-	14
Retinogeniculate Wallerianlike degeneration	-	-	-	3	32	12
Vestibulocochlear Wallerianlike degeneration§	-	-	-	-	1	1

* Findings compiled from the studies in laboratory A (MSD-Chibret, France), including the alpha-tocopherol supplementation experiment.
† One dog showed CNS signs but recovered and was clinically normal when presented for necropsy.
‡ Indicates change not present.
§ A total of 2 of 16 dogs in 1 study.

overtly normal and clinically affected dogs given 60 to 180 mg/kg/day lovastatin (Tables 4, 5). The incidence was dose dependent. The axonal degeneration occurred with similar intensity from 3 to 4 mm retrolaminar in the orbital optic nerve to the terminal optic tract near the lateral geniculate nucleus. Electron microscopy showed that the changes in axons and myelin sheaths conformed essentially with those described previously in experimental WD.[37,38] No endothelial degeneration was seen in optic tract. Slight WD was present infrequently in central projections of the eighth cranial nerve of dogs given 180 mg/kg/day lovastatin (Table 4). The root of the vestibulocochlear nerve, the trapezoid body, vestibular fibers, and myelinated fibers reaching the cochlear nuclei were affected in two dogs.

Eye and Optic Nerve Head (ONH)

In 50-μ sections of ONH from control dogs (Figure 7A), there was minimal retention of HRP within vessels in the prelaminar and retrolaminar optic nerve, and no detectible reaction product within nerve fascicles. At the level of the lamina choroidalis and lamina scleralis, HRP was present in perivascular spaces and within fascicles, as previously noted in normal animals of other species.[39]

In dogs treated with 180 mg/kg/day lovastatin, retrolaminar segmental edema of the ONH was indicated by focal extravasation of HRP (Figure 7B,C) extending to approximately 3.5 mm behind the lamina scleralis (Table 5). Walls of vessels in the retrolaminar pia and within the optic nerve had dense deposits of

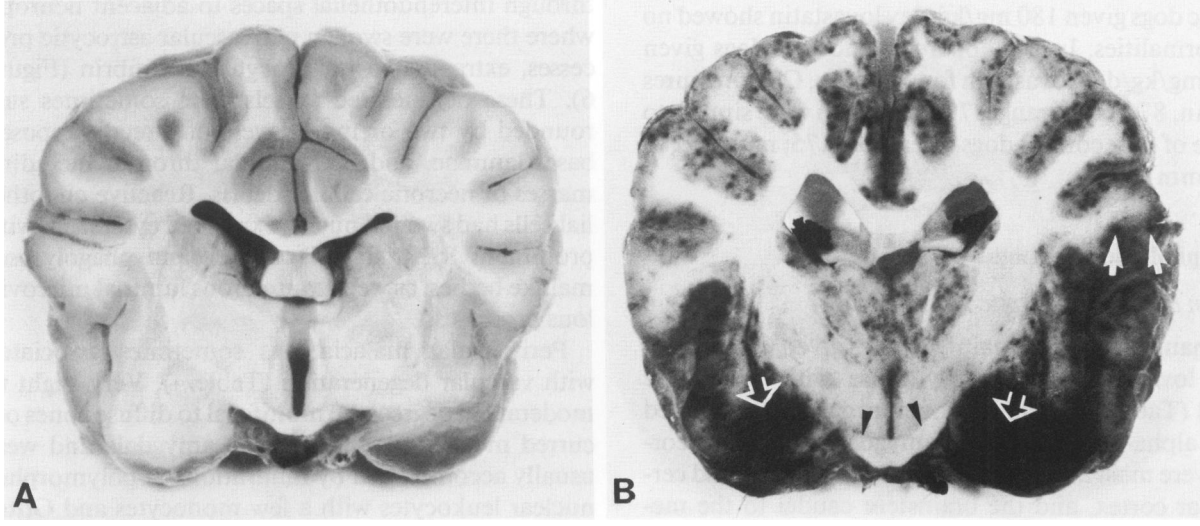

Figure 3—Coronal, 3 to 4 mm slices of perfusion-fixed brain after diaminobenzidine incubation, from dogs injected intravenously with HRP 55 to 65 minutes before anesthesia and perfusion. **A**—Control. There is no evidence of HRP leakage into neuropil. **B**—A dog killed with nervous signs after 12 days treatment with 180 mg/kg/day lovastatin, there is locally extensive leakage in the region of the amygdala (open arrows), and multifocal leakage in the cerebral cortex (closed arrows). White matter appears less affected. The optic chiasma and tract is free of extravasated HRP (arrowheads).

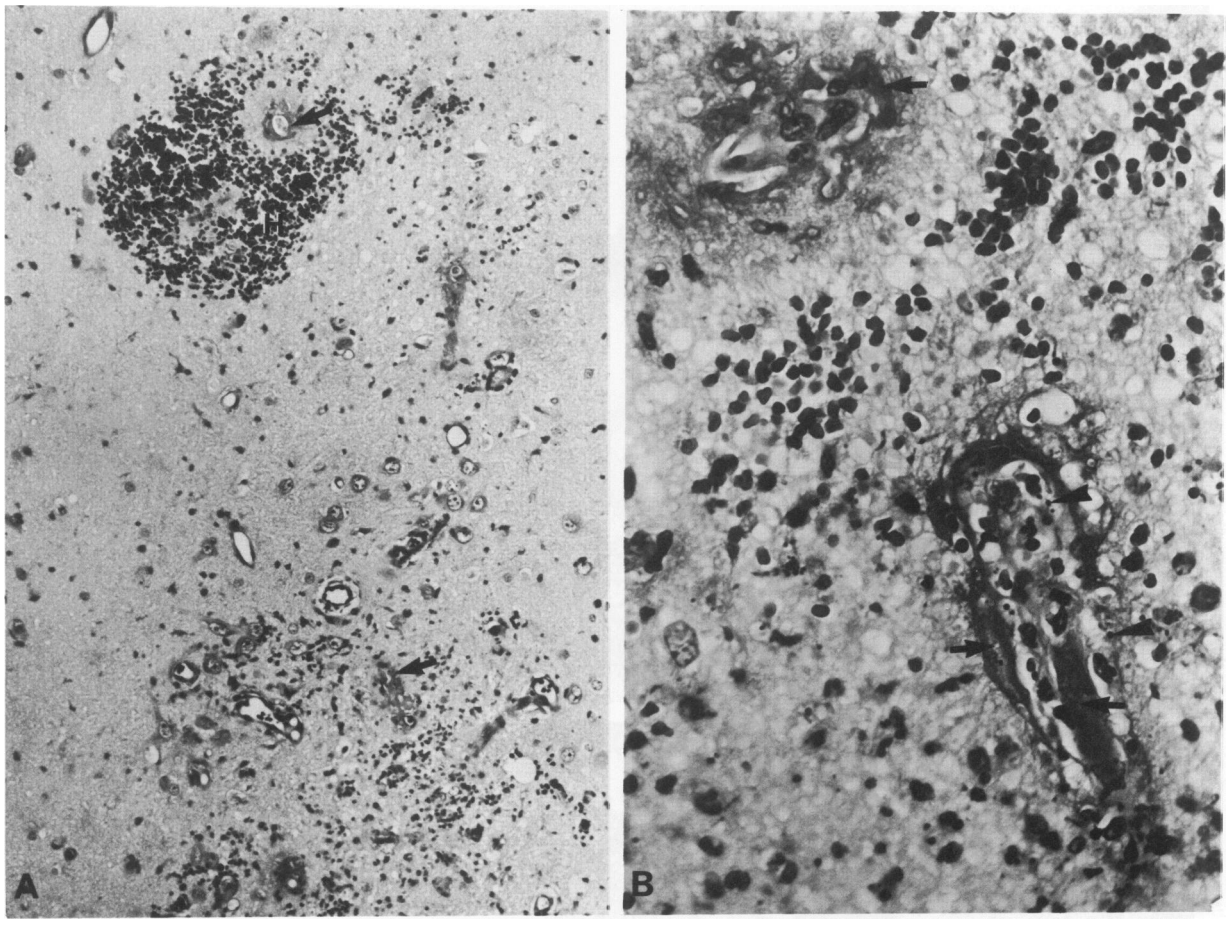

Figure 4—Amygdala of a dog with nervous signs after 21 days treatment with 180 mg/kg/day lovastatin. **A**—Perivascular hemorrhage (H) and fibrinoid degeneration of several small vessels (arrows). **B**—Detail of advanced vascular degeneration with eosinophilic, luminal, mural, and perivascular fibrinoid deposits (arrows). Pyknotic nuclei are present (arrowheads). There is perivascular hemorrhage, vacuolation of neuropil and polymorphonuclear leukocyte infiltration. (H&E, A, ×112; B, ×455)

