The clinical features of the long-term (3-year) natural history of experimental histoplasmic choroiditis in primates are documented in this report. The acute choroiditis resolved into four types of lesions: chorioretinal adhesions (atrophic scars) (2%); retinal pigment epithelial window defects (21%); subclinical lesions (19%); and “disappearing” lesions (58%). It was noted that the most obvious, acute lesions tend to disappear by clinical examination with long-term follow up. No subretinal neovascularization or spontaneous “reactivation” was observed. Invest Ophthalmol Vis Sci 25:801-809, 1984

Presumed ocular histoplasmosis (POH) is a well-described clinical syndrome consisting of hemorrhagic macular disciform lesions associated with peripheral, paramacular, and peripapillary atrophic choroidal scars.1-3 Epidemiologic and clinical evidence point to an association between prior exposure to Histoplasma capsulatum and this syndrome.4-6 The exact pathogenesis of the late macular lesions is not known, but reinfection, subretinal neovascularization, immunologic and vascular decompensatory mechanisms have all been implicated.4-7-10

To aid in studying the pathogenesis of the macular lesion, we developed an animal model of ocular histoplasmosis.11,12 The clinical features of the acute histoplasmic choroiditis produced in primates by intra-carotid injection of live yeast phase H. capsulatum organisms have been previously reported.12 The resolution of acute choroiditis has now been studied over a 3-year period; the clinical features of the long-term natural course of experimental ocular histoplasmosis in this primate model are reported in this paper.

Materials and Methods

Animals*
The stumptailed monkey (Macaca speciosa) and rhesus monkey (Macaca mulatta) were selected for study on the basis of availability, ease of handling, and well-studied macular regions.13 All animals were observed over a 30-day quarantine period; tuberculin skin tests, stool tests for ova and parasites, and other routine laboratory examinations were done and only healthy primates were utilized in this study. One animal was excluded because of retinal abnormalities noted on baseline ocular examination.

Forty-nine eyes were used in this study; all eyes were followed clinically for at least 1 year, and 18 eyes were followed for 2-3 years.

Clinical Examinations
All animals were weighed and had an external eye examination including fundus examination with the binocular indirect ophthalmoscope. Using the Zeiss fundus class III camera, baseline fundus photography and fluorescein angiography (0.7 cc of 5% sodium fluorescein) were performed. Histoplasmin skin tests

* The animals used in this study were maintained in animal care facilities fully accredited by the American Association of Laboratory Animal Science.
Fig. 1. Fundus maps. Location and fate of each lesion was determined by making serial fundus maps on transparent overlay sheets. A, Vasculature of each eye was first drawn onto paper. B, Location and extent of each lesion noted on fundus and fluorescein angiography was then transferred onto overlay sheet. Superimposition of transparent sheets at any follow-up period allowed exact determination of resolution of any lesion.

(Parke-Davis & Co.; Detroit, MI) were administered to all animals; all were negative on baseline examination.

Fungus

H. capsulatum, Campbell strain G184B, was used. This same strain was used in earlier rabbit studies. Stock cultures of yeast phase H. capsulatum were kept refrigerated and transferred at monthly intervals on blood-glucose-cystein agar.

Inocula for injecting animals were prepared as previously reported from fungus grown on slants of blood-glucose-cystein agar incubated at 37°C for 48 hr. Inoculum was injected within 2 hr of preparation; 80% viability at the time of injection was ascertained by dye exclusion test (Janus green B). After injection, the remaining inoculum was cultured on blood agar and Sabouraud's glucose agar as a final test of organism viability and purity.

Surgical Technique

The internal carotid artery was injected with suspended inoculum (5000 organism/lb) via a 25-gauge butterfly needle using the dissection techniques previously reported. Anesthesia was induced with ketamine hydrochloric acid, acepromazine, and atropine and sustained with intravenous pentobarbital or gaseous halothane; there were no deaths due to anesthesia. No wound dehiscences or wound abscesses related to H. capsulatum were noted.

Follow-Up Clinical Examination

Primates were restudied with indirect ophthalmoscopy, fluorescein angiography, and fundus photography at 10, 20, 50, and 110 days after injection, and every 2 months thereafter for up to 3 years. Histoplasmin skin testing was repeated within the first month after injection. For follow-up procedures, animals were fasted overnight and sedated as described for injection. All eyes were dilated with 10% Neo-Synephrine and 1% tropicamide.

