

# Neonatal inclusion conjunctivitis in Australia

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Trachoma has been known to occur in Australia since the latter part of the 19th century. Laboratory confirmation was first made by Rodger and Priestley (1915), who studied cases of trachoma in Western Queensland and found characteristic inclusions within conjunctival epithelial cells. This work was reported 8 years after the original description of cell inclusions in trachoma by Halberstaedter and von Prowazek (1907). Although previously common, the infection has now largely disappeared from the white population, but a large reservoir of infection continues to exist among the Aborigines, as shown by investigations conducted in Western Australia (Mann, 1955, 1960), the Northern Territory (Flynn, 1957), and South Australia (Moore, Howarth, Wilson, Derrington, and Surman, 1965; Hardy, Surman, and Howarth, 1967).

The trachoma agent was first isolated by T'ang, Chang, Huang, and Wang (1957) in Peking. These workers used embryonated eggs, inoculated by the yolk sac route and then incubated at 35°C. This work was confirmed by Collier and Sowa (1959) in London. Subsequently, isolations were made from patients with trachoma in Australia by Perret and Mann (1960) in Perth, and by Howarth and his colleagues in Adelaide (Howarth, 1966). These workers found a high incidence of infection among Aboriginal children in some districts of Western Australia and South Australia.

Inclusion conjunctivitis is due to a micro-organism which by laboratory methods is indistinguishable from that which causes trachoma, and identical inclusions are produced within conjunctival epithelium. The causative agents of these infections have been grouped together as TRIC agent. Inclusion conjunctivitis occurs in newborn infants and the infection is usually derived from the cervix of the mother during birth. Sometimes children and adults are infected, usually from contaminated water in swimming pools.

Neonatal inclusion conjunctivitis has not been reported from Australia, despite attempts to demonstrate this infection. However, recent evidence obtained at this hospital suggests that inclusion conjunctivitis does occur. During a study of conjunctivitis in newborn babies, cases of severe purulent ophthalmia were seen, from which cultures for bacteria yielded negative results, but further investigations revealed the presence of typical inclusions within conjunctival epithelial cells (Hansman, 1969). This article describes further cases; in most of these infants it has been possible to follow the course of the infection.

## Material and methods

Specimens of conjunctival exudate were collected by heat-sterilized cotton-wool swabs and inoculated on plates of blood agar and heated blood agar. In most cases specimens were plated immediately

after collection. Cultures were incubated in a jar with added "Carbogen" for at least 2 days. The criterion for the diagnosis of bacterial conjunctivitis was significant growth of a potential pathogen.

Conjunctival smears were collected with a stainless steel ophthalmic spud and films prepared on glass slides which had been previously cleaned in a mixture of potassium dichromate and sulphuric acid. After air-drying, the smears were fixed with methanol for 3 minutes and then stained overnight with dilute Giemsa. After staining, the smears were washed in running tap water, dried in an incubator, and mounted before examination. As evidence of inclusion conjunctivitis, conjunctival smears were examined for the presence of basophilic, "initial body" inclusions, and Halberstaedter-Prowazek (HP) inclusions.

When possible, cervical smears were collected from the mother of the affected infant and these were fixed and stained by the method described.

## Results

From February to May, 1968, a period of 4 months, nine cases of severe purulent conjunctivitis were observed, in which bacterial pathogens could not be implicated. The findings are summarized in the Table. The conjunctivitis was unilateral in four cases and affected both eyes in five. In three infants both eyes were involved simultaneously, but in two (Cases 3 and 7) the conjunctivitis was at first unilateral with intervals of one and 8 days, respectively, before the contralateral eye was affected.

**Table** Summary of nine cases

Case no.	Age at onset (days)	Distribution	Treatment before collection of conjunctival smears	Giemsa-stained conjunctival smear
1	6	Bilateral	"Neosporin" Penicillin Chloramphenicol	HP inclusion*
2	6	Bilateral	"Neosporin"	Inclusions not seen
3	4	Bilateral	None	HP inclusions
4	5	Bilateral	"Neosporin" Chloramphenicol	Inclusions not seen
5	7	Unilateral	"Neosporin"	HP inclusions
6	2	Unilateral	"Neosporin"	Inclusions not seen
7	6	Bilateral	"Neosporin"	HP inclusion
8	8 (see text)	Unilateral	"Neosporin"	Basophilic inclusions
9	6	Unilateral	"Neosporin" Chloramphenicol	Basophilic and HP inclusions

\*HP = Halberstaedter-Prowazek

Conjunctival smears showed numerous neutrophils with many mononuclear cells which resembled monocytes and lymphocytes; degenerate leucocytes were often found in large numbers. HP inclusions were detected in smears from four cases, basophilic inclusions in one, and both types of inclusion in specimens from another infant. In three cases, despite a careful search, inclusions were not seen, so that the cause of the conjunctivitis in these babies must remain in doubt. The age at onset in the six inclusion-positive cases was from 4 to 8 days.

Cervical smears were collected from five of the mothers and examined for inclusions, all with negative results. Examination of conjunctival smears from the infants of these mothers had revealed inclusions in two cases.

