Ocular tissues from two enucleated and one trabeculectomized human eyes with siderosis bulbi were studied by electron microscopy. Ferritin particles were demonstrated in various cell types of the three eyes. Other types of iron were not identifiable. The ferritin particles were scattered throughout the cytoplasm, with a few particles in the nucleus and extracellular space. More ferritin particles were seen in tissues near the iron foreign body. The cells which contained numerous ferritin particles also had siderosomes, most of which were thought to be conglomerates of the ferritin particles in the secondary lysosomes. Cells with numerous ferritin particles, particularly in the siderosomes, showed vacuolar degeneration. The present study indicated that in siderosis bulbi, iron released from the iron foreign body is deposited as ferritin scattered throughout the cytoplasm and is sometimes accumulated as siderosomes, in the affinitive cells. In siderosis bulbi, the cells become damaged by the deposition of ferritin in the cytoplasm, especially in the form of siderosomes. Invest Ophthalmol Vis Sci 27:226-236, 1986

Among intra-ocular foreign bodies, those of iron are the most numerous ones seen, as a result of industrial accidents. The retention of an iron foreign body in the eye almost always results in progressive iron deposition throughout the eye, inducing degeneration of the retina, cataract formation, and secondary glaucoma, in the late stage. This condition is known as siderosis bulbi, but the pathophysiology of this condition remains obscure. Little is known of the transformation of the iron foreign body and its redistribution in ocular tissues nor of the relationship between iron and its toxicity to ocular tissue. Leber proposed that metallic iron was dissolved by carbon dioxide in the tissues and, having diffused throughout the eye as a bicarbonate of suboxide of iron, entered the cells, where it was oxidized and precipitated in insoluble form. Mayou thought that intraocular iron was absorbed as colloidal ferric hydroxide and was re-precipitated as the albuminate of iron in the cells. He considered colloidal iron had a toxic effect upon the cells. Some authors attributed the cytotoxic effect of the iron to a disturbance in enzymatic activity, and others reported that the oxidation of iron may play a detrimental role. Cibis et al suggested that inadequate blood supply, which may be induced by linkage of iron to siderophilic matter such as acid mucopolysaccharides in the eye, exerts deleterious effects on intraocular structures.

Materials and Methods
A total of five eyes, three with siderosis bulbi and two controls, was examined. Of three eyes with siderosis bulbi, two were enucleated and their ocular tissues, cornea, anterior chamber angle tissue, iris, ciliary body, choroid, retina, and sclera were studied histologically. The third eye had undergone trabeculectomy and the anterior chamber angle tissue and iris were studied. One control eye was enucleated from a 27-yr-old woman with neuroepithelioma of the left zygomatic and orbital region. The other was obtained from an 81-yr-old man diagnosed as right orbital tumor (histological diagnosis was adenocarcinoma). Cornea, anterior chamber angle tissue, iris, ciliary body, choroid, retina, and sclera from the control eyes were examined.

The ocular tissues were fixed in cold 4% glutaraldehyde with 0.1 M phosphate buffer (cases 1 and 3, and one control eye from a 27-yr-old woman), or in cold 4% glutaraldehyde with 0.1 M cacodylate buffer (case 2 and the other control eye), and postfixed in cold 1% osmium tetroxide with the same buffer for 1 hr. These tissues were dehydrated in graded concentrations of ethanol and embedded in Epon. One-micron thick sections were stained with toluidine blue for light microscopy, and ultrathin sections cut on an ultramicrotome and studied under an electron microscope without staining. Some sections were observed after staining for lead or uranyl acetate.
with uranyl acetate and lead citrate, or lead citrate only. Sections ranging from 0.1–0.3 μm were used for analytical electron microscopy (ORTEC, EEDS).

