Chloroquine retinopathy in the rhesus monkey

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Chloroquine was administered intramuscularly 5 days a week to rhesus monkeys for as long as 4½ years. No clinical, fluorescein angiographic, or electrophysiological evidence of retinal damage was observed. Yet chloroquine/chloroquine byproduct analysis of the ocular tissues revealed an enormous binding capacity of the pigmented tissues of the eye (choroid plus RPE, ciliary body, and iris) with eventual accumulation observed in the retina. Despite the normal ophthalmic appearance and function, extensive pathological changes occurred in the retinas and choroids of these experimental monkeys. The chloroquine caused an initial dramatic effect on the ganglion cells, with the photoreceptors affected shortly thereafter. Patchy degeneration of the ganglion cells and photoreceptors then progressed over several years, with the choroid and pigment epithelium ultimately deteriorating as well.

Key words: chloroquine, rhesus monkeys, retinopathy, electroretinogram, chemical analysis, electron microscopy, membranous cytoplasmic bodies, photoreceptors, ganglion cells, retinal pigment epithelium, choroid

The clinical picture of chloroquine retinopathy has become a well-known entity since its first description by Hobbs et al. Subsequent histopathology of two advanced human cases of chloroquine retinopathy has shown that chloroquine causes widespread destruction of photoreceptors and neuroretina. Thus, since the publication of the initial papers on this subject, the ophthalmologist has been repeatedly called upon to determine from electrophysiological and clinical data whether or not an individual patient's retina was being damaged by the drug therapy. It becomes important to understand the mechanism of chloroquine toxicity on the retina and the relationship between the histopathology and the clinical abnormalities of the retina.

Experiments in which chloroquine was administered to a variety of animals in an attempt to produce the clinical picture have caused controversy over the mechanism of chloroquine's toxicity on the retinal pigment epithelium (RPE), retina, and choroid. However, the obvious animal model, the primate, had never been used. It is the purpose of this paper to report our findings of a collaborative clinical, electrophysiological, and electron microscopic study in which rhesus monkeys were given parenteral chloroquine hydrochloride for varying periods up to 4½ years.
Chloroquine retinopathy in monkey

Material and methods

Five days each week 20 mg/kg chloroquine hydrochloride were administered intramuscularly to 14 rhesus monkeys. Two additional monkeys were given lower doses of chloroquine hydrochloride, i.e., 7 mg/kg/day (5 days/week) and 20 mg/kg once weekly. Each monkey was from 6 months to 1 year of age when introduced into the experiment. There was no evidence of weight loss while on chloroquine therapy.

Clinical tests. Fundus photography and fluorescein angiography with the Kowa portable fundus camera were performed at 1, 3, and 6 weeks; 3, 6, and 9 months; and 1, 1½, 2, 2½, 3, 3½, and 4½ years. Both pupils were dilated with 1% tropicamide. Fluorescein angiography was performed.

Fig. 1. ERG B wave latencies and amplitudes to stimulus intensity W16, and flicker ERG amplitude (30 Hz) to stimulus intensity W16, during course of parenteral chloroquine administration.
Electroretinography. Electroretinograms (ERGs) were performed at intervals similar to those of the photographic studies. The animals were sedated with 2 to 3 mg of phencyclidine, administered intramuscularly and subsequently with an intraperitoneal injection of 20 to 30 mg of sodium pentobarbital. Then 1% tropicamide and 10% phenylephrine hydrochloride were applied to produce full pupillary dilation, and 0.5% proparacaine hydrochloride was used to anesthetize the cornea before the insertion of a plastic contact lens with an embedded silver electrode.

A Grass photostimulator (Model PS1) with Grass photoflash (tube PSl 2100) suspended with plastic diffusion face 22 cm above the eye with an interposed 0.9 to 3.9 log unit crossed polarized variable neutral-density filter was used to stimulate the eye. A Hewlett Packard oscilloscope (Model 130 BR) was used to record the ERG, with internal sweep synchronized to start with the stimulus trigger. Flash intensities used were W1, 2, 4, 8, and 16 with the 0.9 log unit filter and W1 with 1.2, 1.8, 2.7, and 3.9 log unit filter settings. A 30 Hz flicker flash was performed with W16 and 0.9 log unit settings. One eye of each animal was tested per session. A control animal was tested at each session. Results obtained with the following intensities were analyzed: W1, 1.8; W2, 0.9; and W16, 0.9.

