ELECTRON-MICROSCOPIC STUDY OF CHLAMYDOZON TRACHOMATIS*†

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NOTHING is known with certainty regarding the electron-microscopic finding of the trachoma virus, *Chlamydozoon trachomatis*. Trials to demonstrate the virus by means of the electron microscope have been made by several Japanese investigators during the past few years. The only finding with a suspicious virus shadow, however, was that obtained by Itô and others (1951), and that obtained by one of us (Tsutsui, 1951).

It was then supposed by another of the present authors (Y. M.) that the failure might have been due to an inadequate selection of conjunctival materials. It is not easy to get more than 100 mg. of conjunctival tissue from a single case of trachoma; in addition, the causative agent is believed to exist only in the epithelial layer of this tissue. An electron-microscopic examination cannot be performed adequately, therefore, unless materials with the highest concentration of the virus have been used. Inclusion bodies apparently indicate the virus, so that cases with innumerable inclusions should be used for the investigation, but despite the high trachoma index in Japan such cases are comparatively rare.

A few such cases were encountered in the course of a study of the mass treatment of trachoma with terramycin (Mitsui, Tanaka, and others, 1951).

The material was sent for electron-microscopic examination to the third author (J. T.) together with some control material in 50 per cent. glycerol.

Electron-Microscopic Identification of Trachoma Virus

The findings in all these cases were uniform, the electron micrographs from Case 1 being the most distinct (Figs 1 and 2). The virus appeared more or less round in shape, but some elongated virus individuals, suggesting a pre-division stage, also occurred. The size varied widely between about 80 and 350 millimicrons.

FIG. 3.—Inclusion bodies in a scraping slide. Arrow indicates early-stage colony of the virus developed from a single mother element.

* Received for publication May 29, 1952.

† Supported by a Scientific Research Grant from the Japanese Ministry of Education.

FIG. 1.— Electron micrograph from Case 1 ($\times 20,000$).

FIG. 2.— Electron micrograph from Case 1. Arrow indicates early-stage colony or morula ($\times 20,000$).

The size and form of the virus appearing in the electron micrograph seem to correspond, so far as the elements in the visible range are concerned, with those of the elementary and initial form of Prowazek's inclusion bodies. A pleomorphism is the criterion which has been considered characteristic of the *Chlamydozoaceae* viruses.

One appearance (Fig. 2, arrow) is of particular interest; it may be compared with those often found in scraping slides (Fig. 3, arrow), and consists of an early-stage

colony of the virus developed from a single mother virus element—the so-called morula or matrix.

These elements were rarely seen in specimens from trachomatous conjunctiva with few inclusion bodies. None of these findings was obtained from any of the control material.

Purification of the Virus

The virus was purified and concentrated by chemical technique according to the procedure recommended by Cox and others (1947):

- (i) Biopsied conjunctiva emulsified with saline solution (1:10 dilution).
- (ii) Emulsion kept in refrigerator for 24 hrs.
- (iii) Freezing at -11° to -18° C. and melting at 30° C. repeated five times.
- (iv) Material centrifuged for 30 min. at 4,000 r.p.m.
- (v) Supernatant fluid mixed with an equal part of ether to eliminate fat substances.
- (vi) Mixture centrifuged for 30 min. at 4,000 r.p.m. to separate ether.
- (vii) After separation from ether, supernatant fluid re-centrifuged for 30 min.
- (viii) 50 per cent. methanol (pure methanol mixed with equal part of phosphate buffer at pH 7.0) dropped into fluid until final concentration of methanol became 25 per cent.
- (ix) Mixture kept at -4° C. for 3 hrs (solution opaque).
- (x) Mixture centrifuged at 4,000 r.m. for 30 min. at 2° C.
- (xi) Sediment thus obtained refined by phosphate buffer (m/100, pH 5.6), *i.e.*, it was added to buffer solution and centrifuged as given in (x).
- (xii) Sediment added to phosphate buffer (m/100, pH 7.6). Freezing and melting repeated thrice as in (iii).
- (xiii) Mixture centrifuged at 3,000 r.p.m. for 30 min. at 2° C.

The supernatant fluid then contained the refined virus, and this constituted the final material for electron microscopy.

FIG. 4.—Inclusion body in scraping from Case 1.

Conjunctival Materials

Case 1, female, aged 14, with chronic trachoma of about one year's duration, the clinical appearances being most severe. A scraping examination revealed a large number of typical inclusion bodies (Fig. 4). More than ten inclusions were demonstrated histopathologically in each section of conjunctiva. Histopathological changes other than inclusions were also typical of trachoma. The upper fornix conjunctiva of the left eye was biopsied for histopathological examination and the lower fornix of the same eye was cut for the electron-microscopic examination. Care was taken to get the latter sample as thin as

possible since the existence of the virus is thought to be limited to the epithelial layer.

Case 2, female, aged 13, chronic trachoma of several years' duration. Clinical and histopathological findings were similar to those of Case 1. The lower fornix conjunctiva of the left eye was used for electron-microscopic examination.

Case 3, female, aged 14, chronic trachoma of several years' duration. Inclusions were most abundant in the scraping. The conjunctivae of both fornices of the left eye were used for the study.

Case 4, male, aged 19, acute trachoma of 3 weeks' duration. The conjunctivae of both fornices of the right eye were used for the examination.

Control Cases.—Several examples of normal conjunctiva, bacterial conjunctivitis, and trachoma with few inclusion bodies were selected as controls.

Summary

By electron-microscopic examinations, a phenomenon supposed to be the virus of trachoma was demonstrated from typical trachoma cases showing numerous inclusion bodies. No similar appearance was obtained from any of the non-trachomatous control cases.

The publication of this paper has been made possible through the kindness of Sir Stewart Duke-Elder.

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