COMMUNICATIONS

EXPERIMENTAL CORNEAL HISTOPLASMOSIS*†

BY

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WOODS and Wahlen (1959) proposed *Histoplasma capsulatum* as a cause for an ophthalmoscopic entity in man. The epidemiological significance of this suggestion is apparent when one considers that over 30 million Americans are histoplasmin-positive (Silverman, Schwarz, Lahey, and Carson, 1955). An investigation of ocular histoplasmosis was therefore initiated in this laboratory, and primary ocular infections have been induced to date in the domestic pigeon (Smith and Jones, 1962), rabbit, and two primate species (Smith and Singer, in press) by anterior chamber injections of *H. capsulatum*. This is the first report of experimental ocular infections from inoculation of *Histoplasma capsulatum* by the corneal route.

Methods and Procedure

1:10 and 1:100 dilutions of yeast phase *H. capsulatum* with normal saline were prepared by techniques outlined in previous reports. Injections were given into the right eye of rabbits, anaesthetized with topical 0.5 per cent. proparacaine hydrochloride, using tuberculin syringes and 30 needles. Control injections of saline were given into the left eye of each animal. Two routes of corneal infection were studied. Four rabbits with histoplasmin-negative skin-tests were given 0.02 ml. diluted organisms by injection into the corneal stroma. Three other histoplasmin-negative rabbits were given intra-epithelial inoculations of *H. capsulatum* by placing one drop of 1:10 yeast phase organisms on the cornea, and then streaking the cornea with a 30 needle as one would a blood agar plate. The left cornea was similarly streaked with saline. Atropine 1 per cent. drops were instilled into each eye daily to facilitate ophthalmoscopy. The eyes were examined daily with focal illumination and serial colour photographs were made. Biomicroscopic and indirect ophthalmoscopic examinations were made at appropriate intervals.

Findings

Stromal Corneal Histoplasmosis.—Immediately after organisms or sterile saline had been injected into the corneal stroma, a glistening infiltrate could be seen at the injection site which had a fractured or splintered appearance due to interlamellar dissection of fluid. The injected fluid was promptly resorbed, however, and by 48 to 96 hours the corneae appeared perfectly normal and the eyes showed little reaction. By 7 to 14 days, however, slight stromal opacification and beginning vascularization could be seen grossly at the injection site. Injections given close to the limbus produced a lesion which closely resembled a phlycten in man during the first 2 weeks. By 3 weeks, these lesions often developed a daughter lesion of raised, pinkish, vascularized tissue. Vascularization progressed slowly after 3 weeks, and the entire clinical picture was quite different from the fulminating granulomatous iridocyclitis noted in rabbits given the organisms by anterior chamber injection.

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Most animals were found to have become histoplasmin-positive after one month. Histological examination of the stromal keratitis revealed a diffuse infiltration of inflammatory cells with corneal thickening, and with the Grocott modification of the Gomori methenamine silver stain, organisms were readily seen in the corneal lesions. Figs 1-4, 7, and 8 (opposite) and Figs 11 and 12 (overleaf) show the typical course and pathology of intrastromal corneal histoplasmosis.

Intra-epithelial Corneal Histoplasmosis.—The group of animals which received intra-epithelial inoculations of *H. capsulatum* was of interest in that the clinical course was again quite different not only from that in the anterior chamber injections but also from that of the stromal corneal lesions noted above. Thus, for about 2 weeks after epithelial inoculations, the corneae were so white and quiet that initially it was suspected that an avirulent organism had been used on that day. However, after 2 weeks, biomicroscopic examination revealed small, greyish, rounded lesions in the streaked areas, which were more easily noted after fluorescein staining. These lesions progressed at an extremely slow rate, so much so that after 3 months' observation vascularization of the epithelial lesions has been virtually absent. The corneal epithelium was cultured in one rabbit Figs 5 and 6 (opposite) and 9 and 10 (overleaf) at 11 days and a profuse growth of *H. capsulatum* was obtained. Some variation was noted in the corneal infections. Lesions which closely resembled dendritic keratitis and also disciform keratitis were produced in some animals. The amount of vascularization noted in the cornea appeared dependent to a large degree upon the depth of the primary inoculation, for in two eyes in which moderate limbal vascularization was noted, microscopic examination revealed superficial stromal involvement. Again the animals became histoplasmin-positive after about one month. Histological examination revealed numerous *H. capsulatum* organisms confined mainly to the anterior layers of the cornea. In all instances the control eyes remained negative to external and histological examinations. No significant intra-ocular involvement was seen in these eyes, with the exception of one in which the anterior chamber had been entered at the time of the primary inoculation. This eye pursued a clinical course similar to that of the other anterior chamber injections. Chorio-retinal lesions have not so far been produced when *H. capsulatum* was given by the corneal stromal, corneal epithelial, subconjunctival, or anterior chamber routes, but they have been produced with intravitreal injections, and these constitute the subject of another report.