Case Reports

Case 1. A 48-yr-old man was first seen in 1960 with right intraocular foreign body. Ophthalmoscopically, the foreign body, which was thought to be iron, was recognized on the retina just beneath the optic disc. There was not much intracocular bleeding. An attempt to remove the foreign body by Riesenmagnet was unsuccessful. In 1974, he again visited our clinic complaining of severe pain.

Visual acuity of the right eye (OD) was 0 and the left eye (OS) was 20/16, with spectacles. Ocular tension was OD: 17.3 mm Hg and OS: 14.5 mm Hg. Slit-lamp examination revealed keratic precipitates and a small quantity of floaters in the right anterior chamber. The right disc was pale without glaucomatous cupping. The retina was degenerated. The electroretinogram was extinguished in the right eye. His right eye was enucleated following a diagnosis of siderosis bulbi associated with intractable endophthalmitis.

Case 2. This 56-yr-old woman had an intraocular foreign body in the left eye since 1962, as removal had been unsuccessful. Sight in the left eye gradually decreased. In 1983, she visited our clinic complaining of left ocular pain.

Visual acuity was OD: 20/20 with spectacles and OS: 0. The ocular tension was OD: 13 mm Hg and OS: 30 mm Hg. The left eye showed ciliary injection and moderate anterior chamber floaters. Gonioscopically, the anterior chamber angle was closed completely. Mild ruberosis was seen on the iris. Severe cataract prevented fundus examination.

Her left eye was enucleated following a diagnosis of siderosis bulbi with secondary angle-closure glaucoma. An iron foreign body was present in the lens. There was no bleeding or opacity in the vitreous body, as determined using a biomicroscope.

Case 3. This 31-yr-old man, with no abnormalities before having gradually advanced visual disturbance in the right eye, visited our clinic in 1975. Visual acuity of the right eye was perception of hand movement. The lens had a brown opacity with rust spots of brown pigmentation on the surface. Head X-ray which showed a foreign body at the ciliary body confirmed the diagnosis of right intra-ocular iron foreign body with complicated cataract. Removal of the iron foreign body by Riesenmagnet followed by extracapsular lens extraction led to an improvement of visual acuity to 20/20, with use of a contact lens.

About 3 yr later, in 1979, he again visited our clinic complaining of right ocular pain with visual disturbance. Visual acuity was OD: perception of hand movement and OS: 20/14. The ocular tension was OD: 45 mm Hg and OS: 14 mm Hg, and the aqueous outflow rate was OD: 0.04 mm²/min/ mm Hg and OS: 0.26 mm²/min/mm Hg. The right cornea had a light brown opacity, diffusely, but the anterior chamber was clear. The iridocorneal angle was open without abnormality. Severe glaucomatous cupping was found in the right optic disc. The retinal vessels were thin. Goldmann perimetry revealed severe glaucomatous loss in the right visual field. The retina of case 1 showed atrophic change with gliosis. In some places, the retinal pigment epithelial cells had disappeared. Electron-dense particles were demonstrated mainly in the cytoplasm of the retinal pigment epithelial cells (Figs. 1A, 3) and Müller cells (Fig. 5). Some of these cells with the particles also had elliptical electron-dense deposits in the cytoplasm (Figs. 1A, 3). In the retinal pigment epithelial cells, some deposits included pigment granules as well as the electron-dense particles (Fig. 1A), and others contained shed photoreceptor outer segment discs and particles, within the same membrane. Several cells had large cytoplasmic vacuoles which included both electron-dense and electronlucent parts, showing degenerative change (Fig. 6). The neural cells of the retina, including cones and rods, horizontal, amacrine, bipolar and ganglion cells, showed few electron-dense particles and deposits (Fig. 5). These findings were observed throughout posterior, equatorial, and peripheral portions of the retina.