HRP that filled perivascular spaces and extended into adjacent nerve fascicles. In the most severely affected nerves, there were increased laminar and prelaminar concentrations of HRP (Figure 7C). By EM, vascular endothelial degeneration was confirmed occasionally in retrolaminar small vessels. In those showing advanced degeneration, the vessel wall consisted of apposed layers of basal laminae only, devoid of endothelium. Luminal thrombi consisted of necrotic cell debris and tightly packed erythrocytes, some in the process of diapedesis (Figure 8).

Focal degeneration of the ONH (Table 5) occurred as discrete retrolaminar (Figure 9A–C) or less frequent laminar (Figure 9D) or prelaminar (Figure 9E) foci of fascicular vacuolation or swollen axons among extravasated HRP (Figure 7C). Retrolaminar degenerative foci occasionally were multiple and coalescing, extending across the optic nerve. Electron microscopy showed that the fascicular extracellular space of degenerate foci was expanded and sometimes contained HRP. Vacuoles corresponded to swollen axons with dispersed organelles or clear contents and thin myelin sheaths, or nonmembrane-bound spaces apparently derived from degenerate cellular processes. Some swollen axons within foci of degeneration contained dense accumulations of normal or disrupted mitochondria, membranous organellar debris, and central, normally-oriented microtubules. Other axons had electron-dense, amorphous contents and disrupted or collapsed myelin sheaths. Light microscopy of the most advanced focal lesions showed fibrinoid degeneration and regenerative hyperplasia of small vessels, hemorrhage, polymorphonuclear leukocyte infiltration, and gliosis, with expansion of interfascicular septae and focal loss of myelinated axons.

In the ONH of all dogs there were occasional individual degenerate axons, not always associated with nearby focal degeneration or extravasated HRP (Table 5, Figure 10). These nonmyelinated or myelinated axons manifested the variety of ultrastructural morphologies found in degenerate foci (Figure 10D). In addition, other individual prelaminar axons con-

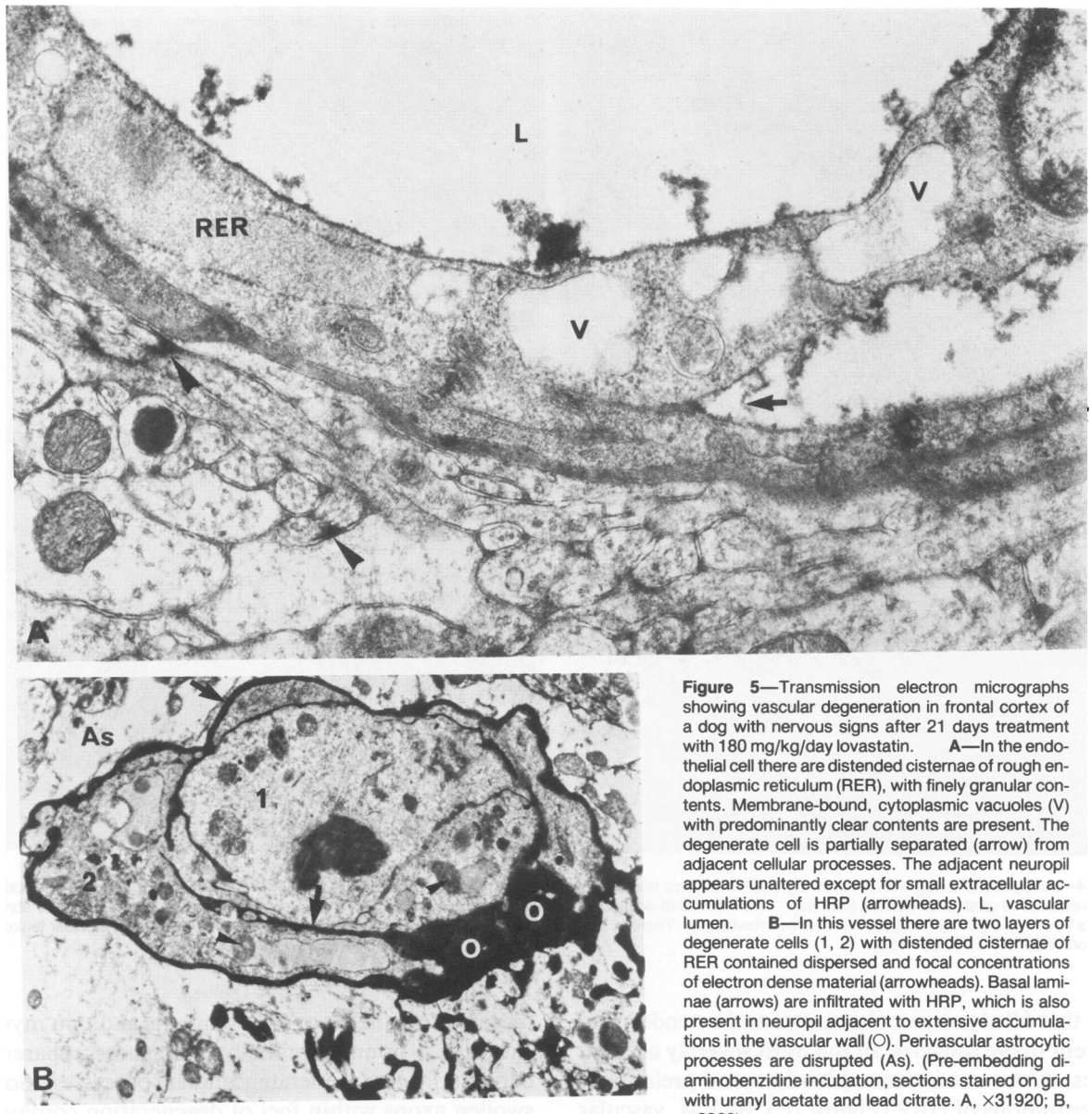

Figure 5—Transmission electron micrographs showing vascular degeneration in frontal cortex of a dog with nervous signs after 21 days treatment with 180 mg/kg/day lovastatin. **A**—In the endothelial cell there are distended cisternae of rough endoplasmic reticulum (RER), with finely granular contents. Membrane-bound, cytoplasmic vacuoles (V) with predominantly clear contents are present. The degenerate cell is partially separated (arrow) from adjacent cellular processes. The adjacent neuropil appears unaltered except for small extracellular accumulations of HRP (arrowheads). L, vascular lumen. **B**—In this vessel there are two layers of degenerate cells (1, 2) with distended cisternae of RER contained dispersed and focal concentrations of electron dense material (arrowheads). Basal laminae (arrows) are infiltrated with HRP, which is also present in neuropil adjacent to extensive accumulations in the vascular wall (O). Perivascular astrocytic processes are disrupted (As). (Pre-embedding diaminobenzidine incubation, sections stained on grid with uranyl acetate and lead citrate. A, ×31920; B, ×6960)

tained enlarged mitochondria and membrane-bound vacuoles apparently derived from them (Figure 10B), or accumulations of microtubules and neurofilaments. Others were distended with varying proportions of degenerative or maloriented forms of these organelles (Figure 10C). Central chromatolysis of occasional retinal ganglion cells was present in paraffin and H&E-stained sections of eyes of dogs given 180 mg/kg/day lovastatin (Table 5). The retina was not examined by EM.