Chemical analysis. At 3 weeks, 3 months, 6 months, 9 months, 20 months, 2½ years, 3½ years, and 4½ years, chemical analysis of chloroquine and chloroquine byproducts was performed on the cornea, sclera, lens, iris, ciliary body, choroid with RPE, retina, and optic nerve. A thin-layer chromatographic method developed by Windham and Huxsoll was employed. One animal which never received chloroquine was also used as a control—time 0.

In two animals chloroquine and chloroquine...
byproduct analyses of ocular tissue were performed 6 months after discontinuation of the drug. The duration of drug administration prior to termination of the drug was 3 and 6 months.

**Electron microscopy.** One or both eyes of the experimental animals were enucleated under deep barbiturate anesthesia. The eye was hemisected behind the limbus, and the eyecup was rapidly cut into nine pieces with a razor blade. A piece of central retina, 6 by 6 mm, which included the edge of the optic nerve and the fovea, was retained for electron microscopy, and the remainder of the retina and other ocular tissues were used for chemical analysis of chloroquine content. The choroid and retina were separated from the sclera and placed in one of the following fixatives: ice-cold 2% osmic acid in 0.06M Na cacodylate buffer (pH 7.4) for 2 hr, or 3.5% glutaraldehyde in 0.05M phosphate buffer (pH 7.3) usually overnight but occasionally for as long as 25 days. The glutaraldehyde-fixed tissue was washed in buffer and post-fixed in 2% osmic acid buffered with cacodylate or phosphate. All tissues were dehydrated, bloccstained with 2% uranyl acetate in 70% alcohol, further dehydrated, and embedded in Epon. Sections (50 μm) of the retinal pieces were cut on a sliding microtome. Selected areas in the 50 μm sections could be cut out and mounted on blank plastic capsules for ultramicrotomy. Sections (1 μm) for light microscopy were stained with azure II and methylene blue in 1% Na borate.

**Results**

**Fundus photography and fluorescein angiography.** No clinical change in the macula, posterior pole, or peripheral retina was observed in any of the animals receiving chloroquine. Similarly, fluorescein angiography did not reveal any change in the retinal vessels, RPE, or choroidal vasculature. Small retinal hemorrhages were observed spontaneously in the macula at varying intervals throughout the study in animals that were administered the drug, but these could not be consistently attributed to a drug effect.

**Electroretinography.** Fig. 1 shows analyses of both the flicker ERG and the ERG taken at the W16, 0.9 light intensity. Clearly, in animals receiving the high-dose chloroquine (20 mg/kg/day) there was no consistent alteration in the ERG response over 4½ years of continuous drug administration. In fact, the variability of the control values encompassed almost the entire range of values measured. Apart from a transient significant decrease in flicker amplitude at 3 to 6 months (analysis of variance), no consistent statistically significant alteration was observed for any of the values.

Animals receiving low-dose or weekly chloroquine showed no changes in either the B wave amplitudes or latency over the entire period of drug administration (Fig. 1).

**Chemical analysis of chloroquine content.** Fig. 2 displays the change in chloroquine content of the various ocular tissues as a function of time. Table I enumerates the findings for two animals which had been on the drug for 3 and 6 months but in which the drug had been discontinued 6 months prior to the analyses.

