CHLAMYDIAL INFECTIONS

LENNOX KANE

INTRODUCTION

Halberstaedter and von Prowazek (1907) described intracytoplasmic inclusions in the conjunctival scrapings of orang-utans that had been infected with material from patients with trachoma. Subsequently, they described similar inclusions in conjunctival epithelial cells from infants with ophthalmia neonatorum (Halberstaedter and von Prowazek, 1909) and these findings were confirmed by others. Hayman (1910) observed inclusions in cells from the cervical smear of the mother, and the urethral smear of the father, of an infant suffering from what today is known as inclusion blenorrhoea. The inclusions were thought to be a form of the organism responsible for the disease (Schachter and Dawson, 1978). The name "chlamydia" originated from the description of Halberstaedter and von Prowazek (1907) of "mantled animals". There is, in fact, no mantle but the name chlamydia has remained.

Chlamydiae are ubiquitous organisms and today account for the majority of non-specific genital infections.

TAXONOMY AND GROWTH CYCLE

The chlamydiae were, formerly, classified as *Rickettsiae*, being very small, immobile, spherical or rodlike parasitic organisms which resemble bacteria, which, like viruses, could not reproduce outside the cells of the host. All psittacosis – lymphogranuloma – trachoma (PLT) agents are now gathered under one genus – Chlamydia (Page, 1966).

The chlamydial growth cycle is complicated and much is unknown. The infectious particle is the small elementary body (0.3 μm): it is metabolically inactive, and has a hard rigid envelope which is similar to that of Gram-negative bacteria. It is taken into the host cell by phagocytosis, reorganising to form, within twelve hours, the initial or reticulate body (0.5-1 μm), which is intracellular and metabolically active.

This particle grows rapidly, within a membrane-bound vacuole divided from the host cell surface membrane. It divides repeatedly by binary fission to form many reticulate bodies which eventually organise into the infectious elementary bodies. Within 48 to 72 hours the host cell bursts, freeing the infectious bodies into the extracellular space ready to invade other cells (Taylor-Robinson, 1980).

LATENCY AND INAPPARENT INFECTIONS

Latency, or the persistence of the organism in an inapparent infection, is characteristic of chlamydial infections. The agent of lymphogranuloma venereum (LGV) persist in the human host and may become demonstrable many years after

the initial infection (Siegal, 1962). People who have left trachoma-endemic areas and who have not had *C. trachomatis* infection since childhood, may develop trachoma in their sixties and beyond (Thygeson, 1963).

Jawetz *et al.* (1967) suggested that the incidence of sub-clinical trachoma is high in endemic areas. Inclusion conjunctivitis is usually a mild and self-limiting infection, although the active disease may persist in chronic form. Chlamydiae may prove difficult to isolate or to observe in such chronic infections but administration of cortisone (Grayston and Wang, 1975) or trauma (Hanna *et al.*, 1968) has sometimes reactivated the infectious process resulting in detectable organisms. The same consideration may apply to the genital tract (Jones, 1964; 1977), sub-clinical salpingitis persisting to be reactivated later. Chlamydiae are obligatory intracellular parasites and do not persist on mucous membranes or cell surfaces (Mordhust, 1967), nor as extracellular elementary bodies in the intact non-replicatory form (Schachter and Dawson, 1978). Inapparent infection reflects, perhaps, the presence of intracellular reticulate bodies, checked by host defence mechanisms at low levels of multiplication (Lee and Moulder, 1981). Chlamydiae may exist within cells in cryptic form (Moulder *et al.*, 1980) being converted periodically into reticulate bodies that multiply and differentiate into infectious elementary bodies in a conventional growth cycle.

DIAGNOSIS

Chlamydiae may be detected in smears from scrapings of epithelial cells, by culture, and by serological methods.

