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EXPERT COMMITTEE ON TRACHOMA

Third Report

CORRIGENDA

Page 23, Table 3, left hand column

delete Other corneal opacities

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Page 23, Table 3, footnote, lines 1 and 3

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This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

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EXPERT COMMITTEE ON TRACHOMA

Geneva, 29 August - 4 September 1961

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EXPERT COMMITTEE ON TRACHOMA

Third Report

The WHO Expert Committee on Trachoma met in Geneva from 29 August to 4 September 1961. The meeting was opened by Dr P. Dorolle, Deputy Director-General, who noted the growing importance of trachoma in the plans of the World Health Organization and stressed the increasing need for well-trained medical and auxiliary personnel to participate in trachoma studies and control programmes.

Dr J. C. Snyder was elected Chairman; Professor Ida Mann, Vice-Chairman; and Dr J. Graham Scott and Professor A.-M. Larmande, Rapporteurs.

1. THE PRACTICAL APPLICATION OF RECENT ADVANCES IN BASIC RESEARCH TO THE PROBLEMS OF TRACHOMA CONTROL

1.1 The taxonomic relationship of the agents of trachoma and inclusion conjunctivitis

It is of some practical importance to recognize that the agents of trachoma and inclusion conjunctivitis, which as yet have not been distinguished from each other in the laboratory, are members of a large group of atypical viruses known as the psittacosis-lymphogranuloma-trachoma (PLT) group which are intermediate between the large typical viruses, such as the pox group, and the rickettsiae. Like typical large viruses, the members of the PLT group form inclusion bodies and have visible elementary bodies, but unlike the typical large viruses they are susceptible to sulfonamides and some antibiotics, and have a cycle of intracellular multiplication with large forms (initial bodies) approaching the size of bacteria. By custom, however, these agents are called viruses, and in the future will probably continue to be included in considerations on viruses. A much clearer understanding of the biological properties of the PLT viruses may be expected to emerge from the intensive investigations now in progress in many laboratories. The Committee decided, therefore, to defer consideration of specific nomenclature for the causative agents of trachoma and inclusion conjunctivitis, referring to them for convenience hereafter in this report simply as the Tr-IC viruses.

1.2 Improvements in techniques of isolation of trachoma virus

Recent studies from several regions indicate that in certain areas the yolk sac culture technique may be a more sensitive means of detecting trachoma virus than the demonstration of inclusion bodies. It seems certain that with improvements in technique, yolk sac culture will have diagnostic value in pilot projects and in areas where clinical diagnosis of mild, early forms of trachoma has been difficult. The most significant improvements in technique that have been described are as follows:

Prevention of contamination of cultures (1) by collecting material only from the upper fornix or upper tarsus where bacteria are less numerous; (2) by using streptomycin, polymyxin B, or in some instances neomycin or ristocetin, as indicated by the local flora; and (3) by allowing the antibiotic-treated culture suspension to stand at +4°C for several hours prior to egg inoculation; this is indicated when heavy contamination has previously been encountered.

Improvement of isolation rate (1) by using an incubation temperature of +35°C with high relative humidity; (2) by holding eggs for from 10 to 12 days before transfer, unless death occurs sooner; and, in some instances, (3) by inoculating eggs directly from patients in areas where past failures may have been due to loss of viability of the virus during prolonged storing or transporting of scrapings.

1.3 Differentiation between trachoma and inclusion conjunctivitis

Viruses isolated from inclusion conjunctivitis of the newborn and the adult, and from the cervices of mothers of babies with ophthalmia neonatorum, are indistinguishable in the laboratory from the viruses isolated from patients with trachoma. This has inspired a re-examination of the trachoma/inclusion-conjunctivitis relationship. A few investigators hold that trachoma and inclusion conjunctivitis form the two ends of a spectrum of disease induced by a single virus; the majority, however, holds that the two diseases have such different clinical courses and epidemiologic patterns that they constitute separate and distinct entities. Trachoma workers in the field should be familiar with this conflict of ideas and on the alert to make clinical and epidemiologic observations which might resolve it.

1.4 Laboratory animals

Primary transmissions of trachoma have so far been made only to simian species, but it is noted that a few strains of trachoma virus have been adapted to mice. That three strains have induced pannus and scars in a limited number of Taiwan monkeys (*Macaca cyclops*) suggests that these

animals may prove to be useful in differentiating trachoma virus from inclusion conjunctivitis virus. The value of monkeys for trachoma research has greatly increased since egg-propagated viruses became available; a much more severe and more readily diagnosed disease develops than that produced by tissue scrapings.

