Experimental Endogenous Endophthalmitis Caused by 
*Haemophilus influenzae* Type b

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Experimental endogenous endophthalmitis was produced in infant rats by either intranasal or intraperitoneal inoculation with *Haemophilus influenzae* type b at 5 days of age. The ocular disease occurred in about 50% of bacteremic animals who survived to age 12 days and probably represents metastatic bacterial infection secondary to hematogenous seeding. The lesion was a highly destructive supplicative endophthalmitis that ultimately progressed to panophthalmitis and was followed by organization of the exudate and phthisis bulbi.

Metastatic infection of the eye was a common complication of a variety of bacteremic infections in the pre-antibiotic era (5). Streptococci were the most frequently involved organisms usually in association with bacterial endocarditis. However, encapsulated pyogenic bacteria such as pneumococci (1, 2, 12, 17, 18, 27) and meningococci (6, 11, 14) were next most frequent. Although *Haemophilus influenzae* type b infection was not commonly associated with metastatic endophthalmitis, three cases are cited by Duke-Elder (5), and Selenkowsky and Woizechowsky (30) include *H. influenzae* in their list of etiological agents. Indeed, almost any bacterium capable of causing bacteremia may result in metastatic ocular infection (5, 8, 15, 16). Renewed interest in clinical and experimental metastatic ocular infection has recently been aroused by the association of endogenous endophthalmitis with disseminated candidiasis (7).

Much of the prior experimental work in endogenous bacterial endophthalmitis was done in the early years of this century. The classical experiments of Selenkowsky and Woizechowsky (30) demonstrated that intravascular injection of bacteria could cause localized ocular infection. They showed that two of the major variables were the degree of bacteremia and the presence of nonpenetrating trauma. Since 1940, most experimental work on bacterial endophthalmitis has utilized exogenous intraocular implantation of bacteria, including gonococci (4, 23), meningococci (25, 26), *Leptospira* species (34), *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (19, 20, 31).

Recently, a reproducible experimental model of *H. influenzae* type b meningitis was described using infant rats infected by the intraperitoneal (i.p.) and intranasal (i.n.) routes (24, 33). The rats were shown to have intense and prolonged bacteremia. By both routes, about 40% of animals with meningitis had histopathological changes of mild purulent panophthalmitis. However, no details about the pathology, evolution, or microbiology of the ocular lesions were given in either report. In the course of our studies on the immune response of rats surviving experimental *Haemophilus* meningitis, we noted that a significant percentage of rats developed ocular opacities. This report describes our investigation of this phenomenon, including subsequent experimentation to show that the lesion was reproducible and represented metastatic purulent endophthalmitis caused by direct bacterial invasion, probably from hematogenous dissemination.

**MATERIALS AND METHODS**

**Animals** Litters of 5-day-old, pathogen-free, albino Sprague-Dawley rats were purchased from Hilltop Lab Animals, Scottsdale, Pa. Three of the litters used in the second experiment were bred in our animal quarters. All animals were housed in a single room in the animal quarters of the Children's Hospital of Pittsburgh.

**Bacteria.** The strain of *H. influenzae* type b used (Pekala 3–9) was originally isolated from the spinal fluid of a child with purulent meningitis. The isolate was subsequently passaged once through a 5-day-old rat by i.p. infection, recovered from a culture of blood, grown overnight in Levinthal base, and stored in 1.0-ml portions at −70°C. One day before animal inoculation, a stored portion was thawed and cultured on chocolate agar with 6% Fildes enrichment (Difco, Detroit, Mich.). After overnight incubation in a candle jar, several colonies were inoculated into Levinthal broth. A 6-h culture at 37°C resulted in approximately $5 \times 10^9$ colony-forming
units/ml. Intranasal inoculation utilized 0.01 ml of this broth culture. For i.p. inoculation, dilutions were made in Trypticase soy broth, and 10⁴ live bacteria in 0.1 ml were inoculated by a 26-gauge needle and a tuberculin syringe.