Discussion

The rate-limiting enzyme in cholesterol synthesis, HMG CoA reductase, and its product mevalonate, play key roles in mammalian cell metabolism. Mevalonate-dependent synthetic paths lead not only to cholesterol, a major membrane component and steroid hormone precursor,[40] but also ubiquinone, a respiratory chain coenzyme,[41] dolichol, an isoprenoid intermediary of protein glycosylation,[42] isopentenyl adenine, a transfer RNA constituent,[43] and recently discovered isoprenoid proteins important in maintenance of cell shape, at least *in vitro*.[44] In the regulation of these paths and the flux of available mevalonate between them, *in vitro* studies suggest multivalent feedback regulation of HMG CoA reductase,[45] with high mevalonate affinity for synthesis of small quantities of the crucial nonsterol isoprenes. The virtual absence of clinical side effects or disturbances in nonsterol isoprene metabolism following therapy of hypercholesterolemic humans with HMG CoA re-

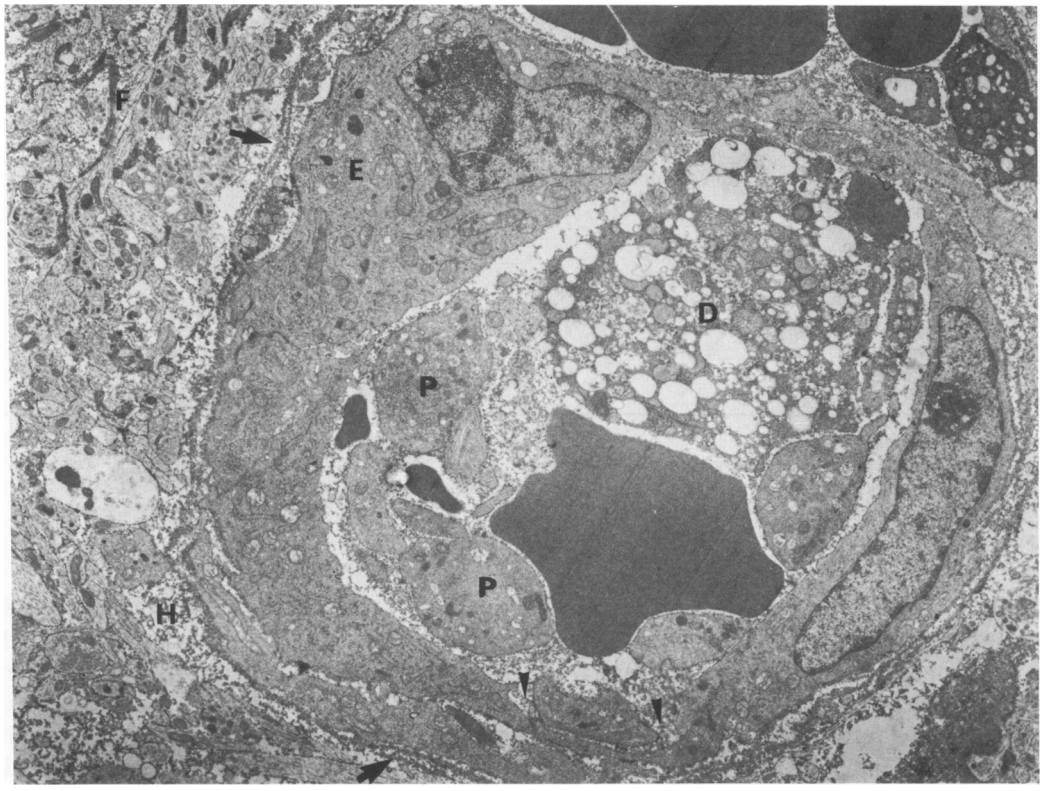

Figure 6—Transmission electron micrograph of advanced vascular degeneration in amygdala of dog with nervous signs after 24 days treatment with 180 mg/kg/day lovastatin. The vascular lumen contains platelets (P), erythrocytes, and masses of organellar debris (D). Flocculent, dark luminal deposits of HRP extend into disrupted inter-endothelial spaces (arrowheads). There are two or more layers of basal laminae (arrows) without intervening cellular processes. Endothelial cells (E) have extensive cytoplasm with prominent organellae. The perivascular extracellular space is expanded and contains HRP (H) and fibrin (F). (Pre-embedding diaminobenzidine incubation, section stained on grid with uranyl acetate and lead citrate. ×6750

ductase inhibitors suggests insufficient inhibition of the reductase to significantly restrict nonsterol mevalonate demands.

In this investigation, normocholesterolemic beagle dogs received up to 180 times the maximum human therapeutic dose of lovastatin, leading to peak mean plasma levels of total inhibitory activity (2249.5 ± 1694.9 ng/ml) approximately 30 times the equivalent values in humans (71.5 ± 37.7 ng/ml; Dobrinska MR, personal communication). Treated dogs had minimum mean total serum cholesterol concentrations as much as 88% below normal, and neurologic disturbance occurred in up to 37% of dogs given 180 mg/kg/day of lovastatin, but in no dogs given 60 mg/kg/day or less. Mean plasma levels of active drug, the degree of cholesterol-lowering, and incidence of neurologic changes were greater in dogs treated in laboratory A than in laboratory B. The reason for this disparity is not known precisely, but the finding of the highest plasma drug levels in dogs offered feed within a few minutes of treatment may indicate greater drug absorption in these animals.

Dogs that manifested neurologic signs apparently were normal until the sudden onset of tremor and convulsions after 11 to 91 days of treatment. These clinical signs suggested disturbance confined primarily to the CNS,[34] where vascular endothelial degeneration was the fundamental pathologic change. All dogs with nervous signs and occasional overtly normal animals treated with 180 mg/kg/day lovastatin had CNS vascular lesions that were most frequent in the amygdala. The multifocal to diffuse extravasation of HRP indicated widespread breakdown of the blood–brain barrier,[36] consequent to altered vascular endothelium.[46,47] The presence of capillaries and other small vessels in apparently normal neuropil but with degenerated endothelium indicated a distinct pathologic effect in these cells. Horseradish peroxidase extended through occasional disrupted inter-endothelial spaces and this was probably the major route of vascular leakage. Necrotic endothelial cells *in situ* were not found in the brain, but masses of organelle debris in luminal thrombi may have been remains of sloughed, degenerated endothelial cells. Multiple, directly-apposed layers of basal laminae probably indicated repetitive intimal damage with re-endothelialization, or residua of transmural injury to multilamellar small vessels.[48,49]

Segmental retrolaminar extravasation of HRP in the ONH, distinct from normal laminar accumulations,[39] was present in six of eight dogs given 180 mg/kg/day lovastatin independent of the occurrence of neurologic signs or vascular changes in the brain. Endothelial degeneration, necrosis, and sloughing were occasionally confirmed in small vessels in retrolaminar zones of extravasated HRP. Thus, like in the

Table 5—Morphologic Changes in Optic System of Eight Dogs given 180 mg/kg/day Lovastatin

	Dog number							
	94M*	96M	78M	04M	98M	93F	81F	92M
No. of days treatment	11	12	15	15	24	28	87	88
Neurologic signs	+†	+	−	−	−	+	−	−
Morphologic changes								
ONH‡ retrolaminar HRP leakage	++§	++	−	−	++	+++	+	+
ONH Focal degeneration	++	+	−	−	−	+++	+	+
ONH individual axonal degeneration	++	+	+	+	+	+	+	+
Retinogeniculate Wallerianlike degeneration	+++	++	++	+	++	++	++	+
Retinal ganglion cell chromatolysis	+	+	+	−	−	+	−	−

* M, male; F, female.

† + or − indicates presence or absence of neurologic signs.

‡ ONH, optic nerve head − optic papilla and 4 mm of retrolaminar optic nerve.