Detection of Inclusions in Smears

The intracellular inclusions of *C. trachomatis* differ from those of *C. psittaci*. They are compact, contain synthesised glycogen and, thus, are stained by iodine. Staining with dilute Lugol's iodine solution is simple, rapid and inexpensive, but is relatively insensitive and suitable only for field studies. Giemsa also stains *C. trachomatis* inclusions and is twenty per cent more sensitive than iodine. The staining properties of chalmydiae with Giemsa differ with the stage of development, elementary bodies staining purple, initial bodies staining blue, and fully formed intracellular inclusions staining deep purple. Thus, interpretation is skilled and the examination of many fields is tedious (Grayston and Wang, 1975). The fluorescent antibody (FA) staining technique is an immunochemical method which stains inclusions specifically. It requires immediate fixation in cold acetone and storage at −60 °C (Darougar *et al.*, 1970; Darougar, 1975). It is more sensitive than Giemsa's stain and may be suitable for study of specimens from the genital tract but it requires expensive reagents, specialised fluorescent microscopes and skilled microscopists. It has been used successfully in field studies to demonstrate trachoma (Darougar *et al.*, 1980). The application of human LGV antibody followed by fluorescein-labelled anti-human globulin to stain inclusions in McCoy cells inoculated

with urethral or cervical swabs, promises rapid diagnosis of genital *C. trachomatis* (Thomas *et al.*, 1977) but has yet to be evaluated thoroughly. Simplified methods yielding acceptable isolation rates are available (Harper *et al.*, 1982).

CULTURE

Inoculation of the yolk sac of embryonated hens' eggs has been used to isolate *C. trachomatis* from genital specimens, having been used first by Bedson *et al.* (1930) to culture *C. psittaci*. *C. trachomatis* was first isolated by T'ang *et al.* (1957) and subsequently by Collier and Sowa (1958). Culture in fertile hens' eggs was replaced by monolayer cell culture of the McCoy cell line which was derived from human synovial fluid. McCoy cells are made non-replicating by irradiation with 4,500 rads from a cobalt source, four to five days before use. This inhibits division but allows the cytoplasm to increase in size so that the inclusions can be identified easily (Gordon and Quan, 1965). Taylor-Robinson and Thomas (1980) reviewed nineteen different methods of culture; all are modifications of the basic technique.

Irradiation of McCoy cells followed by centrifugation at 2,700 g for one hour increases their ability to take up chlamydial inclusions and thus improve the yield (Darougar *et al.*, 1970). Since 1972, chemical inhibitors of cell metabolism have been used as an alternative to irradiation; these include idoxuridine (Wentworth and Alexander, 1974), cytochalasin B (Sompolinsky and Richmond, 1974) and cyclo-heximide (Ripa and Märdh, 1977).

Serological Methods

The complement fixation test detects antibody to the heat-stable lipoprotein carbohydrate antigen common to all members of the genus *Chlamydia*. This test is used routinely in the diagnosis of lymphogranuloma venereum (LGV) and psittacosis as these infections are generally systemic and thus produce high titres. Sensitivity is low in superficial chlamydial infections, where the antigenic stimulus is slight and subsequent antibody response is poor. In men with proven chlamydial infections of the genital tract, complement fixation antibody titres rarely exceed 1:8 or 1:16 but in patients with lymphogranuloma venereum titres above 1:256 are obtained (Schachter, 1977). Complement fixation detects group antigens shared by all members of the genus and is of little value as a sero-diagnostic aid in chlamydial oculogenital infections. Fifteen immunotypes of chlamydiae are known. Type specific antigens are best detected by immunofluorescence.

The micro-immunofluorescence test was introduced by Wang and Grayston (1970) to serotype strains of *C. trachomatis*. The test is highly sensitive and specific but it is complicated. Antigen spots fixed to a slide, are overlaid with serial diluitions of serum. By the use of fluorescein conjugated anti-human globulins, specific antichlamydial antibodies can be measured in serum and in secretions from the eye or genital tract. A modification of the test was introduced by Wang *et al.* (1975), and by Treharne *et al.* (1977 a), using pooled antigens. A two-fold reduction in sensitivity was compensated for by cost reduction and a tenfold increase

in the number of sera which could be examined. Both type-specific and group-specific antibodies are detected by the test, which can be used to assess the prevalence of chlamydial infections within the general population. Rising titres are demonstrable in active infections. Stephens *et al.* (1982 a) have described the development and use of monoclonal antibodies to *C. trachomatis* using the hybridoma technique to develop over forty independent hybrid cell lines that produce antibodies to *C. trachomatis* surface antigens. Stephens *et al.* (1982 b) have also used monoclonal antibodies in a fluorescent technique to recognise species specific antigen. Inclusions were detected after eighteen hours. This interesting procedure is more sensitive than Giemsa staining. Further studies are required to evaluate this rapid method of diagnosis.