Serial Fundus “Maps”

Foci of choroiditis were monitored at each follow-up examination. In order to determine more precisely the fate of specific areas of acute choroiditis and to provide a method for following the long-term course of such specific lesions, composite drawings of the posterior pole were made of each eye and the location and extent of each lesion noted at 10–14 days, 6 months, 1 year, and 2–3 years after intracarotid injection. These drawings were made from original color photographs and fluorescein angiograms obtained at these follow-up intervals. Super-imposition of drawings at any interval allowed exact determination of the site of lesions (required for “disappearing” lesions) (Fig. 1). Drawings did not include the peripheral retina where, in fact, additional lesions were observed clinically but which could not be photographed reliably in all animals at all time intervals.

Results

Acute Histoplastic Choroiditis

In the 49 eyes studied, all but two developed funduscopic or fluorescein angiographic evidence of an
acute choroiditis after injection with live *H. capsulatum* organisms.

The features of acute histoplasmic choroiditis have been described previously and will be summarized here. Acute choroiditis usually became apparent 3–4 days following *H. capsulatum* injection. Some early foci were not clinically visible but fluorescein angiography showed early blocking of fluorescence in the areas of choroiditis, resulting in late staining. Within 5–7 days of injection, multiple foci of acute choroiditis became clinically obvious as discrete, round, poorly circumscribed, yellowish lesions. Severity of disease ranged from a few apparent lesions to confluent choroiditis and serous detachment. Histoplasmin skin test became positive in all animals within 2–4 weeks after injection.

By 6 weeks after infection, it was no longer possible in any eyes to demonstrate organisms by histologic techniques. With the disappearance of organisms, the disease entered the resolution phase by our classification.

Resolution Stage (6 weeks to 6 months after infection)

Thirty-two eyes were followed for a 6-month period. The resolution phase was characterized by four distinct clinical patterns that evolved from the acute experimental histoplasmic choroiditis (Table 1): (1) atrophic scars (chorioretinal adhesions); (2) retinal pigment epithelial window defects; (3) subclinical lesions identified only by fluorescein angiography; and (4) “disappearing” lesions characterized by “normal” fundus examination and normal fluorescein angiography.

**Atrophic scars (chorioretinal adhesions):** These lesions typically developed at the posterior pole in the macular and paramacular regions. Scars were focal, circumscribed, round or oval, yellowish-white, with slightly hazy margins (Figs. 2A, B); they were usually one-half disc diameter or less in size. After the acute phase, and throughout the follow-up period, the hypopigmented portion of these lesions did not change appreciably in size or appearance. Fluorescein angiography typically revealed an early window defect and fluorescence that persisted into the late phase (Figs. 2C, D). The region immediately surrounding the depigmented foci, however, occasionally developed pigmentary changes, which most typically consisted of a halo of increased pigmentation. This increased pigmentation was observed initially as early as 20–40 days after injection and continued to increase in size for as long as 1 year. In other cases, the surrounding fundus became slightly hypopigmented and contained scattered pigment clumps giving a mottled appearance to the region.

**Subclinical lesions identified only by late fluorescein changes:** In still other animals, many acute lesions resolved with no abnormalities detectable on ophthalmoscopy. However, some lesions could still be detected on fluorescein angiography, called subclinical lesions by our classification, appearing as areas of faint, late staining (Figs. 4C, D). In most cases, the lesions evolved from clinically obvious lesions (Fig. 4A) to subtle hypopigmented areas by the end of the first month. Gradual fading then occurred with clinical disappearance of the lesions in some cases by 2–4 months after injection (Fig. 4B). Other lesions disappeared clinically within a month of injection. In general, such variability in resolution depended upon the size of the lesion during the acute phase, with larger lesions fading more slowly.

**“Disappearing” lesions characterized by “normal” fundus and fluorescein angiographic patterns:** Some lesions totally disappeared by both clinical and fluorescein angiographic examinations; these we call “disappearing” lesions. In these instances, no abnormalities were detectable at follow-up examinations. All evidence

