TRIC AGENT*

CHARACTERISTICS AND DETECTION
BY LABORATORY METHODS

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TRIC is the term proposed (Gear, Gordon, Jones, and Bell, 1963) for agents of the trachoma-inclusion conjunctivitis group. Although certain isolates can be differentiated from others by laboratory tests, there is, as yet, no evidence that this differentiation corresponds to the traditional division of the associated clinical illnesses into different syndromes. TRIC agent is known to cause trachoma (T'ang, Chang, Huang, and Wang, 1957; Collier, Duke-Elder, and Jones, 1958, 1960; Collier and Sowa, 1958; Jones and Collier, 1962; Thygeson, 1962; Bernkopf, 1962; Jawetz, 1964; Jones, 1964) and other syndromes of infection of the eye in the adult (Jones, 1961), and inclusion blennorrhoea of the newborn (Jones, Collier, and Smith, 1959; Hanna, Zichosch, Jawetz, Vaughan, and Thygeson, 1960; Mordhorst, 1964). Detailed studies have now shown the presence of TRIC agent in so-called non-specific genital infection which may be associated with infection of the eye by this agent (Al-Hussaini, Jones, and Dunlop, 1965; Dunlop, Jones, and Al-Hussaini, 1965; Jones, Al-Hussaini, and Dunlop, 1965). It has been recovered from the genital tracts of the parents of babies suffering from inclusion blennorrhoea (Jones and others, 1959; Al-Hussaini, Jones, and Dunlop, 1964; Dunlop, Jones, and Al-Hussaini, 1964; Jones, Al-Hussaini, and Dunlop, 1964; Mordhorst, 1964; Al-Hussaini and others, 1965; Jones and others, 1965) and from the genital tracts of patients presenting because of “non-specific” urethritis (Dunlop, Al-Hussaini, Garland, Treharne, Harper, and Jones, 1965).

TRIC agent is a member of the Bedsonia (Meyer, 1953; Andrewes, 1964) or psittacosis-lymphogranuloma-TRIC group of agents; it has never been cultured in or on non-living media, but has been grown in the living cells of the yolk sacs of embryonated eggs (T'ang and others, 1957; Thygeson, 1962; Bernkopf, 1962; Jawetz, 1964) and in certain tissue culture systems (Gordon, Quan, and Trimmer, 1960; Gordon and Quan, 1965; Pollard, Starr, Tanami, and Moore, 1960; Furness, Graham, Reeve, and Collier, 1960). The term “agent” rather than “virus” is preferable for members of the Bedsonia group because they differ from true viruses in certain important respects; briefly, they reproduce by binary fission (Litwin, 1962; Armstrong and Reed 1964), they contain both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) (Zahler and Moulder, 1953; Ross and Gogolak, 1957; Jenkin, 1960), their multiplication is inhibited by sulphonamides (Early and Morgan, 1946) and tetracyclines (Wong and Cox, 1948), and they possess enzymes (Allen and Bovarnick, 1957; Colón, 1960; Moulder, 1962).

The results of studies of inclusions (Gordon and Quan, 1962), growth requirements (Ossowski, Becker, and Bernkopf, 1965), and antigenic properties (Hanna and Bernkopf, 1964; Katzenelson and Bernkopf, 1965) suggest that certain members of the Bedsonia group, including the 6BC agent of psittacosis, differ in the laboratory from certain other members; the latter group includes the agent of lymphogranuloma venereum and TRIC agent. Two sub-groups of the Bedsonia may thus be formed.

Studies of TRIC agent using light and electron microscopes have provided knowledge of structure and multiplication (Halberstaeder and Von Prowazek, 1907; Lindner, 1910; Thygeson, 1934 a, b; Collier and Sowa, 1958; Mitsui, Suzuki, Hanabusa, Minoda, Ogata, Fukushima, and Miura, 1958; Gordon and others, 1959; Furness and others, 1960; Pollard and others, 1960; Bernkopf, Mashia, and Becker, 1962; Litwin, 1962; Mitsui, Kazima, Nishimura, and Konishi, 1962). The infective particle or elementary body, which is stained reddish-purple with Giemsa’s stain, is 0·2–0·4 microns in diameter. It has an electron-dense...
nucleoid surrounded by an enveloping membrane (Fig. 1); after entering a susceptible cell, the particle enlarges to form an initial body (Fig. 2), which stains blue with Giemsa's stain, and divides. The resulting particles do likewise and binary fission has been observed to occur during the process. The aggregation of particles that results stains blue with Giemsa's stain, is pleomorphic, contains RNA and DNA, and is called an initial body inclusion. As the process continues, a varying number of smaller particles appear; these are typical elementary bodies and are embedded in material rich in glycogen (Rice, 1936).

The mature inclusion is composed mainly of elementary bodies, contains RNA and DNA, and is known as a Halberstaedter-Prowazek inclusion body (Fig. 3, opposite). TRIC agent is known to grow in the epithelial cells and it is believed that it does not invade the deeper tissues (Thygeson, 1950). The superficial epithelial cells constitute the material which should be tested for the presence of the agent (Al-Hussaini and others, 1964) and not the overlying pus or the underlying deep tissue.

TRIC agent shares a heat-stable group antigen with other members of the Bedsonia group (Collier and Sowa, 1958; Jones and others, 1959; Murray, Bell, Hanna, Nichols, and Snyder, 1960). This explains the cross-reactions in serological tests which occur between different strains and also between TRIC agent and other members of the Bedsonia group when, for example, using the complement-fixation test for lymphogranuloma venereum (LGVCFT).