Those ocular structures containing pigment (i.e., choroid plus RPE, ciliary body, and iris) showed high accumulation of chloroquine by 1 month, with continued elevation in chloroquine concentration upon additional exposure to the drug. Even with a low concentration or with a weekly administration, the pigmented tissues showed great affinity for the drug at both 6 and 42 months. Prolonged exposure to the drug resulted in accumulation in the retina itself (Fig. 2). Extreme care was taken to ensure that no obvious pigmented tissue adhered to that retina retained for chloroquine analysis. However, we cannot be absolutely sure that slight con-

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**Table I. Chemical analysis of chloroquine + chloroquine byproducts* in the ocular tissue of rhesus monkeys in which chloroquine had been discontinued for 6 months**

<table>
<thead>
<tr>
<th>Time on drug</th>
<th>3 mo.</th>
<th>6 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lens</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Iris</td>
<td>3,216</td>
<td>1,680</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>2,070</td>
<td>7,440</td>
</tr>
<tr>
<td>Sclera</td>
<td>144</td>
<td>342</td>
</tr>
<tr>
<td>Retina</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Choroid</td>
<td>5,920</td>
<td>14,294</td>
</tr>
<tr>
<td>Optic nerve</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Reported as chloroquine base, μg/gm wet tissue.
tamination of the retina with RPE tissue did not occur.

The optic nerve, lens, and cornea showed no elevation in chloroquine concentration over the period studied. That chloroquine could be demonstrated in the sclera in increasing amounts with time can probably be explained by contamination from choroidal pigment.

Particularly remarkable was the observation that the pigmented tissues (i.e., choroid plus RPE, ciliary body, and iris) will continue to hold the drug for prolonged periods after discontinuance of drug administration even with as short exposure to the drug as 3 months (Table I).

**Electron microscopy.** Human chloroquine retinopathy is reported to be primarily a lesion of the macula region, with the first ophthalmoscopically evident signs being macular mottling, which finally develops into the "bull's eye" of pigmentation which covers the parafoveal region. Initially, therefore, we centered our attention on the foveal and parafoveal (as defined by Polyak15) areas of the experimental monkey retinas. Surprisingly, we found that the changes due to chloroquine toxicity were more advanced the farther from the fovea at every time interval investigated. Thus most of the observations to be described here come from perifoveal (ganglion cells one to four deep)15 central retina of the rhesus monkey. All the histological findings relative to particular doses and duration of chloroquine administration are summarized in Table II; particular retinas are referred to by number in the figure legends.

**Long-term, high-dose experiments**

**Ganglion cells.** The first pathological changes in the retina of the rhesus monkey oc-

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**Table II. Histological findings**