Experimental methods. In the first experiment, the experimental animals were challenged i.n. Ten microliters of the bacterial suspension was given with a 50-μl precision syringe (Hamilton Co., Whittier, Calif.) attached to the plastic tubing of a pediatric infusion set (Butterfly Short 25-G, Abbott Laboratories, North Chicago, Ill.). One person handled the syringe and another person held the bevel of the needle adjacent to the nares to direct the flow and avoid trauma. Blood for culture was obtained at 48 h after challenge by cardiac puncture. Three drops of blood were streaked onto antiserum-agar plates (22). Recovery of any colonies with typical morphology surrounded by a precipitin halo was considered a positive culture for H. influenzae type b. The majority of positive cultures yielded colonies that were too numerous to count (>10⁴ bacteria/ml of blood). Rats who died were usually not recovered due to cannibalization by the mother. Littermate control animals were inoculated with saline. Control and experimental litters were housed in separate cages until age 4 weeks, when the rats were weaned and further divided into cages containing four rats of each type. All animals were observed for 4 months. Four animals with ocular disease were unilaterally enucleated at various ages. Aspiration of the anterior chamber was carried out on enucleated eyes using a 26-gauge needle and a tuberculin syringe. Specimens were planted on antiserum-agar plates. Determination of the presence of soluble type b capsular polysaccharide antigen was carried out on aspirated samples using countercurrent immunoelectrophoresis as previously described (21). This technique can measure as little as 30 ng of antigen per ml. Enucleated eyes were fixed in 10% neutral buffered formalin for at least 48 h, processed through graded alcohols and xylene, and embedded whole in paraffin. Histological sections (6 μm) were prepared and stained with hematoxylin and eosin, the periodic acid-Schiff stain, and, in selected instances, the Brown and Brenn (Gram) stain.

For the second experiment, a group of nine litters comprising 114 animals were used. At age 5 days, six litters were inoculated i.n. and three were inoculated i.p. Blood cultures were taken at 48 h, and nonbacteremic rats were discarded. The rats were not further manipulated until their lids began to open, which occurred between days 10 and 12. Ocular disease was detected solely by simple gross inspection by one of us. Rats with unilateral or bilateral abnormalities were immediately bled for culture and for determination of antigenemia by countercurrent immunoelectrophoresis. They were then sacrificed using chloroform, enucleated bilaterally, and decapitated. Anterior chamber aspirates from enucleated eyes (affected and unaffected) were cultured and tested for type b antigen. All eyes and heads were fixed in formalin for at least 48 h. Eyes were processed as described in the first experiment. Heads were skinned and fixed in 10% neutral buffered formalin for at least 48 h. One coronal section at the level of the external ear and one midsagittal section from the remaining rostral fragment were decalcified and prepared for histology. Four of the eyes were serially sectioned at 250-μm intervals in order to identify the optic nerve in section. Rats without ocular abnormalities were followed until age 1 month and then bled, sacrificed, enucleated, and decapitated. Specimens were processed identically to those of rats with clinical eye disease.

RESULTS

Table 1 summarizes the data on the incidence of ocular infection in both experiments.

First experiment. Of 83 rats who were challenged i.n. with H. influenzae type b, 40 (48%) were bacteremic at 48 h postchallenge. Only 12 rats survived long enough to open their eyes (age 10 to 12 days), and 20 (43%) of these 47 rats were bacteremic. Twenty-five control rats were similarly challenged with saline. Ocular disease developed in six (30%) of the 20 bacteremic survivors. Ocular lesions were already apparent by the time the animals first opened their eyes. All six animals had unilateral lesions when first detected, but two animals subsequently developed bilateral disease within a week. No clinically detectable eye disease was noted in any of the challenged but nonbacteremic rats or in the saline control rats.