- FIG. 1.—Cornea immediately after injecting 0.02 ml. 1:10 yeast phase *H. capsulatum* into stroma near limbus. Note splintered appearance of interlamellar fluid.
- FIG. 2.—Cornea 8 days after intrastromal injection (same rabbit as in Fig. 1). Note that all injected fluid has resorbed and eye shows virtually no reaction.
- FIG. 3.—Cornea 27 days after intrastromal injection of *H. capsulatum* (same eye as in Fig. 2).
- FIG. 4.—Cornea 36 days after intrastromal injection of *H. capsulatum*. (same eye as in Fig. 3). Note gradual clearing of primary corneal stromal histoplasmosis.
- FIG. 5.—Cornea 11 days after intra-epithelial injection of *H. capsulatum*. Organisms were demonstrated in this cornea both by culture and by histopathological demonstration on the same day as this photograph was taken.
- FIG. 6.—Cornea 37 days after intra-epithelial injection of *H. capsulatum*. Compare vascularization of epithelial histoplasma keratitis, with that noted in the stromal form (Fig. 7).
- FIG. 7.—Cornea 8 days after intrastromal injection of yeast phase *H. capsulatum* near limbus. Note similarity of lesion to phlyctenular keratitis.
- FIG. 8.—Trichrome section of corneal lesion seen in Fig. 7. $\times 12$.

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FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9.—Section of intra-epithelial histoplasma keratitis. Same eye as in Fig. 5. Haematoxylin and eosin. $\times 24$.

FIG. 10.—Grocott-Gomori stain of intra-epithelial histoplasma keratitis, showing organisms in superficial cornea. Same eye as in Figs 5 and 9. Oil immersion. $\times 360$.

Discussion

Anterior chamber inoculation of *H. capsulatum* into the rabbit causes a reproducible clinical course characterized by a fulminating granulomatous iridocyclitis leading to rapid corneal vascularization, secondary glaucoma, and finally phthisis bulbi in about 6 weeks. Our findings with yeast phase organisms have been similar to those reported by Day (1949), who gave the mycelial phase to rabbits by the anterior chamber route. The corneal infections, however, present a strikingly different course in that the lesions are much more slowly progressive and are characterized by much less vascularization. Intra-epithelial inoculations of *H. capsulatum* produce discrete colonies on the corneal surface which appear to offer a good means for studying the effects of various drugs, for the response to topical applications may thus be directly observed and photographed. Studies of the effects of various agents on the natural course of experimental corneal histoplasmosis are now in progress. The experimental production of lesions similar in appearance to phlyctenular keratitis in man with *H. capsulatum* is of interest. The phlycten is usually considered as

FIG. 11.—Stromal histoplasma keratitis. Same eye as in Fig. 7 sectioned at 15 days. Haematoxylin and eosin.

FIG. 12.—Grocott-Gomori stain of stromal histoplasma keratitis, showing organisms in corneal stroma. Same eye as in Fig. 7. Oil immersion. $\times 360$.

due to sensitivity to the tubercle bacillus, but the production of a similar lesion with another organism causing granulomatous reaction is not altogether unexpected. This might indicate that all the granulomatous skin tests, (e.g. tuberculin, histoplasmin, coccidioidin, and blastomycin) might properly be investigated in patients with phlyctenular keratitis rather than the tuberculin test alone. It should be pointed out, however, that the eye is probably not the portal of entry of *H. capsulatum* in the

naturally-acquired disease, but it has served as an excellent site for investigation of tissue responses and drug effects with this organism.

Summary

The natural course of experimental corneal histoplasmosis in the rabbit is presented. The clinical picture produced by this organism is quite different when it is administered at different ocular sites. Lesions resembling phlyctenular keratitis and interstitial keratitis have been produced by stromal injections, and lesions resembling dendritic and disciform keratitis have been produced by intra-epithelial inoculations with *Histoplasma capsulatum*.

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