1.5 Related bacterial and viral infections of the conjunctiva

Improved methods of cultivating and differentiating the conjunctival bacteria are now available, but the smear and scraping techniques are still useful in distinguishing carrier states from conjunctival infection. Recent studies in Saudi Arabia have shown a frequent association of adenoviruses of several different types with trachoma in children under the age of three years. The association has been seasonal and temporary, however, and it is not known what effect adenoviruses may have on the clinical aspects or transmission of trachoma. In other areas, unsuccessful attempts to isolate viruses other than trachoma from the conjunctiva have been reported.

1.6 Miscellaneous associated allergic conditions

Vernal catarrh has long confused the clinical picture of trachoma and has aggravated its clinical course. Recent research indicates that vernal catarrh is an atopic disease characterized by circulating antibody to pollens, and that strong solutions of hydrocortisone and other corticosteroids administered topically will suppress the allergic inflammation. Topically applied corticosteroids have also been useful in suppressing the inflammation of phlyctenulosis and luetic interstitial keratitis, both of which have an unfavourable effect on trachomatous keratitis.

1.7 Methods of cytological diagnosis

Recent studies have confirmed the concept that the presence of Halberstaedter-Prowazek (HP)[1] inclusion bodies indicates infection with the trachoma-inclusion-conjunctivitis viruses but does not distinguish between the two. No confusion with psittacosis or lymphogranuloma venereum inclusions has occurred on the human conjunctiva since neither of these viruses has been demonstrated in human conjunctival epithelium. The

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[1] In view of past confusion of trachoma inclusion bodies with non-specific cytoplasmic material, including pigment granules, extruded nuclear substance, various cell granules, etc., the term " HP inclusion body " should be reserved for scientific purposes to indicate a cytoplasmic epithelial-cell inclusion body containing (1) a carbohydrate matrix, and (2) elementary and/or initial bodies.

reliability of the iodine method [1] for staining the carbohydrate matrix of the inclusions has been affirmed. It stains with sufficient contrast to reveal inclusions under low power in widely spread smears, or even in thick smears, of conjunctival epithelium. Other methods of staining which might be useful in field studies are being explored; these include the Lindner contrast stain [2] for the demonstration of immature inclusions, and nucleic acid stains, such as acridine orange, for ribonucleic acid (RNA) and desoxyribonucleic acid (DNA). Work with yolk sac cultures has shown that extracellular elementary and initial bodies can be recognized and distinguished from non-specific cell granules by experienced observers. The recognition of free virus bodies has already had diagnostic value in inclusion conjunctivitis in which they appear in large numbers in exudate smears; studies are now under way to determine whether or not free bodies can be recognized with sufficient frequency and accuracy in trachoma to have diagnostic value.

The cytologic examination of exudate smears, conjunctival scrapings, and follicular expressions has recently been explored as a diagnostic tool. An exudate rich in neutrophils is characteristic of both trachoma and inclusion conjunctivitis and is independent of secondary bacterial infection; this is in sharp contrast to the mononuclear exudate characteristic of conjunctival infections with typical viruses such as the adenoviruses. Other diagnostic features of the cytology of material from trachoma depend on the fact that necrosis is induced by trachoma virus but not by the agents of the other follicular diseases. These features, seen best in follicular expressions, are as follows: (1) poor staining of lymphoid elements, particularly the lymphoblasts; (2) cells with bare nuclei; (3) scattered

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[1] *Method of Rice* (*Amer. J. Ophth.*, 1936, **19**, 1).

(1) Stain unfixed slide with one or two drops of dilute Lugol's solution (1 to 10)
(2) Place thin cover slip over drop
(3) Ring cover slip with melted vaseline to retard evaporation
(4) Observe under low power and oil immersion.

If examination is to be made immediately, vaseline seal can be omitted. Gram's iodine solution can be substituted for the dilute Lugol's solution.

The inclusion body stains reddish-brown owing to the carbohydrate matrix.

[2] *Lindner contrast stain* (*Zbl. Bakt., I. Abt. Orig.*, 1910, **55**).

(1) Allow the smear to dry in air
(2) Fix in absolute alcohol for 5-10 minutes
(3) Stain for one hour in the following solution:
Distilled water — 10 ml;
Giemsa — 5 drops;
1 % solution of acetic acid — one drop.
(4) Quickly rinse with absolute alcohol to clear the preparation. Dry and mount in cedar oil.

Bacteria, inclusions and mast cell granules stain dark blue; nuclei of lymphocytes and leukocytes, blue or blueish; epithelial cells pink, the nuclei staining a little less