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Eye</th>
<th>Chloroquine dosage (mg/kg/day)</th>
<th>Chloroquine duration</th>
<th>Enucleation</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>od</td>
<td>20</td>
<td>7 days</td>
<td>7 days</td>
<td>MCBs</td>
</tr>
<tr>
<td>795</td>
<td>od</td>
<td>20</td>
<td>7 days</td>
<td>7 days</td>
<td>MCBs</td>
</tr>
<tr>
<td>217</td>
<td>od</td>
<td>20</td>
<td>21 days</td>
<td>21 days</td>
<td>MCBs</td>
</tr>
<tr>
<td>217</td>
<td>os</td>
<td>20</td>
<td>21 days</td>
<td>6 mo.</td>
<td>Normal</td>
</tr>
<tr>
<td>081</td>
<td>od, os</td>
<td>20</td>
<td>1½ mo.</td>
<td>1½ mo.</td>
<td>MCBs</td>
</tr>
<tr>
<td>080</td>
<td>od</td>
<td>20</td>
<td>3 mo.</td>
<td>3 mo.</td>
<td>MCBs</td>
</tr>
<tr>
<td>080</td>
<td>os</td>
<td>20</td>
<td>3 mo.</td>
<td>9 mo.</td>
<td>Normal</td>
</tr>
<tr>
<td>771</td>
<td>od, os</td>
<td>20</td>
<td>3 mo.</td>
<td>3 mo.</td>
<td>MCBs</td>
</tr>
<tr>
<td>765</td>
<td>od</td>
<td>20</td>
<td>6 mo.</td>
<td>6 mo.</td>
<td>MCBs; some degen. photr.</td>
</tr>
<tr>
<td>765</td>
<td>os</td>
<td>20</td>
<td>6 mo.</td>
<td>1 yr</td>
<td>Normal</td>
</tr>
<tr>
<td>797</td>
<td>od</td>
<td>20</td>
<td>6 mo.</td>
<td>6 mo.</td>
<td>MCBs</td>
</tr>
<tr>
<td>607</td>
<td>od, os</td>
<td>20</td>
<td>9 mo.</td>
<td>9 mo.</td>
<td>MCBs; few degen. photr; few degen. g. c.</td>
</tr>
<tr>
<td>223</td>
<td>od</td>
<td>20</td>
<td>1 yr</td>
<td>1 yr</td>
<td>Few pykn. photr. and g.c.</td>
</tr>
<tr>
<td>223</td>
<td>os</td>
<td>20</td>
<td>2½ yr</td>
<td>2½ yr</td>
<td>Foci degen. photr; degen g. c.; some RPE degen.</td>
</tr>
<tr>
<td>486</td>
<td>od</td>
<td>20</td>
<td>1 yr</td>
<td>1 yr</td>
<td>Patchy degen. photr. and g.c.</td>
</tr>
<tr>
<td>486</td>
<td>os</td>
<td>20</td>
<td>2 yr</td>
<td>2 yr</td>
<td>Degen. photr.; dead g. c.; hemorrhage RPE and retina</td>
</tr>
<tr>
<td>983</td>
<td>od, os</td>
<td>20</td>
<td>2½ yr</td>
<td>2½ yr</td>
<td>Patchy degen. photr; few dead g. c.; sclerotic retinal b.v.?</td>
</tr>
<tr>
<td>796</td>
<td>os</td>
<td>20</td>
<td>2 yr</td>
<td>2 yr</td>
<td>Foci degen. photr.; some dead g. c.; sclerotic b.v.</td>
</tr>
<tr>
<td>796</td>
<td>od</td>
<td>20</td>
<td>3 yr</td>
<td>3 yr</td>
<td>Foci degen. photr.; some dead g. c.; macroph. at RPE; some RPE degen.</td>
</tr>
<tr>
<td>562</td>
<td>od, os</td>
<td>20</td>
<td>4½ yr</td>
<td>4½ yr</td>
<td>Many pykn. photr.; 50% g. c. degen.; RPE degen.; choroid degen.</td>
</tr>
<tr>
<td>292</td>
<td>od, os</td>
<td>20*</td>
<td>3½ yr</td>
<td>3½ yr</td>
<td>Some pykn. photr.; few degen. g. c.; some RPE degen.</td>
</tr>
<tr>
<td>228</td>
<td>od, os</td>
<td>7</td>
<td>3½ yr</td>
<td>3½ yr</td>
<td>MCBs; few degen. photr.; few pykn. g. c.</td>
</tr>
</tbody>
</table>

*Dosage in mg/kg/week.
Fig. 3. a, Light micrograph of rhesus monkey retina after 6 months of high-dose chloroquine administration (765 od). The ganglion cells and some amacrine cells or displaced ganglion cells (arrow) are filled with dark granular particles. b, Other retina of the same monkey (765 os) 6 months after cessation of chloroquine. The ganglion cells are free of dense particles and the retina appears normal. (Both ×450.)

curred within one week of the onset of chloroquine administration. By light microscopy the ganglion cells appeared to be filled with darkly staining granules. These granules accumulated in larger and larger quantities in the cytoplasm of the ganglion cells primarily, but also in other neurons, so that by 6 months (Fig. 3, a) the whole retina was characterized by densely granular cells and processes. Electron microscopy of such ganglion cells, exemplified by a rhesus retina after 3 months of chloroquine (Fig. 4, a and b), shows that the darkly staining granules are membranous cytoplasmic bodies (MCBs). MCBs appear to be irregular spirals of membrane which are not clearly membrane-bound and may arise from endoplasmic reticulum (arrows, Fig. 4, b).

Up to 6 months, these earliest changes due to chloroquine were reversible. In Fig. 3, a and b, the two eyes of the same rhesus monkey are compared, one at 6 months of continuous chloroquine (Fig. 3, a) and one 6 months later after cessation of drug administration (Fig. 3, b). The darkly staining granules, indicative of MCBs, have completely disappeared from the latter retina (Fig. 3, b). Reference to Table I shows that at
this time there was still massive binding of chloroquine or chloroquine byproducts in the uveal tissues.