## Representative cases

### Case 1

This infant developed conjunctivitis at the age of 6 days, and the condition was at first treated with "Neosporin" drops. Pathogenic bacteria were not isolated. On the next day the baby was stated to show bilateral severe conjunctivitis with oedema and erythema of the lids. Treatment was changed to penicillin. After another 2 days, penicillin was stopped and chloramphenicol administered. At the age of 10 days, after treatment with chloramphenicol for one day, there was slight exudate, lid oedema, and chemosis. Conjunctival smears, collected at this stage, showed numerous neutrophils and some mononuclear cells which resembled lymphocytes and monocytes; an epithelial cell containing a typical HP inclusion was detected (Fig. 1). The conjunctivitis slowly improved. At the age of 13 days, moderate chemosis and slight purulent exudate were found. The infant was discharged from hospital at the age of 18 days. The infant's mother was an unmarried girl of 18 years.

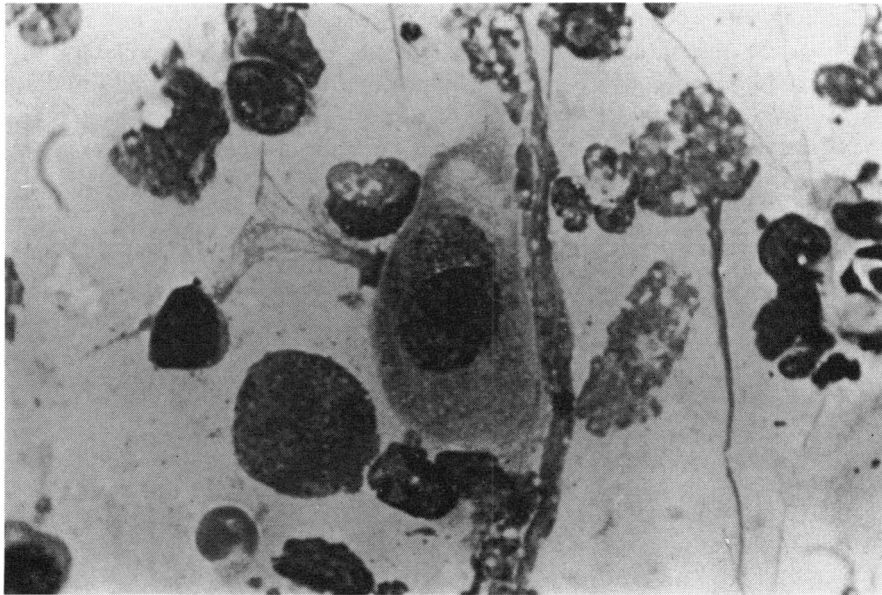


FIG. 1 Case 1 Conjunctival smear stained with Giemsa: epithelial cell containing Halberstaedter-Prowazek inclusion.  $\times 960$

### Case 7

This infant developed unilateral purulent conjunctivitis of the right eye at the age of 6 days. Pathogenic bacteria were not isolated. After treatment with "Neosporin" for one day, the conjunctivitis was worse and the eye showed marked lid oedema and moderate chemosis. Conjunctival smears stained with Giemsa showed "initial body" and "intermediate" inclusions (Fig. 2, opposite) and a "burst" HP inclusion with free elementary bodies. Intermediate inclusions are the stage of development of the TRIC agent between basophilic and HP inclusions.

After treatment with tetracycline ointment for one day, the conjunctivitis was unchanged. Examination of conjunctival smears failed to reveal inclusions. Treatment was changed to chloramphenicol, and after 2 days there was marked improvement.

Eight days after the onset of conjunctivitis, the left eye was involved, showing moderate purulent exudate and chemosis of the lower lid, but the inflammatory changes were not as severe as those seen in the right eye and improved rapidly. The infant was discharged from hospital at the age of 17 days.

The infant's mother was an unmarried girl of 17 years; cervical smears collected 13 days after delivery were examined for inclusions with a negative result.