In the ciliary body, pigmented and nonpigmented epithelial cells, muscle cells, and fibroblasts had both electron-dense particles and deposits in the cytoplasm (Fig. 7). Some cytoplasmatic organelles which were surrounded by mono-layered membrane contained phagocytized materials as well as numerous electron-dense particles (Fig. 7). The cells which comprised the choroid and the iris included diffuse electron-dense particles and small amounts of deposits. In the cornea, anterior chamber angle tissue, and sclera, electron-dense particles were rarely demonstrated, either in the cells or in the extracellular spaces.

Results

Siderosis Bulbi

In the ocular tissue from all three patients, examined without staining, electron-dense uniform particles were demonstrated diffusely throughout the cytoplasm of the cells (Figs. 1A–4). Under a higher magnification, many of the particles were 6–7 nm-sided squares and consisted of 4 subunits about 3 nm in diameter (Figs. 2, 4B). Some of the particles appeared as paired parallel lines, open rings, triads, and pentads (Figs. 2, 4B). A few electron-dense particles were demonstrated in the nucleus (Fig. 5) and in the extracellular connective tissue (Fig. 4A). Some of these cells with diffuse particles also contained electron-dense elliptical deposits, varying in diameter from 0.1 μm to 3 μm (Figs. 1A, 3, 4A).

Usually, the deposits were surrounded by a single membrane (Figs. 1A, 3). In the electron-lucent area of the deposits, isolated electron-dense square particles 6–7 nm long were observed (Fig. 3). Analytical electron microscopy revealed a high content of iron in the electron-dense deposits (Figs. 1B, C). The cells with numerous electron-dense particles and deposits showed vacuolar degeneration (Fig. 4A).

Electroretinogram was markedly reduced in the right eye. His right eye was enucleated following a diagnosis of siderosis bulbi with secondary angle-closure glaucoma. The retina was degenerated. The retinal pigment epithelial cells had both phagocytized materials as well as numerous electron-dense particles (Fig. 1A), and others contained shed photoreceptor outer segment discs and particles, within the same membrane. Several cells had large cytoplasmic vacuoles which included both electron-dense and electronlucent parts, showing degenerative change (Fig. 6). The neural cells of the retina, including cones and rods, horizontal, amacrine, bipolar and ganglion cells, showed few electron-dense particles and deposits (Fig. 5). These findings were observed throughout posterior, equatorial, and peripheral portions of the retina.

The ocular tension was OD: 13 mm Hg and OS: 30 mm Hg. The right cornea had a light brown opacity, diffusely, but the anterior chamber was clear. The iridocorneal angle was open without abnormality. Severe glaucomatous cupping was found in the right optic disc. The retinal vessels were thin. Goldmann perimetry revealed severe glaucomatous loss in the right visual field. The retina of case 1 showed atrophic change with gliosis. In some places, the retinal pigment epithelial cells had disappeared. Electron-dense particles were demonstrated mainly in the cytoplasm of the retinal pigment epithelial cells (Figs. 1A, 3) and Müller cells (Fig. 5). Some of these cells with the particles also had elliptical electron-dense deposits in the cytoplasm (Figs. 1A, 3). In the retinal pigment epithelial cells, some deposits included pigment granules as well as the electron-dense particles (Fig. 1A), and others contained shed photoreceptor outer segment discs and particles, within the same membrane. Several cells had large cytoplasmic vacuoles which included both electron-dense and electronlucent parts, showing degenerative change (Fig. 6). The neural cells of the retina, including cones and rods, horizontal, amacrine, bipolar and ganglion cells, showed few electron-dense particles and deposits (Fig. 5). These findings were observed throughout posterior, equatorial, and peripheral portions of the retina.

