of unlabeled RBP, for a PE concentration of 8 mg. dry weight per milliliter. Results from two typical experiments are reproduced in Fig. 1. From analysis of the figure, it is evident that RBP isolated from the plasma of patients with the recessive form of RP does not demonstrate any appreciable difference from normal human RBP in its capacity to interact with the RBP receptor present on the cell membrane of normal bovine PE.

Present results seem to definitely demonstrate that no impairment of RBP function exists in the most common form of RP. They do not of course rule out the possibility that the RBP receptor on the plasma membrane of PE may be abnormal in this disease.

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Niemann-Pick disease-like inclusions caused by a hypocholesteremic agent. MACHIKO SAKURAGAWA.

AY9944, an inhibitor of cholesterol biosynthesis, was injected into albino rats and the ocular tissue was studied by light and electron microscopy. Abundant lamellar inclusion bodies accumulated in various cells of the eye, especially in the ganglion cells of the retina and glial cells of the optic nerve. Prolonged administration of this drug resulted in degeneration of retinal ganglion cells and oligodendrogial cells of the optic nerve. Micro-organelles of the inclusion body-laden cells were otherwise normal in their appearance. The electron microscopic appearance of these inclusion bodies and their distribution in the ocular tissues closely resembled those of Niemann-Pick disease.

Trans-1,4-bis (2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (AY9944) is one of the most effective hypocholesteremic agents. Suzuki and Zagoren2 have extensively reported electron microscopic and biochemical observations on the central and peripheral nervous systems of developing rats following the injection of this drug. Also, several other authors have described membranous inclusion bodies which abundantly accumulate in the affected cells.3-4

The present experiments have revealed that administration of AY9944 to albino rats causes considerable changes in various cells of the eye and that the accumulation of the inclusion bodies strikingly resembles that in Niemann-Pick disease. The purpose of this communication is to emphasize a possible correlation between the effects of this drug and the pathogenesis of certain sphingolipidoses.

Materials and methods. Sprague-Dawley strain albino rats were used in this study. AY9944 was dissolved in physiologic saline solution (5 mg. per milliliter) and sterilized by ultrafiltration. A daily dose of 50 mg. per kilogram body weight (about 0.35 mg. for a 2-day-old rat) was injected into the peritoneal cavity. Animals were injected commencing on the second postnatal day (Group
I) and on the third postnatal week (Group II). Adult rats were similarly injected. The animals were killed on the next day following injection 1, 3, 5, 7, 10, 12, 15, and 21. Some animals were kept for 1 and 3 weeks after five injections to observe the delayed effect. At least three animals were examined for each time period. Control animals were injected with physiologic saline solution intraperitoneally at identical time intervals.

The eyes and entire optic nerve were fixed in a 4 per cent glutaraldehyde solution in 0.15M phosphate buffer (pH 7.2) and small pieces of the posterior retina, optic nerve, cornea, ciliary epithelium, and lens were excised. Tissues were post-fixed in 1 per cent osmium tetroxide in the same buffer solution for 90 minutes, dehydrated with graded ethyl alcohols, and then embedded in an epoxy resin. Sections cut 1 μm thick were stained with toluidine blue for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by electron microscopy.

Eyes of several animals were embedded in gelatin after formalin fixation and frozen sections were cut 10 μm thick and examined under cross-polarized light for birefringency. Sections were also stained for fat and by the periodic acid-Schiff (PAS) method.

**Results.** Weight gain of the animals treated soon after birth became markedly retarded after four or five injections, and many animals died. All animals in Group I that received more than five injections became ataxic and died within the following 2 weeks. Most animals in Group II gained weight relatively well and did not show severe neurologic symptoms.

**Retina.** Twenty-four hours after the injection of a single dose, electron-dense as well as hyperchromatic inclusion bodies began to accumulate in a few ganglion cells and some fine processes of Müller’s cells of both Groups I and II. The earliest inclusion bodies in these cells measured about 0.15 to 0.2 μm in diameter and appeared usually in the cytoplasmic matrix unconnected with micro-organelles. However, they were eventually bound by membranes and some of them were fused within lysosomes.