§ − absent, + very slight, ++ slight, +++ moderate.

brain, a direct degenerative effect of treatment in vascular endothelium probably accounted for retrolaminar extravasation of HRP in the ONH. Prelaminar and increased laminar deposits of extravascular HRP also occurred in the ONH, but the origin of these accumulations was not resolved. They may have resulted directly from endothelial degeneration and HRP leakage not confirmed morphologically, diffusion of HRP from degenerated retrolaminar vessels or from adjacent choroid vasculature normally permeable to HRP,[39] or perhaps increased papillary HRP extravasation secondary to increased intracranial pressure[50] in dogs with advanced CNS vasogenic edema. No increase in CSF pressure of clinically normal treated dogs was found, but CSF pressure in dogs with neurologic signs and advanced CNS edema was not measured.

Although vitamin E-responsive hemorrhagic encephalopathies occur in a number of species,[51–53] and serum alpha-tocopherol levels in these dogs were lowered proportionally with cholesterol to levels previously associated with canine hypovitaminosis E,[28,29] evidence suggests possible deficiency in these dogs may not have been the primary cause of CNS hemorrhage and endothelial degeneration. Hemorrhagic encephalopathy has not been associated previously with vitamin E deficiency in dogs,[28,29] and the CNS vascular changes were not prevented by prophylactic alpha-tocopherol supplementation at levels used effectively in vitamin E responsive neuropathy of human abetalipoproteinemia.[55] Furthermore, although serum alpha-tocopherol concentrations were lowered in treated dogs, actual brain tissue levels were normal in six dogs after 40 weeks of dosing with 180 mg/kg/day lovastatin.

Although *in vivo* mevalonate-supplementation experiments have not yet been undertaken to examine the issue specifically, results of several *in vitro* studies with HMG CoA reductase inhibitors[44,56–59] suggest endothelial degeneration in the brain and retrolaminar optic nerve of these dogs could have been a direct consequence of inhibition of HMG CoA reductase. Endothelium *in vivo* is proposed to behave like quiescent *in vitro* monolayers of the same cells,[60] so results from *in vitro* studies may have particular relevance *in vivo*. With cultured vascular endothelium and other cell lines, media concentrations of HMG CoA reductase inhibitors equivalent to those found in serum of the high-dose dogs at laboratory A[59] cause mevalonate-responsive disturbance of cell morphology and growth. The reason why degeneration might be morphologically expressed uniquely in CNS vascular endothelium of dogs treated with high doses of lovastatin is unclear. However, pathogenetic factors that might favor morphologic expression of HMG CoA reductase inhibition particularly in these circumstances could be: 1) equivalently higher blood levels of active inhibitor in dogs after dosing than in other species studied (MacDonald JS, unpublished observation); 2) low quiescent endothelial reductase activity with limited capacity for induction following treatment;[57] and 3) special susceptibility of CNS small vessels to mural insudation and degeneration because of functional dependence on interendothelial tight junctions[36] and absence of perivascular space.

The identity of the putative limiting mevalonate product(s) causing endothelial degeneration in the CNS of these dogs is speculative, but restriction of exogenous (serum) cholesterol is not likely to be of primary importance. Cholesterol quantitatively can be

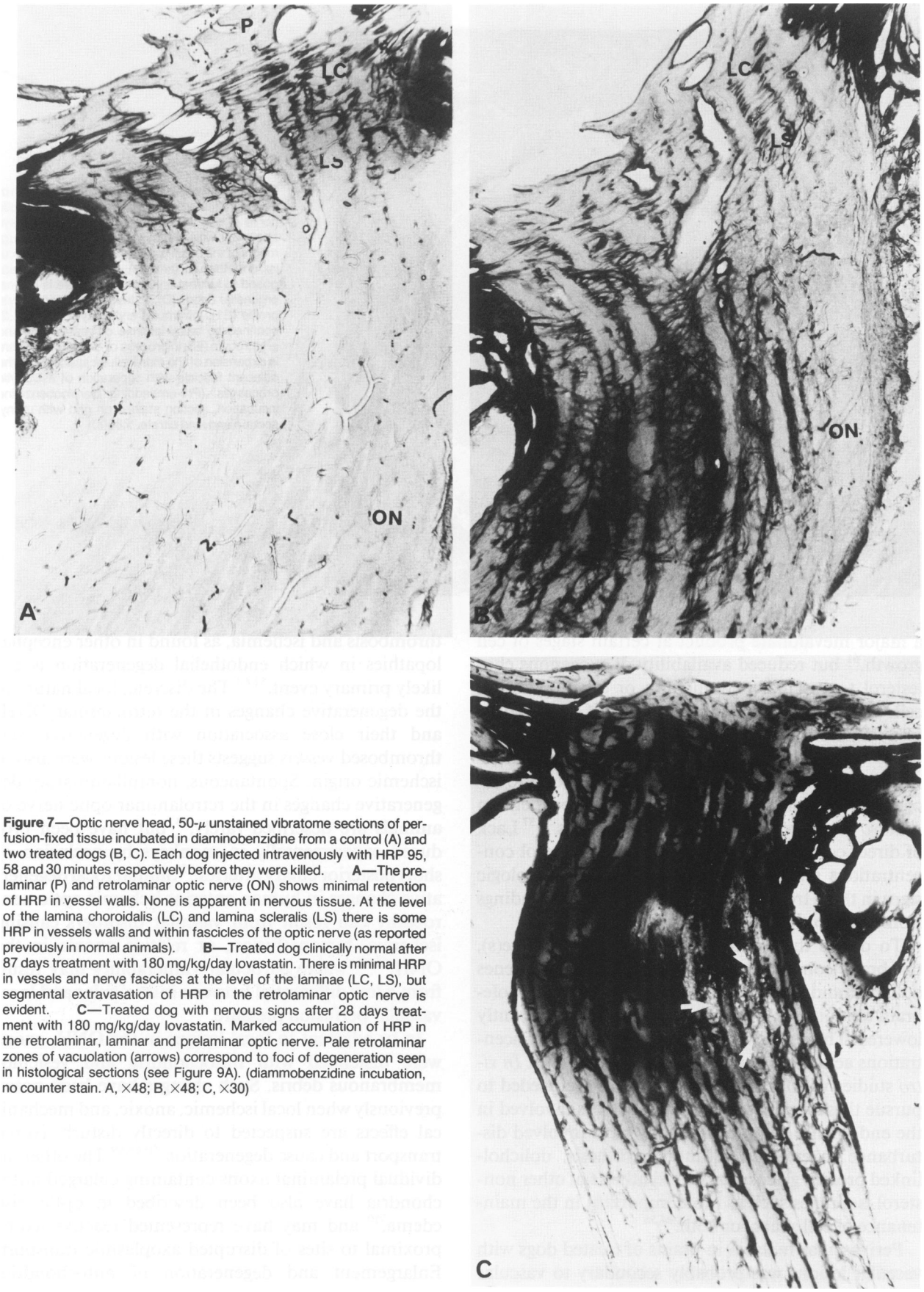

Figure 7—Optic nerve head, 50-μ unstained vibratome sections of perfusion-fixed tissue incubated in diaminobenzidine from a control (A) and two treated dogs (B, C). Each dog injected intravenously with HRP 95, 58 and 30 minutes respectively before they were killed. **A**—The prelaminar (P) and retrolaminar optic nerve (ON) shows minimal retention of HRP in vessel walls. None is apparent in nervous tissue. At the level of the lamina choroidalis (LC) and lamina scleralis (LS) there is some HRP in vessels walls and within fascicles of the optic nerve (as reported previously in normal animals). **B**—Treated dog clinically normal after 87 days treatment with 180 mg/kg/day lovastatin. There is minimal HRP in vessels and nerve fascicles at the level of the laminae (LC, LS), but segmental extravasation of HRP in the retrolaminar optic nerve is evident. **C**—Treated dog with nervous signs after 28 days treatment with 180 mg/kg/day lovastatin. Marked accumulation of HRP in the retrolaminar, laminar and prelaminar optic nerve. Pale retrolaminar zones of vacuolation (arrows) correspond to foci of degeneration seen in histological sections (see Figure 9A). (diammobenzidine incubation, no counter stain. A, ×48; B, ×48; C, ×30)