In the ciliary body, pigmented and nonpigmented epithelial cells, muscle cells, and fibroblasts had both electron-dense particles and deposits in the cytoplasm (Fig. 7). Some cytoplasmatic organelles which were surrounded by mono-layered membrane contained phagocytized materials as well as numerous electron-dense particles (Fig. 7). The cells which comprised the choroid and the iris included diffuse electron-dense particles and small amounts of deposits. In the cornea, anterior chamber angle tissue, and sclera, electron-dense particles were rarely demonstrated, either in the cells or in the extracellular spaces.
Fig. 1. A, A retinal pigment epithelial cell in case 1. Electron-dense uniform particles of ferritin are scattered throughout the cytoplasm. The siderosomes, an aggregation of many electron-dense particles with a surrounding membrane, are observed. Some siderosomes include pigment granules as well as ferritin particles (arrows) (no staining, ×52,000). B, C, Analytical electron microscopy shows a much higher content of iron in a siderosome (B) than in other parts of the cytoplasm (C).
Fig. 2. A higher magnification of electron-dense particles in case 1. The particles show regular squares 6-7 nm long, consisting of 4 subunits (arrows), and several variations, including paired parallel lines (double arrows), open rings (arrowheads), and triads (opened arrow) (no staining, ×300,000).

The retina of case 2 had a fairly normal structure except for reduction in the number of ganglion cells. In the cytoplasm of the retinal pigment epithelial and Müller cells, scattered electron-dense particles were observed (Fig. 8A), while the neural cells were free from the particles. The quantity of the particles was less than that in the anterior part of the eye, and electron-dense deposits were rarely demonstrated. In some retinal pigment epithelial cells, phagosomes located near the basal portion of the cells contained not only discs but also many particles (Fig. 8A), despite the absence of particles in the photoreceptor outer segments and in the intercellular spaces (Fig. 8B). In the choroid and sclera, the cells contained small numbers of electron-dense particles in the cytoplasm. The nonpigmented and pigmented epithelial cells, muscle cells, fibroblasts, and melanocytes of the ciliary body and the iris had both scattered electron-dense particles and elliptical deposits (Fig. 9), some of which included phagocytized pigment granules (Fig. 9A). Some cells had large vacuoles containing many electron-dense particles and electron-lucent areas with a single limiting membrane (Fig. 9A). In the corneal endothelial cells and fibroblasts, electron-dense particles and deposits were seen. Some of the cells showed degeneration with large vacuoles which appeared as enlarged electron-dense deposits.

In the anterior chamber angle tissue of case 3, Schlemm’s canal had an open lumen (Fig. 10), and the trabecular meshwork was compact with narrow intertrabecular spaces. The endothelial cells in the endothelial meshwork or covering trabecular sheets showed degeneration (Figs. 4A, 10). In the section not stained, endothelial cells covering Schlemm’s canal and trabecular sheets and located in the endothelial meshwork, contained electron-dense diffuse particles in the cytoplasm (Fig. 4). Cells with many particles also included oval electron-dense deposits (Figs. 4A, 10). The endothelial cells which had numerous electron-dense particles as well as many large deposits appeared to be degenerated (Fig. 4A). There were a few electron-dense particles and no deposits in the extracellular connective tissues (Fig. 4A). The corneal fibroblasts included in the trabeculectomized specimen had diffuse electron-dense particles and deposits. There were many cells with large cytoplasmic vacuoles. In the iris obtained by iridectomy, the electron-dense particles and deposits...
Fig. 3. Siderosomes in a retinal pigment epithelial cell in case 1. In an electron-thin siderosome, typical figures of the electron-opaque cores of ferritin and single surrounding membrane are demonstrated (no staining, ×150,000).

Fig. 4. A, Endothelial cells of the trabecular meshwork in case 3. There are numerous, diffuse ferritin particles and siderosomes in the cytoplasm. The cell which has a large vacuole (V) shows evidence of destruction. Few ferritin particles are seen in the extracellular tissues (no staining, ×12,000). B, A higher magnification of electron-dense particles shows a square figure 6–7 nm long on one side and consisting of 4 subunits (arrow) and a variation of pentad (arrowhead) (Bar gauge = 10 nm, no staining, ×300,000).

were demonstrated in the cytoplasm of pigmented epithelial cells, melanocytes, and fibroblasts. Some cells containing many particles and deposits showed degenerative changes.