Measurement of Local Antibody

Local antibody to *C. trachomatis* was first described by Bernkopf *et al.* (1966) who demonstrated it in fluid from the conjunctival sacs of patients with trachoma. Treharne *et al.* (1978) examined the sera and cervical secretions of 277 women attending a venereal disease clinic and made a presumptive diagnosis of chlamydial cervical infection in 101 (38 per cent). The diagnosis was based on the presence of chlamydia specific IgG at a titre of $\geqq 1:64$; of chlamydia specific IgM at a titre of $\geqq 1:8$ in sera; and of chlamydia specific IgG or IgA antibodies at a titre of $\geqq 1:8$ in cervical secretions. Culture was positive in 24 out of 104 patients. Richmond *et al.* (1980) were concerned that demonstration of such levels of antibody indicates active infection.

GENITAL CHLAMYDIAL INFECTIONS

Isolation Rates of Chlamydia trachomatis

The epidemiology of non-specific genital infection gives cause for concern (British Medical Journal, 1983). Summarising fifteen studies between 1972 and 1978, chlamydiae were isolated from between 25 and 58 per cent of men with non-gonococcal urethritis: from between 4 and 38 per cent of men with gonococcal urethritis and, surprisingly, from between 15 and 81 per cent of men with post-gonococcal urethritis. Healthy controls showed a 5 to 7 per cent incidence (Kane, 1984).

Serological studies reflect these figures: more chlamydial antibody is found in patients with non-gonococcal urethritis than in those without, and titres are higher amongst those with positive culture (Bowie *et al.*, 1977; Treharne *et al.*, 1977b; Saikku and Paavonen, 1978). Specific IgM antibody may be detectable and a fourfold or greater rise in the titre of specific IgA may be obtained in cases of non-gonococcal urethritis due to *C. trachomatis*.

The culture rate in female partners of infected males lies between 47 and 68 per cent while in the partners of those who are culture negative it is 4 to 18 per cent.

The recovery rate in infections of the lower genital tract in females varies between 12 and 35 per cent in reported studies. In those presenting at gynaecolo-

gical outpatients with a vaginal discharge there is a recovery rate of between 18 and 19 per cent while the incidence in healthy controls (low risk) varies from 1 to 4 per cent. The recovery rate is higher (19 to 48 per cent) if endocervitis is present. The highest incidence is among females (high risk) with cervicitis attending venereal disease clinics and the lowest incidence among women (low risk) attending gynaecological outpatients clinics.

The prevalence of *C. trachomatis* cervical infection in pregnant women varies between 2 and 18 per cent (Harrison *et al.*, 1983).

Martin *et al.* (1982) have demonstrated that cervical *C. trachomatis* infection in pregnant women is associated with an increased risk of prematurity and still-birth, although this was not substantiated by Harrison *et al.* (1983).

Combined infection with *C. trachomatis* and *N. gonorrheae* in women presenting at venereal disease clinics varies between 32 and 63 per cent. Non-gonococcal urethritis may occur up to 3 months after the last sexual contact and chlamydiae can still be cultured (Richmond and Sparling, 1976).

Lycke *et al.* (1980) showed that the transmission of genital *C. trachomatis* was less than that of gonorrhoea. They contacted female and male consorts of patients known to have double infections due to *C. trachomatis* and *N. gonorrhoeae* and found that 45 per cent had *N. gonorrhoeae* and 23 per cent had *C. trachomatis*. This reflects the greater pathogenicity of the gonococcus compared to *C. trachomatis* or suggests that partners without overt chlamydial infection may manifest it at a later stage.

NEONATAL C. TRACHOMATIS INFECTION

Chlomydiae have been cultured from the following sites in neonates: conjunctiva, nasopharynx, middle ear (myringotomy), trachea, lung (biopsy), rectum and vagina.

Dunlop *et al.* (1966) suggested an association between maternal chlamydial cervicitis and puerperal pelvic inflammatory disease. Mordhurst and Dawson (1971) noted pelvic infection with *C. trachomatis* before and after birth in 4 of 16 mothers and Rees *et al.* (1977) demonstrated chlamydial conjunctivits in a premature baby of thirty-five weeks gestation, born by caesarean section, twelve hours after premature rupture of the membranes, showing that ascending infection was possible. Ascending chlamydial infections are not easily demonstrated in the non-gravid state.

Neonatal Inclusion Conjunctivitis is caused by *C. trachomatis* serotypes D to K. It occurs between the third and thirteenth day of life and clinical presentation varies from a mild conjunctivitis "sticky eye" to a purulent ophthalmia, particularly if secondary infection occurs. Some infants, however, show no evidence of clinical infection, despite isolation of *C. trachomatis* from the conjunctival sac.