The clinical appearance of eye lesions was similar in all animals and consisted of varying degrees of anterior chamber clouding (Fig. 1).

<table>
<thead>
<tr>
<th>Expt</th>
<th>No. challenged with H. influenzae type b</th>
<th>Route of challenge</th>
<th>No. bacteremic</th>
<th>Bacteremic survivors*</th>
<th>Survivors with endophthalmitis</th>
<th>No. unilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>83</td>
<td>i.n.</td>
<td>40 (48%)</td>
<td>20 (50%)</td>
<td>6 (30%)</td>
<td>4 (67%)</td>
</tr>
<tr>
<td>2nd</td>
<td>41</td>
<td>i.p.</td>
<td>41 (100%)</td>
<td>10 (25%)</td>
<td>7 (70%)</td>
<td>6 (86%)</td>
</tr>
<tr>
<td>3rd</td>
<td>73</td>
<td>i.n.</td>
<td>29 (40%)</td>
<td>22 (76%)</td>
<td>11 (50%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td>52</td>
<td>24 (46%)</td>
<td>14 (58%)</td>
<td></td>
</tr>
</tbody>
</table>

"Rats who survived to age 12 days."
Two animals developed marked proptosis due to secondary glaucoma and enlargement of the globe. In two animals who were not enucleated, the affected eyes eventually became shrunken with completely opaque white corneas.

Four eyes were enucleated from four animals. Two eyes had already begun to atrophy at the time of enucleation. Aspirated aqueous from each eye was sterile, and neither aspirate had detectable type b polysaccharide antigen. The other two eyes were still clinically acute. Type b antigen was detectable in the aspirated aqueous of both eyes, and one specimen yielded *H. influenzae* type b on culture.

The histopathology of the four specimens revealed the lesions to be in varying stages of development, allowing the formulation of a sequence of histopathological changes. In the earliest stage of infection examined, the main lesion was an acute suppurative iridocyclitis (Fig. 2). The iris and ciliary body were swollen due to capillary congestion, edema, and infiltration by numerous polymorphonuclear neutrophilic leukocytes. Neutrophils were also seen in the aqueous at this stage. The lens became swollen and cataractous and developed focal loss of capsular basement membrane (Fig. 3). The cornea was minimally involved in the
Fig. 3. Another portion of the anterior segment from the same eye shown in Fig. 2, demonstrating changes in the lens (periodic acid-Schiff stain, x67).

early lesion, with scattered neutrophils in the limbal stroma. Small amounts of pus were present in the vitreous. The retina and choroid were not involved at this stage, except for capillary congestion of the retinal and choroidal vessels, with leukocyte margination and perivascular accumulations of polymorphs.

As the lesion progressed (Fig. 4), a diffuse suppurative panophthalmitis developed. The anterior chamber became filled with pus, and the angle was completely filled with fibrin and leukocytes. The corneal stroma was also heavily infiltrated by neutrophils. The lens developed numerous capsular breaks through which extruded cataractous lens material. The retina was detached by a pool of edema fluid and neutrophils (Fig. 5). Frank dissolution of the retina was occurring in several foci. The choroid was diffusely infiltrated by neutrophils. At this stage, extracellular gram-negative bacteria could be visualized (Fig. 6).

As the lesion organized (Fig. 7), the anterior chamber was obliterated by granulation tissue which formed anterior and posterior synchiae. Numerous plasma cells were apparent. The iris and ciliary body were no longer recognizable. The lens epithelium was markedly proliferative. The vitreous space was filled with cellular fibrous tissue and hemosiderin-laden macrophages. Only fragments of retina were recognizable, and these were surrounded by an organized inflammatory exudate. Further organization of the lesion ultimately led to a shrunken and totally disorganized eye (phthisis bulbi).