With the further intoxication, these inclusion bodies began to show lamellar structure, comprising whorled concentric membranes, having a width of about 75 Å without definite periodicity.

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**Fig. 1.** The ganglion cell of the retina of a Group I animal following 12 injections. Irregular electron-dense inclusion bodies are abundantly present in the cytoplasm. Smooth and rough endoplasmic reticulum are enlarged. (×7,600.) **Left inset,** Higher magnification of the marked area. Newly formed inclusion bodies are seen bound by membranes and free in the cytoplasm. (×22,200.) **Right inset,** Higher magnification of an inclusion body which shows lamellar membranes. (×38,700.)
The membranes were often closely packed to form a configuration like that of myelin. The numbers of the lamellar inclusions and the affected cells increased markedly within a short period of time. Following five injections, all ganglion and Müller’s cells, several horizontal and amacrine cells, and some bipolar cells of the retina were involved. Despite the abundance of the inclusion bodies, the cytoplasm of these cells showed no sign of edema or degeneration during this stage. The number of lysosomes might have been increased but all other micro-organelles were normal in appearance.

Deposition of the inclusion bodies in the retina became conspicuous after twelve injections. The larger inclusion bodies, measuring about 0.3 to 0.8 μm in diameter were distributed diffusely throughout the cytoplasm (Fig. 1). The inclusion bodies in the ganglion cells of Group I animals consisted of densely packed membranes and were somewhat irregular in shape, whereas those of the Group II animals were loose and round. Many lamellar bodies formed large agglomerates. Inclusion bodies which were fused in lysosomes showed a moderately positive histochemical reaction for acid phosphatase. Also, they were strongly stained with PAS and Sudan black B and showed brilliant birefringency by cross polarized microscopy (Fig. 2).

The cytoplasm of the ganglion cell began to show some changes at this stage. Enlargement of saccular lumens of smooth and rough endoplasmic reticulum became apparent, but the cells still lacked any sign of degeneration. When poisoned further, however, the ganglion cells became edematous and degenerated. The severely damaged ganglion cells were totally vacuolated. Also, shrunken cells with pyknotic nuclei were present in the bipolar cell layer. These cells eventually disappeared from the retinal tissue.

The myelinoid inclusions in the various cells persisted even when the ganglion cells had begun...
Fig. 4. The postbulbar zone of the optic nerve of a Group I animal following 12 injections. Inset, Light microscopic view of the area. (Toluidine blue stain; x700.) A, Lamellar inclusion bodies are abundantly deposited in glial cells. The cytoplasm is otherwise normal. (x7,700.) B, Higher magnification of an inclusion body. (x52,800.) C, Some inclusion bodies show a positive reaction for acid phosphatase. (x57,600.)

to degenerate. These cells remained normal except for a small number of cells in the bipolar cell layer which degenerated at a later stage. Inclusion bodies were absent in the differentiating photoreceptor cells. Development of the photoreceptor elements was unaffected by the drug administration.

Pigment epithelium. Abundant inclusion bodies were present in the pigment epithelial cells similar to those in the ganglion cells. However, degenerative changes in the pigment epithelium were mild (Fig. 3). The inclusion bodies in the pigment epithelium often showed fine reticular membranes which were arranged in a crystalloid pattern (Fig. 3, insert). At the early stage of the intoxication, a transitional step of transformation of smooth endoplasmic reticulum into lamellar bodies and then crystalloid bodies was evident. The crystalloid inclusion bodies were more frequently found in Group II animals. Following ten injections, the inclusion agglomerated into irregular giant granules.

Optic nerve. Pathologic changes in the optic nerve were considerably variable with the age of the animals. Animals that received the injection prior to myelination (i.e., on the fifth postnatal day), showed extremely severe damages, whereas older animals produced considerably milder changes.

Following a single injection, 3-day-old rats showed deposition of the inclusion bodies in the glial cells, especially astrocytes in the postbulbar zone. After three injections, marked swelling was noted in many axons in these areas.