In addition to complete disappearance of MCBs from the neurons after discontinuation of drug therapy, MCBs became less evident in the ganglion cells by both light and electron microscopy after about 1 year of continuous chloroquine administration (Figs. 5, a, and 6, a). MCBs were replaced by numerous small lysosomal packets and inclusion bodies (circled area, Figs. 6, a, and 7, a). Most of these inclusion bodies were membrane-bound bodies containing parallel arrays of membrane. Others were more uniformly granular and lysosome-like.

Ganglion cell pathology increased progressively over the next several years, so that after 4½ years of high-dose chloroquine, with light microscopy half the ganglion cells appeared to have a dense, shrunken appearance with darkly staining vacuolated cytoplasm (Fig. 5, b). Electron micrographs of such ganglion cells are shown in Fig. 6, b and c. Fairly normal-appearing cells (Fig. 6, c, G1 and G2) surrounded grossly degenerated cells (Fig. 6, c, G3). The latter cells were shrunken, with pyknotic irregular nuclei and electron-dense cytoplasm containing numerous vacuoles. Fig. 6, b shows a ganglion cell in an intermediate stage of degeneration after 52 months of chloroquine. Its nucleus was normal, but the cytoplasm had become electron-dense and the mitochondria vacuolated.
Fig. 5. a, Retina in a monkey after 1 year of high-dose chloroquine (486 OD). Patches of rod and cone nuclei are pyknotic. Notice dark degenerating cone pedicle (black/white arrow) in the OPL. Ganglion cells (G arrows) in comparison with ganglion cells of Fig. 3, a, have no great accumulation of granules. (×500.) b, Light micrograph of perifoveal retina in a rhesus monkey after 4½ years of continuous high-dose chloroquine administration (562). The outer nuclear layer is uneven in thickness, pyknotic nuclei can be seen, and enlarged cone axons or glial elements occupy spare areas of lost photoreceptors. Rod spherules are scarce and cone pedicles are dark (arrows). The ganglion cells are in various stages of degeneration. (×500.)

and interspersed with condensed inclusion bodies.

Clusters of curvilinear tubules (as described by Ramsay and Fine 9) and membrane arrays were a common feature of both normal and degenerating ganglion cells after 4½ years of chloroquine (circled in Figs. 6, c and 7, c).

Photoreceptors. Photoreceptor degeneration began at about 1 year of chloroquine administration and progressed slowly thereafter. At first (Fig. 5, a) the pathology was visible as patches of pyknotic nuclei in the photoreceptor layer, with occasional dark cone pedicles present (white/black arrow Fig. 5, a). After 4½ years of the drug (Fig. 5, b) more extensive patches of rod and cone nuclei were pyknotic, and glial processes now filled spaces formerly occupied by photoreceptor cell bodies and synapses. Dark degenerating cone pedicles could be seen in the outer plexiform layer (arrows, Fig. 5, b). Loss of photoreceptors was reflected by the sparseness of the outer segments (compare Fig. 9, b with 9, a).

Fig. 8, a, b, and c, shows, at higher magnification, the pathological changes which had occurred in the photoreceptors after 4½
Fig. 6. For legend see facing page.
years of chloroquine therapy. A degenerating cone had a pyknotic nucleus and dense vacuolated cytoplasm containing fibrillar material and dark inclusion bodies (arrowed Fig. 8, a). The degeneration was also evident at the level of the cone pedicles (Fig. 8, d). In contrast, the outer segment of the cone did not seem affected. Degenerating rods (dr., Fig. 8, b and c) were interspersed with normal rods (nr.). Like the cones, the rod outer segments appeared normal but the inner segments were vacuolated, with disintegrating mitochondria. Vacuolated debris between the rods (arrows, Fig. 8, b and c) might be the remains of disintegrated photoreceptors or pigment epithelial debris. Thus the pathology of both photoreceptor types appeared to be primarily in the nucleus and cell body, reaching the outer segment only at the end stage.

