Experimental Guinea Pig Ocular Infection by Salmonella typhimurium

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The clinical, microbiologic, and cytologic features of the guinea pig model of keratoconjunctivitis with enterobacteria, *Salmonella typhimurium* were elucidated. Guinea pig eyes were instilled with *S. typhimurium* and the eyes were studied by biomicroscopy, culture, cytology, pathology, and electron microscopy. All animals developed moderate to severe conjunctivitis that was present in 18% of the animals on day 1. It became more intense, appearing in all of the eyes on day 10 and disappeared before day 30. The cultures for *S. typhimurium* were almost all positive on days 1 and 2, declined steadily to 10% on day 10, and were negative after that. A coarse, epithelial punctate keratitis was present in more than 90% of the infected eyes at some time during the experiment. The keratitis had a biphasic clinical course. The first peak correlated with the maximum culture results, but during the second peak only 10% of the cultures were positive. Electron microscopy of the cornea showed the *S. typhimurium* at the epithelial surface within surface epithelial cells during the early phases of infection. The later phase keratitis, with negative culture results, resembles the keratitis of Reiter’s syndrome. Invest Ophthalmol Vis Sci 26:591-594, 1985

*Salmonella* is a pathogenic agent that can affect the eye as a complication of septicemia, especially in infants with leukemia.1 It is also reported to be one of the agents that can initiate Reiter’s syndrome.2

In 1957, Serény reported that the inoculation of *Shigella* in the guinea pig eye was followed by acute conjunctivitis and keratitis.3 Later it was demonstrated that other enteric organisms had the ability to provoke an inflammatory reaction in the guinea pig eye. It was then determined that *Salmonella, Yersinia enterocolitica* and enteroinvasive *E. coli* could cause conjunctivitis or keratoconjunctivitis.4,5 However, the keratoconjunctivitis caused by enteric organisms in the guinea pig has not been studied by ophthalmologic or by microscopic examination.

The purpose of this article is to describe the guinea pig eye infection induced by *S. typhimurium*, using biomicroscopy, culture, cytology, pathology, and electron microscopy (EM).

**Materials and Methods.** Inoculum: A lactose fermenting strain of *S. typhimurium* which is endemic in Sào Paulo city6 was used. The strain was grown on tryptic-soy-agar slants at 37°C for 20 hr. The growth was harvested in saline and the suspension was adjusted to 10⁸ bacteria/ml of saline. Then 0.1 ml of this suspension was instilled in both eyes of the guinea pigs.

**Animals and procedures:** Forty adult albino guinea pigs (250–350 g) were used in this study in accordance with the ARVO Resolution on the Use of Animals in Research. All eyes were biomicroscopically normal and had negative cultures for enterobacteria prior to the experiment. Nine guinea pigs received saline in both eyes and were used as controls. The remaining 31 guinea pigs had both eyes infected. Four of these animals were killed 3, 7, 12, and 24 hr after inoculation, and both corneas of each animal were studied by electron microscopy. Two animals were killed on days 0, 1, 2, 4, 7, 10, 14, 17, 24, and 30 hr after inoculation, and their eyes were studied histologically (hematoxylin-eosin and Gram-Weigert). Before they were killed, the eyes were examined by slit lamp after instillation of fluorescein, and by bacteriologic culture and cytologic (Giemsa) examination of conjunctival smears. The bacteriologic examination included gram-staining of the conjunctival scrapings and plating of the ocular secretion on MacConkey agar and blood agar base, with 5% of sheep blood. The conjunctival response was graded from 0 to 4, according to the severity of hyperemia, edema and discharge. The bacterial colonies observed on the plates were identified by biochemical and serologic methods.

**Results.** All infected eyes developed moderate to severe conjunctivitis with hyperemia, edema and discharge. The frequency of the conjunctivitis is shown in Figure 1. It was present in some animals on day 1, was most intense and frequent on day 10, and disappeared before day 30.

A coarse, epithelial punctate keratitis (Fig. 2) was present in 66% of the infected eyes on day 10 (Fig. 1). More than 90% of the infected eyes showed the keratitis at some time during the experiment, usually between days 7 and 14. Some animals showed keratitis on days 1 and 2 but it was more frequent on day 10. In most eyes the keratitis followed a biphasic clinical course, disappearing after 3 days and recurring a short time later. It disappeared before 24 days (Fig. 1). Intracorneal inflammation was never found. There was no corneal vascularization.