Following the twelfth injection, almost all glia cells in the postbulbar zone contained abundant inclusion bodies, many of which were conglomerated into large masses (Fig. 4, A and B). The appearance of the inclusion bodies was identical to that of the ganglion cell of the Group II animals. A positive acid phosphatase activity was demonstrated within the lamellar inclusion bodies (Fig. 4, C). Degeneration in the axons became apparent at this stage. Nodular swelling of the axon was frequently observed in the postbulbar zone. Swollen and proliferated mitochondria and various electron-dense particles were abundantly packed in the axonal nodules (Fig. 5). The number of nerve fibers was reduced and large spaces were formed between the axons. On further administration, affected glia cells began to degenerate.
Fig. 5. The postbulbar zone of the optic nerve of a Group I animal following 12 injections. A nodularly swollen axon contains various electron-dense inclusion bodies. The lamellar inclusion bodies in the glial cell are shown in the upper left corner. (×10,000.) Inset, Higher magnification of the marked area. (×30,000.)

Other ocular tissues. Inclusion bodies having a similar structure were abundantly present in the stromal and endothelial cells of the cornea, epithelium and fibers of the lens, ciliary and iris epithelium, and endothelium, and pericytes of blood capillaries. The inclusion body-laden cells were otherwise normal in their cytologic appearance.

Discussion. The present investigation has demonstrated that AY9944, a hypocholesteremic drug which blocks δ7-reductase in the cholesterol biosynthetic pathway, results in accumulation of 7-dehydrocholesterol, causes deposition of membranous inclusion bodies in various cells of the eye, especially in the ganglion cells of the retina and glia cells of the optic nerve. Similar changes in the brain and optic nerve have been noted by several investigators, but the main morphologic emphasis has been concentrated on degeneration of oligodendroglia and retardation of myelin formation.

The present study shows that the lamellar inclusion bodies in the AY9944-intoxicated rats are formed only in the cells, the cytologic structure of which is otherwise normal, and at a stage significantly earlier than when the cells begin to degenerate. Although retinal ganglion cells and oligodendroglia of the optic nerve degenerate eventually, many other cells of the eye which accumulate the inclusion bodies remain normal.

The fine structural and histochemical appearance of the lamellar inclusion bodies in this experiment is strikingly similar to those in various sphingolipidoses. In addition, the wide distribution of the inclusion bodies in the ocular tissue is similar to that observed in Niemann-Pick disease reported by Robb and Kuwabara and Libert and associates.

It has been known that the tissue cholesterol as well as the sphingomyelin is increased in Niemann-Pick disease. Recently Brady has suggested the presence of a primary disturbance of cholesterol metabolism in this disease. A preliminary biochemical study of the retina and lens of the rats administered AY9944 in this study has demonstrated moderate decrease of the tissue cholesterol and marked reduction of sphingomyelinase activity at the early experimental stage. Administration of AY9944 seemingly causes inhibition of the sphingomyelinase in various cells resulting in the marked deposit of the lamellar inclusion bodies. Further biochemical and morphologic studies are now under investigation.

Several authors have suggested a possible origin for the inclusions induced by hypocholesteremic drugs as an elaboration from the smooth endoplasmic reticulum. Also, crystalloid bodies have been demonstrated in the adrenal cortex of the mice treated with AY9944, Triparanol, and 20, 25-diazacholesterol. This study has demonstrated the continuity of the newly formed aggregates of membranes (Chen and Yates, type II inclusions) with the smooth endoplasmic reticulum in the pigment epithelium. The aggregated membranes form crystalloid inclusion bodies. However, lamellar inclusion bodies in other cells appear to be formed within the cytoplasmic matrix without any correlation with micro-organelles.

Marked retardation of myelin formation has been demonstrated in the optic nerve of the Group I rats, as previously reported by Rawlins. Also, oligodendrogial degeneration has been consistently demonstrated in the optic nerve after prolonged poisoning. Suzuki and Zagoren have pointed out that this oligodendrogial damage causes the myelin degeneration, leaving axons intact. However, the present study has revealed that
axons in the optic nerve degenerate profoundly and that the number of axons are markedly reduced even at the early experimental stage.

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