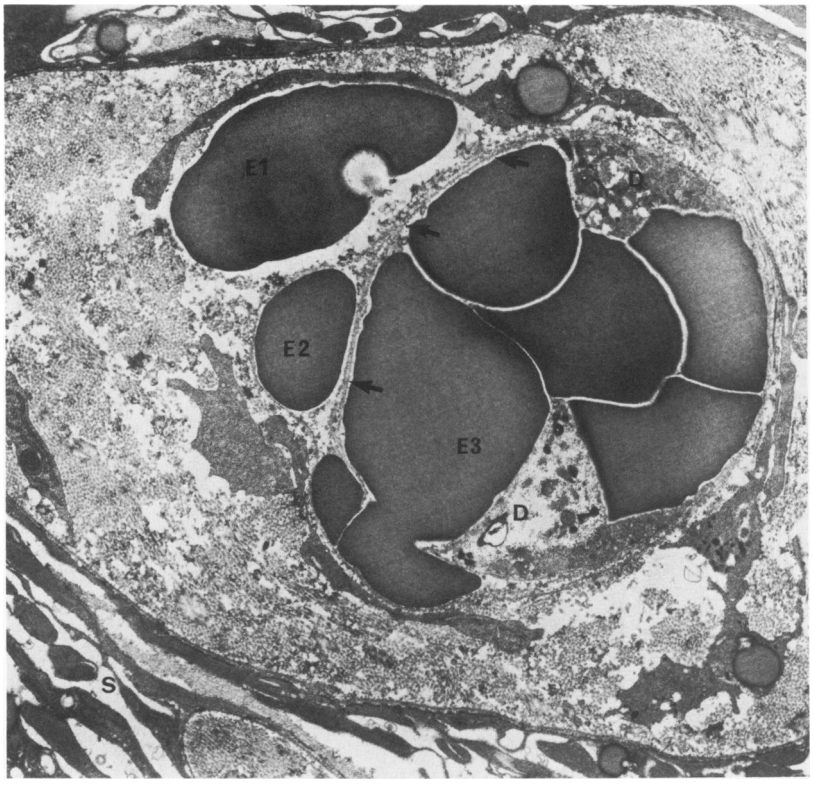

Figure 8—Transmission electron micrograph of degenerative small vessel in retrolaminar optic nerve of dog with nervous signs after 28 days treatment with 180 mg/kg/day lovastatin. Segments of the basal lamina are denuded of vascular endothelium (arrows), and are directly apposed to luminal erythrocytes. There is luminal organellar debris (D), Extravascular erythrocyte profile (E1), intramural erythrocyte profile (E2) confined by basal laminae of vessel wall, and erythrocyte (E3) in process of diapedesis. There is expansion of the extracellular space (S) of the adjacent fascicle with separation of astrocytic processes. (Pre-embedding diaminobenzidine incubation, section stained on grid with uranyl acetate and lead citrate. ×8000)

a major mevalonate product at certain stages of cell growth,[61] but reduced availability of exogenous cholesterol to vascular endothelium or other quiescent cells *in vitro* following inhibition of HMG CoA reductase may not be the fundamental cause of degeneration.[56,57] *In vitro* morphologic changes, although reversed with added mevalonate, do not respond to exogenous LDL-cholesterol[57] and are not dependent on *de novo* cholesterol synthesis in affected cells.[56,57] Lack of direct correlation between serum cholesterol concentrations and the occurrence of overt neurologic signs in these treated dogs may have reflected findings from these *in vitro* studies.

To define the limiting mevalonate metabolite(s), the brain concentration of the nonsterol isoprenes dolichol and ubiquinone were measured. Whole-brain ubiquinone levels were slightly but significantly lowered. These results may not have reflected concentrations actually within endothelium, however. *In vitro* studies with brain endothelial cells are needed to pursue these findings. Speculative factors involved in the endothelial degeneration may have involved disturbance in either ubiquinone synthesis, dolichol-linked protein glycosylation,[62] synthesis of other nonsterol isoprenes such as those important in the maintenance of cell shape, or both.[44,56]

Perivascular malacia in brains of treated dogs with vascular lesions was probably secondary to vascular thrombosis and ischemia, as found in other encephalopathies in which endothelial degeneration is the likely primary event.[51,63] The discrete, focal nature of the degenerative changes in the retrolaminar ONH, and their close association with degenerate and thrombosed vessels suggests these lesions were also of ischemic origin. Spontaneous, noninflammatory degenerative changes in the retrolaminar optic nerve of animals are described rarely,[64] but have been produced experimentally in monkeys by occlusion of short posterior ciliary arteries.[65] Similar focal degenerative changes in human anterior ischemic optic neuropathy can result from vascular disorders causing ischemia, hypoperfusion, or relative anoxia in the ONH,[66–68] where particular vulnerability may result from a low differential between intra-ocular and local vascular perfusion pressure.

In or adjacent to focal lesions in the ONH, axons were distended with mitochondria and vesiculomembranous debris. Such changes have been noted previously when local ischemic, anoxic, and mechanical effects are suspected to directly disturb axonal transport and cause degeneration.[50,65,69] The other individual prelaminar axons containing enlarged mitochondria have also been described in optic disc edema,[50] and may have represented reactive axons proximal to sites of disrupted axoplasmic transport. Enlargement and degeneration of mitochondria,