The infective particle is associated with toxin which kills mice (Murray, Snyder, and Bell, 1959); by immunizing mice with vaccines prepared from different isolates of TRIC agent and subsequently challenging them with live isolates, these have been divided into two main groups (Bell, Snyder, and Murray, 1959; Bell and Theobald, 1962; Chang, Wang, and Grayston, 1962). Fluorescent-antibody techniques also have made possible the division of isolates of TRIC agent into

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**FIG. 1.—** Electron micrograph of elementary body, platinum/iridium shadowed, in purified preparation of isolate obtained from urethral scrapings from a case of "non-specific" urethritis. \( \times 90,000. \)

**FIG. 2.—** Initial bodies. Giemsa \( \times 2,000. \)
TRIC AGENT

Fig. 3.—Halberstaedter-Prowazek inclusion body. Giemsa × 3,000.

Fig. 4.—Halberstaedter-Prowazek inclusion body in cervical scrapings from the mother of a baby with inclusion blennorrhoea. Giemsa × 2,000.

groups, and these partly correspond with the groups obtained by using the mouse-immunization test (Nichols and McComb, 1964; Hanna and Bernkopf, 1964; Katzenelson and Bernkopf, 1965).

Serological tests estimating antibody levels have shown that the titres vary from patient to patient and generally tend to be lower than in lymphogranuloma venereum. The Frei intradermal test is known to be positive in most cases of clinical lymphogranuloma venereum (Bedson, Downie, MacCallum, and Stuart-Harris, 1950) and in some cases of pneumonia caused by other members of the Bedsonia group (Rivers and Horsfall, 1959). In infections due to TRIC agent, the results are variable and at the present time their significance is uncertain.

Monkeys and baboons have been infected successfully with TRIC agent (T'ang and others, 1957; Jones and others, 1959; Collier, 1962). Experimental inoculation of the conjunctiva results in follicular conjunctivitis; Halberstaedter-Prowazek inclusions can be seen in conjunctival scrapings from which TRIC agent can be recovered.

The presence of TRIC agent in scrapings can be detected in the laboratory in two ways: by the finding of a Halberstaedter-Prowazek inclusion in a smear, and by the isolation of the agent in the embryonated egg. The latter method permits further identification.

Scrapings should consist essentially of the superficial epithelial cells in which the agent occurs and details of techniques for collection of material have been described (Smith, Gilkes, and Sowa, 1958; Dunlop and others, 1964, 1965). On microscopical examination of scrapings initial bodies may be seen, but the finding of a single typical Halberstaedter-Prowazek inclusion (Fig. 4) is diagnostic of infection.
by TRIC agent. Inflammatory exudate is usually present, composed of polymorphonuclear leucocytes, mononuclear cells of various types including lymphocytes, and larger cells and an occasional plasma cell; a few phagocytic cells are commonly present. A proportion of the epithelial cells show evidence of degeneration with enlargement of the cell and smudging and vacuolation of the nuclear chromatin (Fig. 5). This mixed cellular response which is usually present in infection due to TRIC agent may suggest the diagnosis but cannot establish it (Al-Hussaini and others, 1964).

Fluorescent-antibody staining is of value in the detection of inclusions (Fig. 6) in conjunctival

**Fig. 5.—Degenerate epithelial cells in cervical scrapings. Giemsa × 2,000.**

**Fig. 6.—Halberstaedt-Prowazek inclusion body in conjunctival scrapings. Stained by fluorescent-antibody technique × 1,000.**
Scrapings (Nichols and McComb, 1962, 1964; Nichols, McComb, Haddad, and Murray, 1963) and a similar technique may prove useful in examination of scrapings from the genital tract.

Scrapings which are to be cultured are collected in a suitable medium, usually containing streptomycin and neomycin in concentrations which have no inhibitory action on TRIC agent, and are inoculated into the yolk sacs of embryonated eggs. In smears of yolk sacs from embryos dead or dying of infection by TRIC agent, elementary bodies can be seen. If the embryos do not die or if no elementary bodies are seen in yolk-sac smears, three additional egg passages should be carried out before the result of the culture is reported as negative.

When elementary bodies have been seen, it is necessary to show that they possess Bedsonia antigen; a complement-fixation technique using differentially centrifuged yolk-sac suspension is frequently employed. Smears of yolk-sac suspension can be stained using fluorescent-antibody techniques, and in this way also antigen can be detected.

Further identification of the isolate as TRIC agent requires the inoculation of material from an early egg passage into the conjunctiva of monkey or baboon with ensuing development of characteristic conjunctivitis, and the demonstration of a typical Halberstaedter-Prowazek inclusion in conjunctival scrapings. Attempts to pass freshly isolated TRIC agent in mouse brains have been unsuccessful, although Hurst and Reeve did succeed in passing two strains after a number of passages in eggs (Hurst and Reeve, 1960); however, inoculation of mouse brains with material from buboes of patients suffering from lymphogranuloma venereum (Miya- gawa, Mitamura, Yaoi, Ishii, and Okanishi, 1935), or with isolates of the agent of lymphogranuloma venereum in yolk sac (Hurst and Reeve, 1960), resulted in signs of disease. Elementary bodies have been observed in smears made from the brains of inoculated mice (Miyagawa and others, 1935); in some cases the agent has been recovered from the brains of such mice by yolk-sac inoculation (Hurst and Reeve, 1960).

Summary

An account is given of some of the characteristics of TRIC agent with a general description of tests for its detection and recognition.

REFERENCES

Les caractéristiques de l'agent TRIC et sa recherche par des méthodes de laboratoire

RÉSUMÉ

Un exposé est donné de certaines caractéristiques de l'agent TRIC suivi d'une description générale des tests pour le découvrir et le reconnaître.