Neither conjunctivitis nor keratitis was seen in the control eyes.

Bacteriologic cultures showed the percentage of eyes with positive growth for *S. typhimurium* as follows: day 1, 100%; day 2, 100%; day 4, 96.4%; day 7, 66.6%; day 10, 10%; and 0% on days 14 to 30 (Fig. 1). The examination of the conjunctival scrapings stained by gram and giemsa showed gram-negative..
bacilli inside or adherent to epithelial cells on days 1, 2, and 4. The gross number of epithelial cells increased from day 1 to 4 and polymorphonuclear leucocytes increased until day 10.

The histologic studies showed a subconjunctival inflammatory infiltration characterized by PMN until day 7 and by mononuclear cells from day 7 to 30.

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**Fig. 1.** Percentage of eyes with conjunctivitis, keratitis, and positive culture on different days of observation.

**Fig. 2.** Experimental *S. typhimurium* epithelial keratitis on day 10 (after staining).

**Fig. 3.** Surface of the infected cornea 12 hr after the inoculation. The arrow shows the bacteria adhered to the microprojections and inside vacuoles in the first cellular row of the epithelium. *Inset,* the arrow indicates a sectioned bacteria adhered to the cornea surface (bar = 0.5 μm).
Fig. 4. The arrow indicates a partially degenerated bacteria inside a vacuole in the first cellular row of the epithelium 12 hr after the inoculation (bar = 0.5 μm).

Studies of the corneal epithelium by transmission electron microscopy showed the \textit{S. typhimurium} at the epithelial surface and inside some epithelial cells. Some bacteria present at the surface appeared to be adherent to the cell surface (Fig. 3). The intraepithelial bacteria showed different phases of lytic changes in cells whose general architecture was normal (Fig. 4).

**Discussion.** This study showed that \textit{S. typhimurium} causes a keratoconjunctivitis in guinea pig eyes during which inoculated eyes develop a moderate to severe conjunctivitis, with highest frequency on day 10 and disappearing before day 30. This is accompanied by a coarse, epithelial punctate keratitis without corneal vascularization. In more than 90% of the infected eyes it lasts up to 10 days, and disappears before 3 weeks without leaving any sequel. It has a biphasic clinical course. The first peak correlates with the maximum number of bacteria recovered in culture and with the largest numbers of bacteria in scrapings. In the second peak, however, only 10% of the cultures are positive and no \textit{Salmonella} occur in scrapings at this time. This late, postinfectious phase suggests that there is a process, similar to that suspected to exist in Reiter's syndrome, in which a noninfectious keratoconjunctivitis is common. It is known that \textit{S. typhimurium} is one of the several agents that appear to initiate this disease.\textsuperscript{2} The persistence and worsening of the keratitis, in spite of the negative culture results, could perhaps be related to a toxic or immunologic reaction to bacterial debris such as peptidoglycan and lipopolysaccharides.\textsuperscript{7}

We stress that it was possible to diagnose the keratitis only by slit-lamp examination with fluorescein staining. The clinical picture is totally different from that of the necrotizing keratitis which follows the inoculation of guinea pig eyes with other Enterobacteriaceae such as \textit{Shigella}.\textsuperscript{3,4} This may explain the negative results obtained with \textit{Salmonella} in previous studies where the eyes were not ophthalmologically examined.\textsuperscript{4,5}

It has been known that \textit{Salmonella} can be found inside conjunctival and intestinal epithelial cells,\textsuperscript{8} but this is the first time that this bacterium has been reported adherent and inside corneal epithelial cells. A few bacteria such as \textit{Listeria monocytogenes} has been described inside corneal epithelial cells.\textsuperscript{9,10}

\textbf{Key words:} \textit{Salmonella typhimurium}, ocular infection, keratitis, conjunctivitis, Reiter's syndrome, bacterial adherence, corneal epithelial phagocytosis
From Escola Paulista de Medicina, Disciplinas de Oftalmologia, Microbiologia, Anatomia Patológica e Anatomia Descritiva, São Paulo, Brasil. Supported by grants provided by CEO Moacyr Alvaro, CNPq, and FINEP. Submitted for publication: October 27, 1983. Reprint requests: Rubens Belfort Jr, MD, Caixa Postal 4086 São Paulo, Brasil.

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