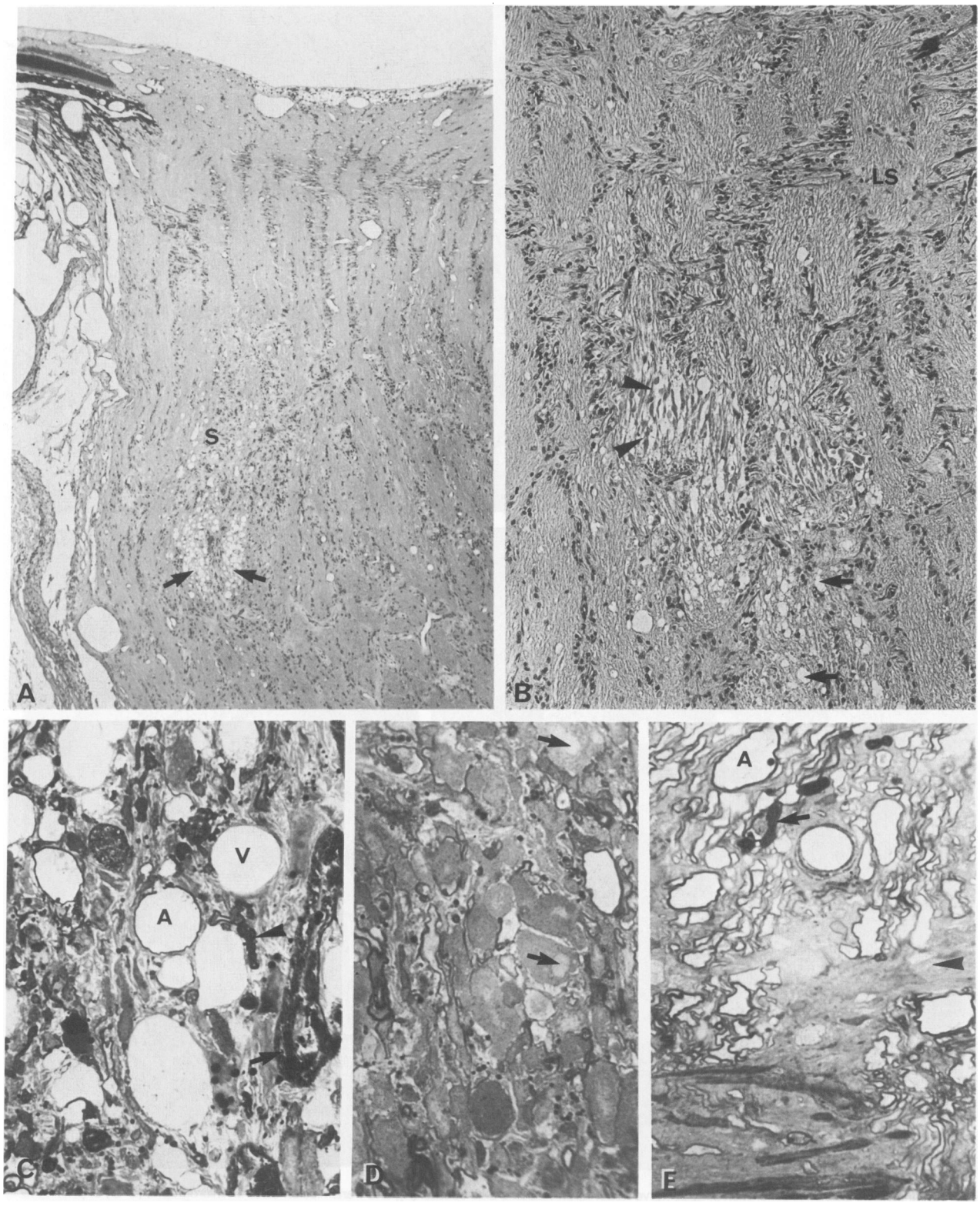

Figure 9—Optic nerve head focal degeneration. Paraffin and H&E (A, B) and Epon-toludine blue (C–E) sections from dogs with nervous signs after 11 to 28 days of treatment with 180 mg/kg/day lovastatin. **A**—Focal retrolaminar vacuolation of adjacent fascicles of optic nerve (arrows) and localized expansion of interfasicular septae (S). Contralateral optic nerve head to that shown from dog in Figure 7C. **B**—Focal vacuolation (arrows) of the retrolaminar optic nerve with adjacent fusiform axonal swellings (arrowheads) closer to the retina. Limit of the lamina scleralis (LS). **C**—Detail of retrolaminar focal degeneration. Dense HRP in the perivascular space (arrow), distended myelin sheaths with clear contents (A) vacuoles (V), and collapsed myelin sheaths (arrowhead). **D**—Optic nerve at level of lamina scleralis and lamina choroidalis. Detail of focus of dense, granular, axonal swellings with paler centres (arrows). **E**—Junction (arrowhead) of lamina retinalis (prelaminar optic nerve, upper half of micrograph) and lamina choroidalis. Occasional swollen pale axons (A) and collapsed myelin sheaths (arrow). (A, ×48; B, ×112; C, ×800; D, ×813; E, ×826)

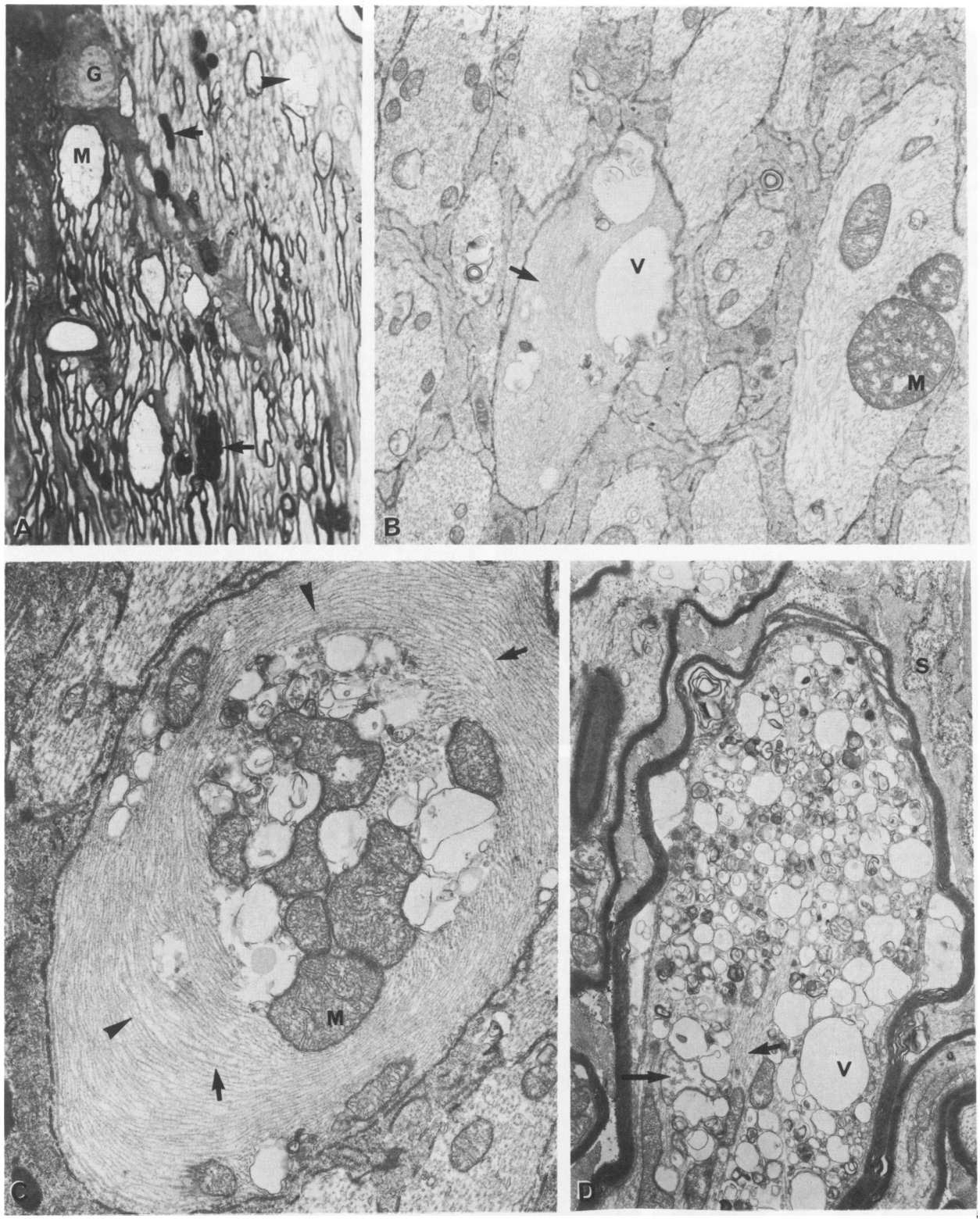

Figure 10—Individual axonal degeneration in optic nerve head. Epon-toluidine blue section (A), and transmission electron micrographs (B–D) of prelaminar (A–C) or laminar (D) optic nerve from dogs showing nervous signs after 11 or 12 days treatment with 180 mg/kg/day lovastatin. **A**—Scattered individual swollen myelinated axons (M) containing vacuoles. Collapsed myelin sheaths (arrows), and other vacuoles (arrowhead) are present. The retinal ganglion cell (G) appears normal. **B**—From a nearby zone of the prelaminar optic nerve, individual nonmyelinated axons contain enlarged mitochondria (M), vacuoles (V), and accumulations of neurofilaments and microtubules (arrow, detail not evident at this magnification). **C**—An adjacent nonmyelinated axon is distended with swirling accumulations of microtubules (arrows), neurofilaments (arrowheads), enlarged mitochondria (M), and degenerative organellae. **D**—Lamina choroidalis (adjacent to the focus of degeneration showing in Figure 9D), there is flocculent HRP in the extracellular space (S), the myelinated axon is distended with normal and degenerative mitochondria (long arrow), vesiculo-membranous debris, and membrane-bound vacuoles (V). Central, normally oriented microtubules (arrow) are evident. (A, epon-toluidine blue. B–D stained on grid with uranyl acetate and lead citrate. A, ×933; B, ×10000; C, ×18130; D, ×11000)

however, sometimes with microtubule and neurofilament accumulations, may also be a nonspecific feature of several toxic axonopathies.[70]

Axonal (Wallerianlike) degeneration with neuronal chromatolysis, as found in the optic system of dogs treated with 60 or 180 mg/kg/day lovastatin, may follow a metabolic effect within a neuronal perikaryon or in an axon itself, or may be due to direct or metabolic proximal separation of an axon from its neuron.[71-74] In this study the exact pathogenesis of optic system axonal degeneration and ganglion cell chromatolysis is not known. A likely hypothesis would be axonal degeneration and neuronal chromatolysis, both secondary to ischemic effects on axons in the ONH where vascular endothelial degeneration could be the primary pathogenetic event. In the absence of proven vascular degeneration in the ONH of all dogs, there also might be a separate or superimposed direct neurotoxic effect. Neurologic changes in these dogs clearly contrasted with those induced in rodents by agents inhibiting cholesterol synthesis after squalene cyclization, however.[23-25] In these dogs there was no evidence of direct disturbance of myelinogenesis or membrane synthesis throughout the nervous system as observed in neonatal rats treated with AY-9444 or triparanol.