Second experiment. This experiment was designed to see if the lesions observed previously were reproducible and to establish the route of eye infection. All 41 5-day-old rats challenged i.p. were bacteremic at 48 h postchallenge, but only 10 survived to the age of 10 days (Table 1). Seven of these 10 developed endophthalmitis. Twenty-nine of 73 (40%) rats challenged i.n. were bacteremic at 48 h postchallenge. Twenty-two bacteremic rats survived to age 10 days, of which 11 developed endophthalmitis. In all, there were 18 animals with endophthalmitis in the second experiment with 26 affected eyes. Ocular disease was apparent at the time of eye opening in 16 of 18 rats. One rat developed disease at age 16 days and one rat developed disease at age 18 days. No clinical disease was detected thereafter. All affected eyes displayed some degree of anterior chamber clouding. Seven eyes also had severe proptosis due to secondary glaucoma. Nine eyes had dense white opacities due to a cataractous lens.
and were already beginning to atrophy.

*H. influenzae* type b was recovered from the aqueous in 24 (92%) of the 26 affected eyes. The two eyes with negative cultures had type b antigen in the aqueous. Six of the seven rats with endophthalmitis who were inoculated i.p. had negative blood cultures at the time of enucleation. However, eight of eleven rats with endophthalmitis who were inoculated i.n. had positive blood cultures at the time of enucleation. This difference is statistically significant (*P* < 0.05 using a two-tailed Fisher's exact test). All animals with endophthalmitis had type b polysaccharide antigenemia at the time of enucleation. All cultures and tests for type b antigen were negative in the aspirated aqueous of clinically unaffected eyes, including the contralateral eye of rats with unilateral endophthalmitis (tested at age 12 days) and the eyes of rats who never developed clinical endophthalmitis (tested at age 1 month).

Histopathological examination showed that all clinically affected eyes had pathological evidence of endophthalmitis. There were two animals with clinically unilateral disease who had histological evidence of bilateral endophthalmitis. None of the rats without clinical disease who were followed until age 1 month had any pathological evidence of active or healed endophthalmitis.

The extent of pathological changes observed in affected eyes varied. Seventeen of 26 affected eyes had extensive disease involving all internal ocular structures. In most of these eyes, no distinction in intensity of inflammation was apparent between the anterior and posterior segments. In a few eyes, the posterior segment seemed more severely affected with extensive vitreous abscess formation and chorioretinitis and less severe iridocyclitis. In the other nine eyes, inflammatory changes were less extensive, but anterior and posterior structures were equally affected.

The composition of inflammatory infiltrate also varied. Eleven of 26 eyes had an equal mixture of neutrophils and fibrin with hemosiderin-laden macrophages, plasma cells, and early granulation tissue. Ten eyes had a predominantly purulent inflammation, although early organization was apparent in all. Five eyes were predominantly in a stage of organization, although some residual acute inflammation was still present. The more extensively involved eyes generally had the greater content of acute inflammatory cells.

The optic nerve was identified in four af-
FIG. 5. High-power view of the retina and choroid of the eye in Fig. 4. The retina (large arrow) is detached from the choroid (small arrow) by a pool of edema fluid and leukocytes, predominantly neutrophils (hematoxylin and eosin stain, x410).

FIG. 6. Extracellular gram-negative coccobacilli (arrows) and neutrophils are present in the edema fluid separating the retina and choroid (Brown and Brenn, 1,480).
fected eyes. No acute or chronic inflammation was apparent in the subarachnoid space in any of these specimens. Six of eighteen animals with endophthalmitis had histological evidence of concomitant healing meningitis, which was characterized by a minimal chronic inflammatory infiltrate at the base of the brain around the pons. Five other animals had chronic inflammatory cells in the endolymphatic space of the cochlea, suggesting prior meningitis. There was no histological evidence of acute rhinitis or sinusitis. There was no meningitis or otitis interna in any of the animals who failed to develop eye disease when examined at age 1 month.