Other retinal changes. For the first year of the drug most of the retinal neurons contained MCBs. As in the ganglion cells, MCBs became replaced by dense lysosomes and inclusion bodies. The inner nuclear layer remained otherwise relatively normal even after 4½ years of chloroquine. The retinal blood vessels appeared somewhat sclerotic in some experimental animals (Table II, 983, 796), but this was not a consistent finding. In one animal (Table II, 486) there was hemorrhage between the RPE and retina, and in another (Table II, 796), macrophages were obvious at the RPE.

Pigment epithelium. The pigment epithelium of the experimental animals appeared completely normal during the first 2 years of high-dose chloroquine administration (Fig. 9, a). The cells had a regular cuboidal shape, normal lipofuscin and phagosome content, and normal-appearing distribution of melanin granules in the apical process (Fig. 9, a). With electron microscopy occasional irregular membrane figures (as described by Babel and Engelbert8) were seen (Fig. 7, b).

After about 2 years of high drug dosage, the pigment epithelium began to deteriorate, and the final appearance at 4½ years is shown in Fig. 9, b. The pigment cells were ragged or rounded off at the apical margin. Some cells appeared pale and vacuolated compared with neighboring cells, which were dark and heavily pigmented. The basement membrane of the pigment epithelium was irregular and thrown into folds in some areas. In all the specimens with pigment epithelium pathology, detachment occurred during histological processing (Fig. 9, b). This may be because epithelial apical processes that normally extended down between the photoreceptor outer segments, anchoring retina to pigment epithelium, were more fragile or had in some way deteriorated due to drug administration.

Electron microscopy indicated that the pigment epithelial cells with the “dark” appearance in the light micrographs were probably at an earlier stage of degeneration than the paler cells. The cytoplasm was electron dense but had many small vacuoles (Fig. 10, a). Although the mitochondria were relatively normal, the number of melanin granules was reduced and those remaining were no longer cigar-shaped. The apical border of the pigment cells was rounded, with loss of elongated extensions; however, phagosomes (arrow, Fig. 10, a) were still present, suggesting outer segment phagocytosis in these cells.

The "pale"-appearing retinal pigment epithelial cells of the light micrographs (Fig. 9, b) are shown in Fig. 10, b. The apical
Fig. 7. Inclusion bodies and lamella figures seen in chloroquine retinopathy of rhesus monkey.

a, Inclusion bodies seen in a ganglion cell cytoplasm after 1 year of chloroquine (223 od). (×37,000.)
b, Irregular lamella figures in the pigment epithelium of a monkey after 1 year of chloroquine (486 od). (×18,000.)
c, c-Tubules in ganglion cells of monkey after 4½ years of chloroquine (562). (×15,000.)
processes of the cell were lost and the membrane broken, to give an extremely ragged appearance. The cytoplasm was highly vacuolated with disintegrating endoplasmic reticulum. The mitochondria had become round instead of elongate, and again, phagosomes were observed in the degenerating cytoplasm of such cells. The basement membrane of the pigment epithelium was thickened, with deposition of collagen fibrils in some areas.

**Choroid.** About the time that pigment epithelial cell damage became apparent with light or electron microscopy (i.e., 2 years and onward of chloroquine administration) the choroid began to show pathology. By 4½ years of chloroquine, electron microscopy showed that some pigment cells were normal but others contained irregular whorls of membrane material (Fig. 11) similar to MCBs but larger. Macrophages were interspersed with melanocytes and contained cellular debris and pigment granules of degenerated melanocytes. The choriocapillaris appeared normal throughout the 4½ years of chloroquine administration.

**Long-term, low-dose experiment.** The rhesus monkey receiving 7 mg/kg chloroquine per day for 3½ years (Table II, 228) had pathological changes in the retina similar to those in an experimental animal on Wi years of the high dose. Several dark, degenerating ganglion cells were found. The pigment epithelium was essentially normal, but several foci of photoreceptor pyknosis were apparent. The choroid looked normal, with the exception of occasional lamellar structures in melanocytes and macrophages.