References

1. Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, Springer J: Mevinolin: A highly potent competitive inhibitor of hydroxy-methyl-glutaryl-coenzyme A reductase and a cholesterol lowering agent. Biochemistry 1980, 77:3957–3961
2. Endo A, Kuroda M, Tsuijita Y: ML-236A, ML-236B and ML-236C, new inhibitors of cholesterogenesis produced by *Penicillium citrinum*. J Antibiot 1976, 29: 1346–1348
3. Tobert JA, Bell GD, Birtwell J, James I, Kukovetz WR, Pryor JS, Buntinx A, Holmes IB, Chao YS, Bolognese JA. Cholesterol-lowering effect of mevinolin, an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, in healthy volunteers. J Clin Invest 1982, 69:913–919
4. The Lovastatin Study Group II: Therapeutic response to lovastatin (mevinolin) in nonfamilial hypercholesterolemia: A multicenter study. JAMA 1986, 256: 2829–2834
5. East C, Grundy SM, Bilheimer DW: Normal cholesterol levels with lovastatin (mevinolin) therapy in a child with homozygous familial hypercholesterolemia following liver transplantation. JAMA 1986, 256: 2843–2848
6. Mabuchi H, Haba T, Tatami R, Miyamoto S, Sakai Y, Wakasugi T, Watanabe A, Koizumi J, Takeda R: Effects of an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase on serum lipoproteins and ubiquinone-10 levels in patients with familial hypercholesterolemia. N Engl J Med 1981, 305:478–482
7. Mabuchi H, Sakai T, Sakai Y, Yoshimura A, Watanabe A, Wakasugi T, Koizumi J, Takeda R: Reduction of serum cholesterol in heterozygous patients with familial hypercholesterolemia: Additive effects of compactin and cholestyramine. N Engl J Med 1983, 308:609–613
8. Kuroda M, Tsujita Y, Tanzawa K, Endo A: Hypolipidemic effects in monkeys of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Lipids 1979, 14:585–589
9. Tsujita Y, Kuroda M, Shimada Y, Tanzawa K, Arai M, Kaneko I, Tanaka M, Masuda H, Tarumi C, Watanabe Y, Fujii S: CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: Tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. Biochim Biophys Acta 1986, 877:50–60
10. Watanabe Y, Ito T, Saeki M, Kuroda M, Tanzawa K, Mochizuki M, Tsujita Y, Arai M: Hypolipidemic effects of CS-500 (ML-236B) in WHHL-rabbit, a heritable animal model for hyperlipidemia. Atherosclerosis 1981, 38:27–31
11. Kroon PA, Hand KM, Huff JW, Alberts AW: The effects of mevinolin on serum cholesterol levels of rabbits with endogenous hypercholesterolemia. Atherosclerosis 1982, 44:41–48
12. Tsujita Y, Kuroda M, Tanzawa K, Kitano N, Endo A: Hypolipidemic effects in dogs of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Atherosclerosis 1979, 32:307–313
13. Kovanen PT, Bilheimer DN, Goldstein JL, Jaramillo JJ, Brown MS: Regulatory role for hepatic low density lipoprotein receptors *in vivo* in the dog. Proc Natl Acad Sci 1981, 78:1194–1198
14. Endo A, Tsujita Y, Kuroda M, Tanzawa K. Effect of ML-236B on cholesterol metabolism in mice and rats: Lack of hypocholesterolemic activity in normal animals. Biochem Biophys Acta 1979, 575:266–276
15. Edwards PA, Lan SF, Fogelman AM: Alterations in the rates of synthesis and degradation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase produced by cholestyramine and mevinolin. J Biol Chem 1983, 258:10219–10222
16. Singer II, Kawka DW, Kazazis DM, Alberts AW, Chen JS, Huff JW, Ness GC: Hydroxymethylglutaryl-coenzyme A reductase-containing hepatocytes are distributed periportally in normal and mevinolin-treated rat livers. Proc Natl Acad Sci 1984, 81:5556–5560
17. Norton WT, Poduslo SE: Myelination in brain: Changes in myelin composition during brain maturation. J Neurochem 1973, 20:759–773
18. Spohn M, Davison AN: Cholesterol metabolism in myelin and other subcellular fractions of rat brain. J Lipid Res 1972, 13:563–570
19. Minsker DH, MacDonald JS, Robertson RT, Bokelman DL: Mevalonate supplementation in pregnant rats suppresses the teratogenicity of mevinolinic acid, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Teratology 1983, 28:449–456
20. Steinberg D, Avigan J: Studies of cholesterol biosynthesis. II. The role of desmosterol in the biosynthesis of cholesterol. J Biol Chem 1960, 235:3127–3129
21. Fumagalli R: Brain and myelin sterols studied using