Control Eyes

There were neither electron-dense particles nor deposits in the cells or in the extracellular areas of the ocular tissues, including retina, choroid, ciliary body, iris, cornea, anterior chamber angle tissue, and sclera.

Discussion

In three patients with siderosis bulbi, many electron-dense particles were observed in the cytoplasm of the cells that comprised the ocular tissues. Most of the particles were uniform, and some were regular squares measuring 6–7 nm on one side and consisting of quadruplets of dense subunits each of which measured about 3 nm in diameter. Others showed several variations, including paired parallel lines, open rings, triads, and pentads. On the basis of these characteristic figures, the particles were interpreted as electron-opaque cores of ferritin, which consists of a homogeneous protein known as apoferritin, associated with micelles of ferric hydroxide-phosphate. Other types of material which were thought to be iron compounds were not observed electron microscopically in any ocular tissue. Tissue with long-lasting bleeding was considered to contain intracellular ferritin particles. In case 1, however, no evidence of much bleeding was recognized by
No. 2 CYTOTOXICITY OF IRON IN SIDERO5IS / Toworo 233

Fig. 6. A Müller cell with heavy accumulation of iron shows vacuolar degeneration in case 1 (lead staining, X13,000).

Fig. 7. A nonpigmented epithelial cell of the ciliary body in case 1. Numerous scattered ferritin particles are seen within the cytoplasm. Phagocytized materials (arrows) as well as ferritin particles are observed within the siderosomes and which are surrounded by a single membrane (no staining, X44,000).

the ophthalmologists clinically. It can hardly be considered that there would be much intraocular bleeding while the eye was left unattended for the 10 yr before enucleation. In case 2, there may have been anterior chamber bleeding because of rubeosis iridis. Ferritin particles, however, were demonstrated not only in the anterior part of the eye but also in the retina, which was thought to be free from bleeding because the metal was located in the lens, and the vitreous body was clear under biocular–microscopic examination at the time of enucleation. In case 3, both the patient and the doctors were unaware of any signs of intraocular bleeding throughout the course. Histologically, in all three cases there were no red blood cells or macrophages which engulfed debris of the red blood cells, and aggregations of ferritin particles were noted in tissues near the iron foreign body rather than in other tissues. These findings suggest that the origin of the ferritin particles is mainly the intraocular iron foreign bodies. The ferritin particles were demonstrated mainly in the cytoplasm of the cells, and a few particles were seen in the nucleus of the cells or extracellular tissues. There were no ferritin particles in the tissues from the control eyes without any intraocular iron foreign body. Thus, the iron released from the metal deposits in the cytoplasm of the cells in ocular tissues, as ferritin. Some authors reported that in cases of siderosis bulbi, iron combines with proteins to form an insoluble protein salt within the cell.1 In the retina of albino rabbits given an intravitreal injection of Ferrobalt, cytoplasmic ferritin particles were demonstrated as well as cytoplasmic ferric cores of Ferrobalt.5 In the spleen, liver, and kidney of experimental animals given iron compounds intraperitoneally, cytoplasmic ferritin particles were detected.14

I found differences in the quantity of deposited ferritin particles among the types of the cells in the same

Fig. 8. A, A retinal pigment epithelial cell in case 2. Scattered ferritin particles are shown in the cytoplasm. A phagosome contains not only discs of the photoreceptor outer segment but also many ferritin particles (no staining, X65,000). B, Outer segment of the photoreceptor cell near Fig. 10A. No ferritin particles are demonstrated in the outer segment and intercellular space (no staining, X65,000).
Fig. 9. A, Fibroblasts and a melanocyte of the iris in case 2. There are numerous scattered ferritin particles and some siderosomes within the cytoplasm. A large vacuole which contains ferritin particles (V) and a siderosome which includes both a pigment granule (arrow) and ferritin particles are shown. Few ferritin particles are observed in the extracellular tissues (no staining, X21,000). B, A higher magnification of electron-dense particles. Typical square figures of the cores of ferritin are diffuse (no staining, X150,000).