Similarly, the rhesus monkey (Table II, 292) on the once-a-week injection schedule (20 mg/kg/week) was observed to have very early pathological changes in the retina. There were several foci of degenerating photoreceptors and an occasional vacuolated pigment epithelial cell. In addition, several ganglion cells exhibited early degenerative changes, although MCBs were not obvious. The melanocytes of the choroid contained lamellar structures.

**Discussion**

The clinical picture of chloroquine retinopathy, with the "bull's eye" degeneration and ERG changes, as seen in man, could not be produced in the rhesus monkey after 4½ years of 20 mg/kg/day (5 days/week) of the drug. If one assumes that the average rhesus monkey weighs 5 kg, this dosage is equivalent to a 50 kg patient receiving 5 gm of chloroquine base per week, or 2.86 times the advocated weekly dose for patients with rheumatoid arthritis or systemic lupus erythematosus. The total accumulative equivalent dose in these monkeys after 4½ years of administration is 1125 gm, far exceeding the dose of 500 to 700 gm, which is the dose reputedly required to produce the retinopathy in man. We suggest that we administered appropriately toxic doses of chloroquine to the experimental animals of this study.

It is significant that despite the normal clinical picture, electron microscopy demonstrated clear drug-induced pathology of the retinas in these experimental monkeys. In comparison to histopathological findings in two human cases of advanced chloroquine retinopathy described in the literature, we conclude that we have achieved an early form of chloroquine retinopathy in rhesus monkeys after 4½ years of drug administration. The pathological changes observed here were definite but patchy, and evidently not yet widespread or severe enough to be apparent by ophthalmoscopy or electroretinopathy. In fact, the lack of ERG findings has an important implication, i.e., ERG changes may not become evident in chloroquine retinopathy until advanced pathology has ensued, a fact suspected from clinical observations.

The binding capacity of the pigmented tissues of the eye for chloroquine has been pointed out by Potts and Gonasum and Potts. The enormous degree to which binding of the drug can occur (i.e., 2% to 4% of the wet weight of the tissue) and can be retained after cessation of drug therapy has once again been demonstrated in this paper. The drug uptake by the uveal tissues appears
Fig. 8. For legend see facing page.
Chloroquine retinopathy in monkey

Fig. 9. Light micrographs of retinal pigment epithelium in rhesus monkeys on high-dose chloroquine. a, Pigment epithelium is normal after 2 years of chloroquine (486 od). (×450.) b, Pigment epithelium is degenerate after 4½ years of chloroquine (562). The basement membrane is thrown into folds and the cells are rounded off and pale (arrow) or dark (double arrows). The RPE detached from the retina in the 4½ year specimens only. The figure is a montage with subretinal space eliminated, but the RPE lies apposed to the correct underlying photoreceptors. (×450.)

to increase with time and is particularly massive in the pigmented tissues: choroid plus RPE, ciliary body, and iris. Even with weekly administration of the drug, large amounts are picked up by the pigmented tissues. However, an important observation is the accumulation of chloroquine in the retina itself, suggesting that the retinal neurons also have the ability to bind the drug in some way.

The initial reaction of the retina to chloroquine is the formation of membranous whorls (MCBs) in the cytoplasm of the ganglion cells. Apparently, the appearance

Fig. 8. a, Electron micrograph of a degenerating cone in the retina of a monkey after 4½ years of chloroquine administration (562). The nucleus is pyknotic and the cytoplasm dark and vacuolated but contains dense inclusion bodies (arrowed). The outer segment appears normal. (×2,700.) b, Degenerating rod (dr) alongside a normal rod (nr). The mitochondria are swollen and the cytoplasm vacuolated in dr. Arrows indicate photoreceptor or RPE debris. (×3,750.) c, Rod in an early stage of degeneration showing (dr) showing broken membranes and vacuolation. The outer segment appears to be normal. Arrows point to probable photoreceptor debris. (×3,750.) d, Degenerating cone pedicle (CP) in the outer plexiform layer. Glial elements (gl) occupy space of lost photoreceptor terminals. (×6,250.)
Fig. 10. For legend see facing page.
of MCBs is a fairly generalized effect which various drugs\textsuperscript{21--24} have in common with chloroquine on ganglion cells of the retina. However, it is particularly interesting that, by 9 to 12 months of chloroquine the ganglion cells appear to have recovered from this initial drug shock and MCBs become replaced by smaller membrane-bound inclusion bodies. Yet longer drug administration causes curvilinear $\alpha$-tubule formation in the ganglion cell cytoplasm, as Ramsay and Fine\textsuperscript{4} described in their report of an advanced case of human chloroquine retinopathy. Ultimately chloroquine causes some ganglion cell death. In the perifoveal area of monkey retina studied here, after 4½ years of chloroquine, we found as many as 50% of the ganglion cells showing signs of morbidity.