### Table 1. Resolution of Specific Lesion Types: 6 Months to 2–3 Years

<table>
<thead>
<tr>
<th>Lesion type</th>
<th>6 mo (32 eyes)</th>
<th>No. of lesions 1 yr (32 eyes)</th>
<th>3–4 yr (18 eyes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean*</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.9</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Range†</td>
<td>0–3</td>
<td>0–3</td>
<td>0–2</td>
</tr>
<tr>
<td>RPE defects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean*</td>
<td>5.4</td>
<td>4.3</td>
<td>3.1</td>
</tr>
<tr>
<td>SD</td>
<td>5.0</td>
<td>4.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Range†</td>
<td>0–19</td>
<td>0–16</td>
<td>0–8u</td>
</tr>
<tr>
<td>Subclinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean*</td>
<td>5.6</td>
<td>4.0</td>
<td>2.8</td>
</tr>
<tr>
<td>SD</td>
<td>6.2</td>
<td>4.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Range†</td>
<td>0–22</td>
<td>0–18</td>
<td>0–9</td>
</tr>
<tr>
<td>Disappearing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean*</td>
<td>3.5</td>
<td>7.8</td>
<td>8.7</td>
</tr>
<tr>
<td>SD</td>
<td>4.0</td>
<td>6.7</td>
<td>7.4</td>
</tr>
<tr>
<td>Range†</td>
<td>0–17</td>
<td>0–20</td>
<td>0–28</td>
</tr>
</tbody>
</table>

* Mean number of lesions per eye.
† Range of number of lesions.
Fig. 2. Atrophic scars, chorioretinal adhesions. Resolution of acute choroiditis (A and C) to atrophic scars (B) was characterized by persistence of hypopigmented portion of acute lesion to form focal, circumscribed, round or oval, yellowish-white depigmented areas with slightly hazy margins. Fluorescein angiography typically revealed early window defect that persisted into late phase (D). Acute period, A and C, 9 days; resolved, B and D, 378 days.

of prior choroiditis disappeared, and the retinal pigment layer had a normal appearance (Figs. 5A–D).

Long-Term Natural History (6 months to 3 years)

The natural history of experimental *H. capsulatum* ocular infections was followed carefully on successive clinical examinations and through preparation of meticulous serial fundus maps. Of the 49 original eyes, 32 were followed for at least 1 year, and 18 eyes were followed for 2–3 years.

Though it was not possible to predict from the appearance of an acute lesion its predominant resolution
pattern or eventual course, a strong tendency was noted for lesions to disappear clinically on successive follow-up examinations (Fig. 6). Of 49 originally infected eyes, an average of 14.9 foci of acute choroiditis per eye were identified by clinical exams 10–14 days after injection of yeast phase H. capsulatum; all of these lesions also were identified on fluorescein angiography.

In 32 eyes, at 6 months after injection an average of only 5.9 lesions per eye remained visible ophthalmoscopically while an average of 11.2 lesions could still be identified by fluorescein angiography. At 1 year, follow-up of these same 32 eyes revealed an average of 4.8 and 8.5 lesions per eye discernable on funduscopic examinations and fluorescein angiography, re-
Fig. 4. Subclinical lesion. A, Acute choroiditis (arrow). B, Resolution resulted in subclinical lesions characterized by return to normal appearing fundus (arrow). C and D, Such lesions could still be detected on fluorescein angiography by late fluorescence (D), although markedly reduced in intensity compared to acute stage (C). Acute period was 21 days; resolved, 454 days.

spectively, and in the 18 eyes followed for 2–3 years, this fell to an average of 3.2 and 6.6 lesions per eye on funduscopic and fluorescein angiographic examination.

The results of the 6 month, 1 year, and 2–3 year follow-up examinations are presented in Table 1 with respect to resolution of acute lesions by specific lesion types. It was noted that while atrophic scars tended to represent permanent changes in the fundus (ie, an average of 0.5 ± 0.9 scars per eye at 6 months versus 0.3 ± 0.6 scars per eye at 2–3 years), the number of retinal pigment epithelial defects and subclinical lesions fell markedly at each follow-up, and contributed substantially to the growing number of clinically "dis-
appearing" lesions (Table 1). In no instance did fluorescein angiography indicate the presence of subretinal neovascularization; similarly, no spontaneous hemorrhage or "reactivation" was noted.

Discussion
The pathogenesis of macular disease in human POH is unknown;4-7-10 ultimately, the rationale for therapy rests on the solution to this problem. Study of primate multifocal histoplasmic choroiditis has the potential for delineating features of the acute and long-term natural course of this entity and also for providing a model for evaluation of naturally occurring or experimentally induced, late reactivation of macular disease. Although subretinal neovascularization or clear-cut reactivation of inactive scars did not occur in our
model, the natural course of this entity, studied over a 3-year period, has demonstrated many features that have relevance to the human disease and provide a basis for understanding some aspects of human POH.