Pneumonia

Clinically, pneumonia presents later than inclusion conjunctivitis, occurring between the fourth and twelfth weeks of life following inhalation of infected ma-

terial at birch. It is a low grade afebrile disorder presenting with partial nasal obstruction, with mucoid discharge, tachypnoea and a paroxymal "staccato" cough. Chest X-ray show hyperexpansion with bilateral diffuse interstitial and patchy alveolar infiltrates. There may be an eosinophilia. Very high IgA, IgM and IgG titres are obtained in this condition – much higher than in adult disease reflecting the initial exposure to maternal vaginal infection.

Approximately half of these infants may have had an earlier inclusion conjunctivitis. Harrison *et al.* (1983) suggest that high titres of local tear anti-chlamydial antibodies predisposes to infection. The delayed onset of this condition combined with very high anti-chlamydial antibody titres and an eosinophilia suggest that a hypersensitivity reaction exists. The condition is uncommon in the United Kingdom but "high risk" mothers should be screened for infection (Hobson *et al.*, 1983).

Treatment

Chlamydial inclusion conjunctivitis should be treated systemically to prevent possible pneumonia developing later. Saline bathing to the eye can be used. Erythromycin ethylsuccinate should be used in divided doses up to 50 mgms/hg body weight/day for 14 to 21 days. Pneumonia should be similarly treated and the parents investigated for *C. trachomatis* infection.

REFERENCES

Bedson S. P., Western G. T., Simpson S. L.: *Lancet, 1,* 235, 1930. – Bernkopf H., Orfila J., Maythar B.: *Nature, 209,* 725, 1966. – Bowie W. R., Wang S. P., Alexander E. R., Floyd J., Forsyth D. S., Pollock H. M., Lin J. S. L., Buchanan T. M., Holmes K. K.: *Journal of Clinical Investigation, 59,* 735, 1977. – *British Medical Journal Leader, 286,* 1500, 1983. – Collier L. H., Sowa J.: *Lancet, 1,* 993, 1958. – Darougar S.: "Advances in laboratory tests for the diagnosis of chlamydial infection. Application to clinical and epidemiological". In: *Genital Infections and their complications,* edited by Danielsson *et al.,* p. 97, Almqvist and Wiksell International, Stockholm, 1975. – DarougarS., Kinnison J. R., Jones B. R.: *Excerpta Medical International,* Series no. *223,* 63, 1970. Proceeding of Symposium, Boston, Mass. – Darougar S., Woodland R. M., Jones B. R., Houshmand A., Farahmandian H. A.: *British Journal of Ophthalmology, 64,* 276, 1980. – Dunlop E. M. C., Al-Hussaini M. K., Freedman A., Garland J. A., Harper I. A., Jones B. R., Race J. W., Du Toit M. S., Treharne J. D., Wright D. J. M.: *Transactions of the Ophthalmological Society of U.K., 86,* 321, 1966. – Gordon F. B., Quan A. L.: *Proceedings of the Society of Biological Medicine, 118,* 354, 1965. – Grayston J. T., Wang S. P.: *Journal of Infectious Diseases, 132,* 87, 1975. – Halberstaedter L., Von Prowazek S.: *Arbeiten aus dem Kaiserlichen Gesundheitsamte, 26,* 44, 1907. – Halberstaedter L., Von Prowazek S.: *Klinische Wochenschrift, 46,* 1839, 1909. – Hanna L., Dawson C. R., Briones O., Thygeson P., Hawetz E.: *Journal of Immunology, 101,* 43, 1968. – Harper I. A., Johnston L., Thompson R. G.: *Journal of Clinical Pathology, 35,* 365, 1982. – Harrison H. R., Alexander E. R., Weinstein L., Lewis M., Nash M., Sim D. A.: *Journal of the American Medical Association, 250,* 1721, 1983. – Heyman B.: *Berliner Klinische Wochenschrift, 47,* 663, 1910. – Hobson D., Rees E., Viswalingam W. D.: *British Medical Bulletin, 39,* 128, 1983. – Jawetz E., Hanna L., Dawson C. R., Wood R., Briones O.: *American Journal of Ophthalmology, 63,* suppl., 1413, 1967. – Jones B. R.: *British Journal of Venereal Diseases, 40,* 3, 1964. – Jones B. R.: "Discussion on non-gonococcal urethritis". In: *Nongonococcal urethritis and related infections,* D. Hobson, K. K. Holmes (Eds.), p. 51, American Society for Microbiologists, Washington D.C., 1977. – Kane J. L.: *Journal of Obstetrics and Gynaecology, 4,* 246, 1984. – Lee C. K., Moulder J. W.: *Infection and Immunity, 32,* 822,