Our data in monkey indicate that chloroquine affects the photoreceptors before the pigment epithelium shows any sign of pathology. Degenerative changes start in the nucleus and cell body of both the rods and cones. Since chloroquine is known to inhibit DNA and RNA synthesis\textsuperscript{25} in addition to inhibiting alcohol dehydrogenase,\textsuperscript{26} it is not surprising that long-term drug administration can cause damage to photoreceptors directly\textsuperscript{10} as long as the drug can get into the retina. The results of the chemical analysis illustrate that considerable amounts of chloroquine were accumulating in the retina over the 4½-year period of drug administration in these experimental monkeys.

We were surprised to find no dramatic changes in the ERG after 2 or more years of drug administration, particularly in light of the pathological changes occurring in the photoreceptors. However, the ERG evaluates a response of the whole retina, whereas the damage observed microscopically, though widespread, was rather patchy. Possibly, focal ERG recordings, had they been performed, would have indicated damaged areas of retina.

In contrast to some of the earlier subprimate animal experiments, in which the RPE was interpreted as being the primary site of chloroquine toxicity, we have found that pigment epithelium damage is a later manifestation of chloroquine toxicity. It is possible that the pigment epithelium is spared early damage because of its capacity to bind the drug in an inactive form on the melanin granules.\textsuperscript{18--20} Presumably, once this binding capacity has been exceeded, direct damage to the cells can occur. The RPE becomes irregular, consisting of a ragged sheet of rounded-off pigment cells, some of which lose melanin. In this study we saw no evidence of pigment-laden cells migrating into the outer retinal layers, as has been observed in the human cases studied by light microscopy,\textsuperscript{23} but then our experiments probably represent only early chloroquine retinopathy as compared with this human material.

Chloroquine-induced pathology of the choroid has not been described before in either of the human cases or the experimental animal studies. We showed here that massive accumulation of the drug occurs with time in the pigmented ocular tissues of these monkeys (Fig. 2). It is not therefore surprising that the drug eventually has a direct effect on the melanocytes storing it, resulting in membrane formations, degeneration of the melanocytes, and invasion of the tissue by macrophages.

We conclude from this collaborative study of chloroquine retinopathy in experimental rhesus monkeys that the drug acts on the ret-

\textbf{Fig. 10.} Electron micrographs of retinal pigment epithelium in a monkey after 4½ years of high-dose chloroquine administration (562). a, "Dark" variety of pigment cell has electron-dense cytoplasm filled with small vacuoles. Pigment granules are round instead of elongate. The apical border of the cell is rounded off. Phagosomes are present (ph, arrow). (×4,000.) b, "Pale" variety of pigment cell represents a later stage of degeneration. The cytoplasm is highly vacuolated, containing round mitochondria, and the cell membrane is broken, giving the cell a ragged border. Notice the presence of phagosomes (ph, arrows). The basement membrane is thick and filled with collagen. (×4,2000.)
ina in a generalized toxic fashion, initially destroying photoreceptors and ganglion cells and later involving the retinal pigment epithelium and choroid. The appearance of MCBs may be a temporary and reversible effect in the ganglion cells signaling an initial drug shock. However, the long-term effects of the drug on the photoreceptors and ganglion cells are presumably responsible for the retinopathy. A clinical lesson which one may learn from these experiments is that widespread early retinal damage may exist without evidence of clinical or electrophysiological changes, and thus particularly careful
monitoring of patients receiving chloroquine is important.

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