In cases of siderosis bulbi, the concentration of the iron is said to differ among the cells. The reasons for this difference remain unknown.

The distribution of deposited ferritin particles varied with the case. In case 1, many more ferritin particles were seen in the cytoplasm of the cells in the retina than in the anterior part of the eye, despite more particles in the anterior part of the eye in cases 2 and 3. This difference in iron distribution is thought to depend on the location of the intraocular iron foreign body, as Declercq et al. reported. When the iron is located in the posterior part of the eye, as in case 1, iron ions spread mainly by diffusion and are concentrated more in the posterior part of the eye. In those with an iron foreign body in the anterior part of the eye, as in cases 2 and 3, iron ions spread by diffusion and with the flow of aqueous humor, and they accumulate mainly in the anterior part of the eye. Therefore, in eyes with an iron foreign body, iron ions, released from the metal probably spread by diffusion and by the flow of aqueous humor, convert to ferric hydroxide-phosphate, and combine with apoferritin to deposit as ferritin in the cytoplasm of certain cells of the ocular tissues.

In cases of siderosis bulbi, cells with numerous scattered ferritin particles also contained intracytoplasmic electron-dense deposits. These deposits are thought to be siderosomes, which have been reported to be an aggregation of iron. In our cases, electron microanalysis showed peaks of iron in the siderosomes, as noted in cases of post-traumatic hyphema, and of siderotic cataract. In relatively electron-thin areas of the siderosomes, characteristic structures of the electron-opaque cores in ferritin were demonstrated. Many siderosomes had a single limiting membrane, and some included phagocytized materials as well as aggregation of ferritin. In the retina, phagosomes of retinal pigmented epithelial cells contained not only discs of the photoreceptor outer segment but also ferritin particles. Neighboring discs and intercellular spaces were free from ferritin particles. From these findings, it is considered that the origin of most of the siderosomes is...
the secondary lysosome in which numerous ferritin particles are accumulated. Though, in experimental siderosis bulbi, Matsuo and Hasegawa\(^5\) attributed the origin of siderosomes to mitochondria, siderosomes are thought to be related to secondary lysosomes, autophagosomes, or heterophagosomes in other tissues.\(^{20-23}\)

Cells which contain numerous ferritin particles, especially as siderosomes, showed degeneration. Histologically, the degenerated cells contained vacuoles that were thought to be enlarged siderosomes. Similar findings of cytoplasmic vacuole-formation were demonstrated in cases of siderotic cataracts.\(^9\) In lysosomal storage diseases, in which congenitally a lysosomal enzyme is missing, secondary lysosomes with many undigested materials (which may have sensitivity to the enzyme), become so large that the cells show vacuolar degeneration.\(^{24-28}\) In silicosis or asbestosis, accumulation of silica or asbestos in the secondary lysosomes of macrophages leads to a permeation of the membrane, the contained lysosomal enzymes are released, and cell damage ensues.\(^{29,30}\) Peters and Seymour\(^31\) found significantly higher activities of the lysosomal enzymes in liver biopsies from patients with iron-overload than in those from other patient groups, thereby suggesting that iron accumulation damages the lysosomal membrane, releasing acid hydrolases into the cytoplasm and initiating cell damage. In siderosis bulbi, accumulation of ferritin in the cytoplasm of the cells, especially in the form of siderosomes, is considered to be one of the causes of cell damage.

**Key words:** siderosis, ferritin, siderosome, human eye, electron microscopy

### Acknowledgments

I thank Prof. H. Inomata for pertinent suggestions, Mr. T. Kanemaru for technical assistance, and M. Ohara and Dr. M. Murakami for comments on the manuscript.

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