1981. – Lycke E., Lowhagen G. B., Hallhagen G., Johannisson G., Ramstedt K.: *Sexually Transmitted Diseases*, 7, 6, 1980. – Martin D. H., Koutsky L., Eschenbach D. A., Daling J. R., Alexander E. R., Benedetti J. K., Holmes K. K.: *Journal of the American Medical Association*, 247, 1585, 1982. – Mordhurst C. H.: *American Journal of Ophthalmology*, 63, 1282, 1967. – Mordhurst C. H., Dawson C.: *American Journal of Ophthalmology*, 71, 861, 1971. – Moulder J. W., Levy W. J., Schulman L. P.: *Infection and Immunity*, 30, 874, 1980. – Page L. A.: *International Journal of Systemic Bacteriology*, 16, 223, 1966. – Rees E.: "Perinatal chlamydial infection". In: *Nongonococcal urethritis and related infections*, D. Hobson, K. K. Holmes (Eds.), p. 140, American Society for Microbiologists, Washington D. C., 1977. – Richmond S. J., Sparling P. F.: *American Journal of Epidemiology*, 103, 428, 1976. – Richmond S. J., Milne J. D., Hilton A. L., Caul E. O.: *Sexually Transmitted Diseases*, 7, 11, 1980. – Ripa K. T., Mardh P. A.: "New simplified cultures technique for *Chlamydia trachomatis*". In: *Nongonococcal urethritis and related infections*, D. Hobson, K. K. Holmes (Eds.), p. 323, American Society for Microbiologists, Washington D. C., 1977. – Saikku P., Paavonen J.: *Journal of Clinical Microbiology*, 8, 119, 1978. – Schachter J.: "Lymphogranuloma and other non ocular *Chlamydia trachomatis* infections". In: *Nongonococcal urethritis and related infections*, D. Hobson, K.K. Holmens (Eds.), p. 91, American Society for Microbiologists, Washington D. C., 1977. – Schachter J., Dawson C. R.: "Genital tract infections". In: *Human chlamydial infections*, p. 121, P. S. G. Publishing Corporation, Littleton, Massachusetts, 1978. – Siegel M. M.: "*Lymphogranuloma venereum*: Epidemiological, clinical, surgical and therapeutic aspects based on a study in the Caribbean Goral Gables, Florida". University of Miami Press, 1, 1962. – Sompolinsky D., Richmond S.: *Applied Microbiology*, 28, 912, 1974. – Stephens R. S., Tam M. R., Kuo C. C., Nowinski R. C.: *Journal of Immunology*, 128, 1083, 1982a. – Stephens R. S., Kuo C.C., Tam M. R.: *Journal of Clinical Microbiology*, 16, 895, 1982b. – T'ang F. F., Chang H. L., Huang Y. T., Wang K. C.: *Chinese Medical Journal*, 75, 529, 1957. – Taylor-Robinson D., Thomas B. J.: *Journal of Clinical Pathology*, 33, 205, 1980. – Thomas B. J., Evans R. T., Hutchinson G. R., Taylor-Robinson D.: *Journal of Clinical Microbiology*, 6, 285, 1977. – Tygeson P.: *Investigative Ophthalmology*, 2, 482, 1963. – Treharne J. D., Darougar S., Jones B. R.: *Journal of Clinical Pathology*, 30, 510, 1977a. – Treharne J. D., Dines R. J., Darougar S.: "Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East". In: *Nongonococcal urethritis and related infections*, D. Hobson, K. K. Holmes (Eds.), p. 249, American Society for Microbiologists, Washington D. C., 1977b.

RUBELLA AND CYTOMEGALOVIRUS INFECTION IN PREGNANCY

CATHERINE S. PECKHAM

Although only about 3% of all congenital defects are known to be caused by infections in pregnancy, these infections are of particular importance because appropriate preventive measures and treatment could result in a significant reduction in these defects.

Clin. Exp. Obst. Gyn. - ISSN: 0390-6663
XIII, n. 3-4, 1986

97