It has been postulated that in benign systemic histoplasmosis infection in humans, there is an acute multifocal histoplasmic choroiditis phase associated with the fungemia. Our laboratory model clearly demonstrates that such an event can, in fact, occur, leading to spontaneously resolving foci of acute choroiditis.

Table 2. Classification of peripheral atrophic scars and other lesions found in patients with presumed ocular histoplasmosis

<table>
<thead>
<tr>
<th>Description</th>
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<tbody>
<tr>
<td>Scars deemed characteristic of presumed ocular histoplasmosis</td>
</tr>
<tr>
<td>1. Slightly irregular, roundish, depigmented, atrophic, discrete, primarily choroidal scar, 0.2 to 0.7 DD size; pigment clump centrally, eccentrically or diffusely present within borders of scar, posterior to equator or in periequatorial region.</td>
</tr>
<tr>
<td>2. Similar to 1, except pigment present only at margins of scar and not within scar.</td>
</tr>
<tr>
<td>3. Similar to 1, except no pigment present.</td>
</tr>
<tr>
<td>4. Peripapillary scar, nodular or confluent, 0.3 to 0.7 DD in breadth, atrophic, with or without light pigmentation in scar or at border.</td>
</tr>
</tbody>
</table>

Fundus scars questionably associated with presumed ocular histoplasmosis

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Similar to 1, except less than 0.2 DD size, with or without pigment in scar or at periphery.</td>
</tr>
<tr>
<td>6. Diffuse confluent chorioretinal scar, greater than 1 DD size with or without pigment.</td>
</tr>
</tbody>
</table>

Fundus scars and other lesions not judged to be associated with ocular histoplasmosis

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Minute macular and paramacular alterations in pigment epithelium.</td>
</tr>
<tr>
<td>8. Pigment flecks in retina.</td>
</tr>
<tr>
<td>10. Senile macular degenerative changes.</td>
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</tbody>
</table>

This spontaneous and relatively rapid resolution was somewhat surprising and emphasized the ability of the host's cellular immunity to handle this infection quite well. Animals converted from negative to positive histoplasmin skin test, indicating systemic sensitization. It is likely that these same events occur in human systemic disease, but with much fewer foci of acute choroiditis than seen with the large bolus given in our experimental primate system. The rapid clinical resolution of acute lesions may account for the lack of clinical findings in patients with known systemic histoplasmosis or in individuals seen several weeks after an epidemic of benign systemic histoplasmosis. The relatively small amount of inoculum reaching the eye during acute, benign, human, systemic infection might result in foci of choroiditis too small to cause symptoms; such foci would be expected to resolve rapidly, leaving the fundus clinically uninvolved just a few weeks after the fungemia. This proved to be the case in many primates whose small, early lesions totally disappeared.

The clinical appearance in primates of the late lesions correlates well with human cases. In the Walkersville study, 24 persons with nondisciform, peripheral scars suggestive of POH were identified. In that study, 163 scars in 42 eyes were analyzed in terms of location, frequency, and appearance. Fundus changes were classified as either “characteristic of POH,” of questionable association,” or “not judged to be associated with POH” (Table 2). A comparison of the late lesions found in our model to those in humans in the Walkersville study was made: (1) pigment epithelial defects and mottling were found frequently in the fundus of humans but the lesions were “not judged to be associated with POH.” Observations of such defects in our primate model suggests that an association may, in fact, be justifiable. (2) Clinical findings classified as “of questionable association with POH,” such as very small atrophic scars or diffuse confluent chorioretinal scars, also were present in our primate model as well as in the human subjects from Walkersville. (3) The most frequently seen atrophic scars seen in the Walkersville study were “nonpigmented chorioretinal adhesions 0.1 to 0.6 DD in size.” This describes exactly the clinically obvious scars typically found in our primate model.

Findings from our laboratory model and a reevaluation of the Walkersville patient data suggest that POH may present a much more varied clinical picture than usually is recognized. The “typical histo scar” is only one of a number of clinically observable features in an involved eye. The clinical situation is further complicated by the fact that some changes in involved eyes may be subclinical and observable only by fluorescein angiography. There is also a tendency for acute lesions to disappear entirely by clinical examination,
including fluorescein angiography. The relevance of subclinical and disappearing lesions is unknown, but they may play a role in the development of so-called *de novo* foci of choroiditis or in the pathogenesis of late active macular disease in patients with prior and apparently totally "normal" macular examinations.

**Key words:** ocular histoplasmosis, primate model, choroiditis, chorioretinal scars

**Acknowledgments**

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**References**