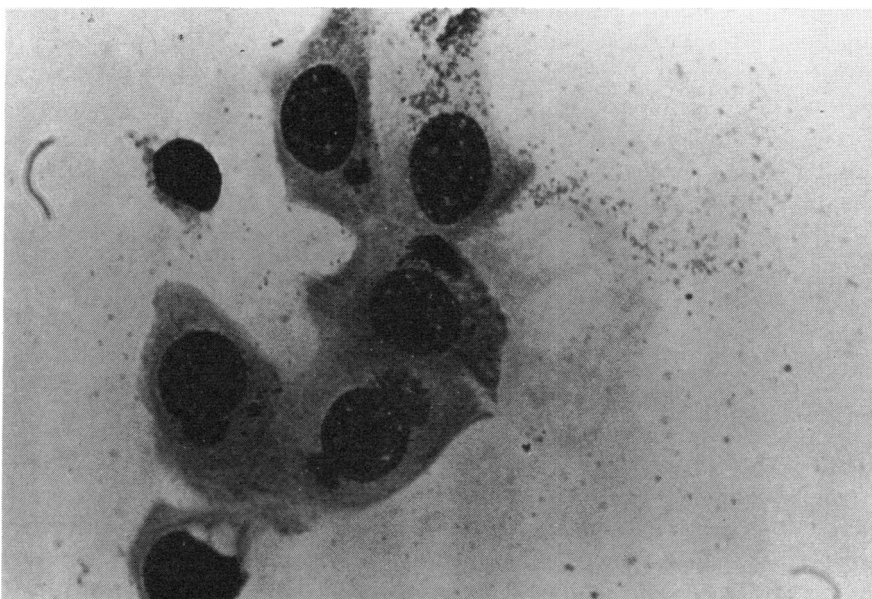


FIG. 2 Case 7 Conjunctival smear stained with Giemsa: group of epithelial cells, some containing initial body and intermediate inclusions, also a "burst" Halberstaedter-Prowazek inclusion with free elementary bodies.  $\times 960$

### Case 8

This infant developed mild conjunctivitis on the day of birth; bacteriological cultures yielded no growth. The conjunctivitis was treated with "Neosporin" for 8 days, and the infant then showed unilateral purulent conjunctivitis with moderate lid oedema and marked chemosis. Cultures were again negative and conjunctival smears stained with Giemsa showed basophilic inclusions. Treatment was changed to chloramphenicol drops which produced a marked improvement after one day, although chemosis of the lower lid persisted for at least a week. The infant was discharged from hospital when aged 17 days.

The infant's mother was an unmarried woman aged 25 years. It was considered that the mild initial conjunctivitis was probably not due to TRIC agent infection, which usually has an incubation period of 6 to 8 days. However, Sowa, Sowa, and Collier (1968) reported the case of a neonate who developed TRIC agent infection at the age of one day.

### Response to treatment

During this investigation, all cases of conjunctivitis were initially treated with "Neosporin" drops (which contain a mixture of three antibiotics: neomycin, polymyxin B, and gramicidin). If the conjunctivitis failed to improve, treatment was changed to chloramphenicol or penicillin drops. In none of the nine cases did the conjunctivitis respond to "Neosporin" and in five (including three of the inclusion-positive cases) the degree of inflammation increased during treatment. Six of the infants were subsequently treated with chloramphenicol and one with penicillin; one was treated with penicillin and subsequently with chloramphenicol; and another with tetracycline and then chloramphenicol. All responded, but improvement was not usually evident until at least 2 days' treatment had been given.

After the acute phase of inflammation had subsided, mild conjunctivitis, manifest by slight exudate from the eye and thickening of the conjunctiva of the lower lid, often persisted. Some cases showed transverse ridging or corrugation of the lower lid conjunctiva, an appearance similar to that described by Allen (1944). In two infants apparent healing had occurred 7 days after onset of the conjunctivitis, but the others showed slower resolution of infection. The mean period in hospital after conjunctivitis began was 26 days, and all except one had recovered sufficiently to be discharged from hospital after 5 weeks. Most of the babies (6/9) were adopted and this tended to increase the duration of their stay in hospital.

In most cases it was not possible to collect conjunctival smears before treatment was begun. Inclusions were found despite previous treatment with "Neosporin" (Cases 5, 7 and 8), "Neosporin" and chloramphenicol (Case 9), and "Neosporin", penicillin, and chloramphenicol (Case 1).

### Discussion

In all cases the babies were born to parents of European descent. Inclusion conjunctivitis has not been observed in Aboriginal babies, only small numbers of whom are born at the hospital where the investigation was carried out. It would appear to be of some importance to establish whether inclusion conjunctivitis is restricted to Europeans in Australia. Inclusion conjunctivitis is said to be unknown among American Indians, while trachoma is common among Indians living upon reservations in the south-west of the United States.

All but one of the infants in this study were born to young unmarried women, whose mean age was 19 years (range 16 to 25). Because of this it was difficult to arrange for the collection of cervical smears after delivery, and we were unable to re-examine the infants after discharge from hospital. It has usually been accepted (Thygeson, 1934; Thygeson and Stone, 1942) that complete and permanent healing occurs in neonatal inclusion conjunctivitis. However, recent evidence (Freedman and ten others, 1967; Watson and Gairdner, 1968) suggests that in some cases corneal involvement, with formation of pannus, and conjunctival scarring develop. Follow-up of these infants is therefore desirable.

Cervical smears from five of the mothers were examined but inclusions were not found. Inclusions may be difficult to find in such material and even when both microscopy and egg inoculation techniques are used negative results are common in women who have given birth to infants with proven infection (Hanna, Zichosch, Dawson, Thygeson, and Jawetz, 1962; Sowa and others, 1968).

Clinically, it is not possible to distinguish inclusion conjunctivitis from severe bacterial conjunctivitis. Although the laboratory diagnosis may be made presumptively on negative bacteriological results, the demonstration of inclusion bodies is essential for a certain diagnosis.

### Summary

Until 1969, neonatal inclusion conjunctivitis had not been described from Australia. Nine cases of purulent conjunctivitis in newborn babies, from whom bacterial pathogens were not isolated, are reported. In six of the nine cases, basophilic or Halberstaedter-Prowazek inclusions were found within conjunctival epithelial cells. All of the babies were born to parents of European descent.

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