DISCUSSION

This study demonstrates that acute endogenous suppurative endophthalmitis can be produced by either i.n. or i.p. inoculation of *H. influenzae* type b in 5-day-old rats. These results extend the earlier observations of Smith et al. (33) and Moxon et al. (24) showing that endophthalmitis is reproducible and represents a form of metastatic infection due to actual seeding of the eye by *H. influenzae* type b, probably secondary to bacteremia. Other possible routes of ocular infection seem much less likely. Direct spread along the optic nerve via the subarachnoid space as a complication of meningitis is unlikely, since none of the rats who developed endophthalmitis had active leptominusingitis at the time when endophthalmitis was acute, and no suppuration was found around the optic nerve in the four eyes in which the nerve was examined histologically. Spread to the orbit as a complication of sinusitis is also unlikely since there was no histological evidence of rhinitis or sinusitis, and rats infected i.p. developed endophthalmitis as frequently as those infected i.n.

In the second experiment, both the i.p. and i.v. routes produced disease in about half of bacteremic rats who survived to the age of 12 days. The mortality from i.n. induced bacteremia (24%) is considerably less than from i.p.-induced bacteremia (75%). But this advantage was counterbalanced by the fact that bacteremia only occurred in about 40% of rats inoculated i.n. compared with 100% of rats inoculated i.p. The ratio of affected animals to the total originally challenged is thus about the same for either route of inoculation. Of course, the true incidence of ocular disease may have been higher for either or both groups, since some of the animals who died before eye opening could have had endophthalmitis.

Rats inoculated i.n. who developed endo-
phthalmitis had more prolonged bacteremia than rats inoculated i.p. One explanation for this difference may be that i.p. inoculation results in higher grade bacteremia shortly after inoculation, and only animals who can more rapidly clear bacteria from the bloodstream survive to age 12 days. Intranasal inoculation may result in less intense bacteremia, allowing even animals who fail to rapidly clear bacteremia to survive. Indeed, Smith et al. (33) found that rats made bacteremic by i.p. inoculation had $7 \times 10^8$ bacteria/ml of blood at 24 h after inoculation, whereas Moxon et al. (24) found that bacteremic rats inoculated i.n. had about $6 \times 10^4$ bacteria/ml of blood at 24 h.

Previous animal models of endogenous bacterial endophthalmitis have utilized streptococci (3, 13, 28) and various organisms isolated from infected teeth (10, 32). All of these investigators used rabbits and none were able to produce disease as often or as consistently as in the present study. The major disadvantage of the present model is the necessity for using infant rats. The small eyes of these animals are not as easily observed or manipulated with the detail that one can use with the eyes of adult rabbits. Schnearson and Robbins (29) have demonstrated that 4- to 6-week-old rabbits are susceptible to intravenous infection with \textit{H. influenzae} type b. None of their animals, however, were reported to have eye disease, although no eye cultures or histopathology was done.

Several aspects of this model remain to be studied. The time course of the infection must be examined and will require the histopathological examination of eyes before the time when clinical evaluation is possible. Such studies may allow us to answer whether the initial lesion is in the anterior uveal tract (iridocyclitis) or in the posterior uveal tract (choroiditis) and/or retina. Our initial observations suggested that iridocyclitis (Fig. 2) was probably the initial event. However, subsequent observations suggested that the posterior structures of the eye may be initially infected. Other experimental models have given varied results on this subject. The experiments using streptococci (3, 10, 13) generally produced iridocyclitis. However, experimental models using organisms other than bacteria, e.g., \textit{Candida albicans} (7) or \textit{Toxoplasma} and \textit{Besnoitia} (9), have produced choroidal lesions almost exclusively.

Finally, the most obvious application of this model to the study of human disease would appear to be its potential usefulness in studying the role of antibiotics in endogenous ocular infection. This model would allow the study of antibiotic penetration into normal and diseased eyes without having to cause intraocular infection by exogenous inoculation of the eye (20, 31).

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\section*{LITERATURE CITED}