specific inhibitors of sterol synthesis. Advan Exp Med Biol 1971, 13:53–61
22. Roux C, Dupuis R, Horvath C, Talbot JN: Teratogenic effect of an inhibitor of cholesterol synthesis (AY-9944) in rats: Correlation with maternal cholesterolemia. J Nutrition 1980, 110:2310–2312
23. Smith ME, Hasinoff CM, Fumagalli R: Inhibitors of cholesterol synthesis and myelin formation. Lipids 1970, 5:665–671
24. Susuki K, Zagoren JC: Degeneration of oligodendroglia in the central nervous system of rats treated with AY9944 of triparanol. Lab Invest 1974, 31:503–515
25. Suzuki K, De Paul LD: Myelin degeneration in sciatic nerve of rats treated with hypocholesterolemic drug AY9944. Lab Invest 1972, 26:534–539
26. Bilheimer DW, Grundy SM, Brown MS, Goldstein JL: Mevinolin and colestipol stimulate receptor-mediated clearance of low density lipoprotein from plasma in familial hypercholesterolemia heterozygotes. Proc Natl Acad Sci USA 1983, 80:4124–4128
27. Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousdian S, Kannel WB: Incidence of coronary heart disease and lipoprotein cholesterol levels: The Framingham study. JAMA 1986, 256:2835–2838
28. Hayes KC, Nielsen SW, Rousseau JE: Vitamin E deficiency and fat stress in the dog. J Nutr 1969, 99:196–209
29. Hayes KC, Rousseau JE, Hegsted DM: Plasma tocopherol concentrations and vitamin E deficiency in dogs. J Am Vet Med Assoc 1970, 157:64–71
30. Wentz PW, Cross RE, Savory J: An integrated approach to lipid profiling: Enzymatic determination of cholesterol and triglycerides with a centrifugal analyzer. Clin Chem 1976, 22:188–192
31. Mills GL, Lane PA, Weech PK: The isolation and purification of plasma lipoproteins, Laboratory Techniques in Biochemistry and Molecular Biology: A Guide Book to Lipoprotein Technique. Edited by RH Burdon, PH Van Knippenberg. Amsterdam, Elsevier, 1984, pp 78–91, 178–180
32. Taylor SL, Lamden MP, Tappel AL: Sensitive method for tissue tocopherol analysis. Lipids 1976, 11:530–538
33. Greenspan MD, Lo C-YL, Hanf DP, Yudkovitz JB: Separation and identification of triglycerides, cholesterol esters, cholesterol 7-dehydrocholesterol, dolichol, ubiquinone, alpha-tocopherol and retinol by HPLC with a diode array detector. J Lipid Res 1988, in press
34. Delahunta A: Small animal neurologic examination. Vet Clin North America 1971, 1:191–206
35. Duncan JR, Oliver JE, Mayhew IG: Laboratory examinations, Veterinary Neurology. Edited by JE Oliver, BF Hoerlein, IG Mayhew. Philadelphia, WB Saunders, 1987, pp 57–58
36. Reese TS, Karnovsky MJ: Fine structural localization of a blood–brain barrier to exogenous peroxidase. J Cell Biol 1967, 34:207–217
37. Cook RD, Ghetti B, Wisniewski HM: The pattern of Wallerian degeneration in the optic nerve of newborn kittens: An ultrastructural study. Brain Research 1974, 75:261–275
38. Cook RD: Observations on glial cells within myelin sheaths in degenerating optic nerves. J Neurocytology 1974, 3:737–751
39. Tso MOM, Shih CY, McLean IW: Is there a blood–brain barrier at the optic nerve head? Arch Ophthalmol 1975, 93:815–825
40. Bloch K: The biological synthesis of cholesterol. Science 1965, 150:19–28
41. Olson RE: Biosynthesis of ubiquinone in animals, Vitamins and Hormones. Edited by RS Harris, IG Wool, JA Loraine. New York, Academic Press, 1968, p 551
42. Gough DP, Hemming FW: The characterization and stereochemistry of biosynthesis of dolichols in rat liver. Biochem J 1970, 118:163–166
43. Faust JR, Brown MS, Goldstein JL: Synthesis of Δ^2-isopentenyl tRNA from mevalonate in cultured human fibroblasts. J Biol Chem 1980, 255:6546–6548
44. Schmidt RA, Schneider CJ, Glomset JA: Evidence for post-translational incorporation of a product of mevalonic acid into Swiss 3T3 cell proteins. J Biol Chem 1984, 259:10175–10180
45. Brown MS, Goldstein JL: Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J Lipid Res 1980, 21:505–517
46. Brightman MW, Broadwell RD: The morphological approach to the study of normal and abnormal permeability. Adv Exp Med Biol 1976, 69:41–54
47. Rosenblum WI: Biology of disease: Aspects of endothelial malfunction and function in cerebral microvessels. Lab Invest 1986, 55:252–268
48. Cancilla PA, Frommes SP, Kahn LE, Debault LE: Regeneration of cerebral microvessels: A morphological and histochemical study after local freeze-injury. Lab Invest 1979, 40:74–82
49. McKinney RV, Panner BJ: Regenerating capillary basement membrane in skeletal muscle wounds: ultrastructural and histochemical study. Lab Invest 1972, 26:100–113
50. Tso MOM, Hayreh SS: Optic disc edema in raised intracranial pressure. III. A pathologic study of experimental papilledema. Arch Ophthalmol 1977, 95:1448–1457
51. Dror Y, Budowski P, Bubis JJ, Sandbank U, Wolman M: Chick nutritional encephalopathy induced by diet rich in oxidized oil and deficient in tocopherol, Progress in Neuropathology, vol 3. Edited by HM Zimmerman. New York, Grune & Stratton, 1976, 343–357
52. Keeler R, Young S: Role of vitamin E in the etiology of spontaneous hemorrhagic necrosis of the central nervous system of fetal hamsters. Teratology 1979, 20: 127–132
53. Chiswick ML, Johnson M, Woodhall C, Gowland M, Davies J, Toner N, Sims D: Protective effect of vitamin E on intraventricular haemorrhage in the newborn, Biology of Vitamin E. Ciba Foundation Symposium 101. London, Pitman, 1983, pp 186–200
54. Van Fleet JF: Experimentally induced Vitamin E-selenium deficiency in the growing dog. J Am Vet Med Assoc 1975, 166:769–774
55. Muller DPR, Lloyd JK, Wolf OH: The role of vitamin E in the treatment of the neurological features of abetalipoproteinaemia and other disorders of fat absorption. J Inher Metab Dis 1985, 8 (Suppl 1):88–92
56. Schmidt RA, Glomset JA, Wight TN, Habenicht AJR, Ross R: A study of the influence of mevalonic acid and

its metabolites on the morphology of Swiss 3T3 cells. J Cell Biol 1982, 95:144–153
57. Cohen DC, Massoglia SL, Gospodarowicz D: Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in vascular endothelial cells. J Biol Chem 1982, 257:11106–11112
58. Fairbanks KP, Witte LD, Goodman DWS: Relationship between mevalonate and mitogenesis in human fibroblasts stimulated with platelet-derived growth factor. J Biol Chem 1984, 259:1546–1548
59. Fairbanks KP, Barbu VD, Witte LD, Weinstein IB, Goodman DWS: Effects of mevinolin and mevalonate on cell growth in several transformed cell lines. J Cell Physiol 1986, 127:216–222
60. Huttner I, Gabbiani G: Vascular endothelium: recent advances and unanswered questions. Lab Invest 1982, 47:409–411
61. Chen HN: Role of cholesterol metabolism in cell growth. Federation Proc 1984, 43:126–130
62. Jago MV, Payne AL, Petterson JE, Bagust TJ: Inhibition of glycosylation by corynetoxin, the causative agent of annual rye grass toxicity: A comparison with tunicamycin. Chem Biol Interactions 1983, 45:223–234
63. Kurtz HJ, Short EC: Pathogenesis of edema disease in swine: pathologic effects of hemolysin, autolysin, autolysate, and endotoxin of *Escherichia coli* (0141). Am J Vet Res 1976, 37:15–24
64. Wilcock BP: The eye and ear, Pathology of Dosmetic Animals, 3rd Ed, Vol. 1. Edited by KVF Jubb, PC Kennedy, N Palmer. Sydney, Academic Press, 1985, p 385
65. McLeod D, Marshall J, Kohner EM: Role of axoplasmic transport in the pathophysiology of ischaemic disc swelling. Br J Ophthalmol 1980, 64:247–261
66. Henkind P, Charles NC, Pearson J: Histopathology of ischemic optic neuropathy. Am J Ophthalmol 1970, 69: 78–90
67. Hayreh SS: Anterior ischemic optic neuropathy. Arch Neurol 1981, 38:675–678
68. Böke W, Voigt GJ: Circulatory disorders of the optic nerve. Ophthalmologica, Basel 1980, 180:88–100
69. Samuelson DA, Williams LW, Gelatt KN, Gum GG, Merideth RE: Orthograde rapid axoplasmic transport and ultrastructural changes of the optic nerve. II. Beagles with primary open-angle glaucoma. Glaucoma 1983, 5:174–184
70. Spencer PS, Schaumburg HH: Central-peripheral distal axonopathy: The pathology of dying-back polyneuropathies, Progress in Neuropathology, vol 3. Edited by HM Zimmerman. New York, Grunes & Stratton, 1976, pp 253–295
71. Blackwood W: The neuron, Greenfield's Neuropathology, 3rd ed. Edited by W Blackwood, JAN Corsellis. London, Edward Arnold, 1976, p 11
72. Lowndes HE, Baker T: Toxic site of action in distal axonopathies, Experimental and Clinical Neurotoxicology. Edited by PS Spencer, HH Schaumburg. Baltimore, Williams and Wilkins, 1980, 193–205
73. Asbury AK, Brown MJ: The evolution of structural changes in distal axonopathies, Experimental and Clinical Neurotoxicology. Edited by PS Spencer, HH Schaumburg. Baltimore, Williams & Wilkins, 1980, pp 179–192
74. Jones HB, Cavanagh JB: Comparison between the early changes in isoniazid intoxication and the chromatolytic response to nerve ligation in spinal ganglion cells of the rat. Neuropathol Appl Neurobiol 1981, 7:489–501

Acknowledgments

The authors thank Drs. C. F. Hollander, M. J. Van Zwieten, J. D. Burek and D. L. Bokelman for helpful support, Drs. L. Cork, J. T. McGrath and R. L. Sidman for consultation, Dr. M. K. Axthelm for the immunohistochemistry for canine distemper virus, and Dr. T. Nolan for measuring CSF pressures. They also thank Hoffman La Roche, Basel, Switzerland for their generous gift of alpha-tocopherol acetate, C. Graulière and the technical staff at Chibret and West Point for expert technical assistance, and N. Delorme for typing the manuscript. The ultrastructural studies were performed in the Service de Microscopie Electronique (Directeur, Dr. Bohatier), Faculté des Sciences, Université de Clermont-Ferrand.