TRACHOMA INCLUSIONS AND PENICILLIN*

BY

M. J. GILKES, C. H. SMITH, AND J. SOWA

Trachoma Research Group, Medical Research Council

AFTER the release of penicillin for general medical use after the second world war, immediate interest was awakened in its possibilities in the therapy of trachoma. The subject was extensively investigated but results tended to be equivocal and attention became diverted to the newer antibiotics as these became available. In consequence, no definite assessment of the potentialities of penicillin in this disease appears to have been made.

However, developments in the technique of virus isolation by tissue culture methods have raised the question of the action of penicillin on trachoma to a new importance, in view of its use, with or without other antibiotics, to prevent the contamination of cultures. Recently Tang, Chang, Huang, and Wang (1957), reporting the culture in hen eggs of a virus, probably that of trachoma, have reported the inhibition of viral growth by such use.

The following observations of the disappearance of HP inclusions from the conjunctival scrapings of a small series of cases of trachoma, previously inclusion-positive on repeated examination, after treatment with penicillin, may therefore be of interest.

Our attention was drawn to this in the course of work on the aetiology of trachoma in Jordan, when a tendency for inclusion-positive cases to become inclusion-negative, after minimal local or general therapy with sulphonamides or antibiotics, was observed. Having regard to the likelihood of such disappearance occurring in the later stages of the disease, even in the absence of therapy, little weight was attached to this.

After this work was transferred to Gambia, the observations reported below in Case 1 were made, and in view of the very definite changes noted it was decided to attempt their reproduction in a small series.

Methods

All patients were African of Mandingo or Jola extraction. With the exception of Case 1 they had been followed-up by repeated conjunctival scrapings for some months before the present observations were made. Inclusions were found to be present at every examination.

Patients were admitted to hospital and conjunctival scrapings were taken from

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the upper tarsae every second day, using alloy scrapers without the previous administration of local anaesthetic—in our experience this procedure is well tolerated. Clinical details were confirmed with the slit lamp (except in Case 4). In most cases bacteriological cultures were taken before each scraping and organisms identified by cultural characteristics and microscopy.

After admission, penicillin was administered to Cases 2 to 5 in the form of aqueous suspension of procaine penicillin (Avloprocil), one injection of 900,000 units daily for 5 days. No other local or general therapy was given.

Inclusions were identified by the iodine-staining method of Rice (1936) as modified by Gilkes, Smith, and Sowa (1958). Where necessary such identifications were confirmed by re-staining with Giemsa.

Case Reports

Case 1, a male infant aged 4 months, had clinically early Trachoma Stage I. There were many small follicles and papillae, but, as is commonly found in Gambian trachoma, no evidence of any corneal involvement.

27.5.57: He was admitted to hospital for repeated conjunctival scrapings to observe the alleged developmental cycle of the inclusion body. Culture was sterile. Scrapings showed inclusions in vast numbers (as many as nineteen in one 1/12" objective field).

30.5.57: Scrapings showed inclusions greatly reduced in number. The majority of those seen were increased in size and vacuolated in appearance. Epithelial cells were noted as containing iodine-staining fragments suggestive of disrupted inclusions.

3.6.57: Clinical signs fewer and conjunctival congestion reduced. Scrapings showed no inclusions, but a few leucocytes containing stained granules.

5.6.57: Lids clinically normal. Scrapings showed no inclusions.

5.7.57: Lids clinically normal. Culture showed few *Corynebacterium* and *Staph. albus*. Scrapings were normal, with epithelial cells only.

On investigating this sudden disappearance of inclusions, it was discovered that from 29.5.57 to 31.5.57 the child had received three daily intra-muscular injections of procaine penicillin 900,000 units for treatment of a concurrent scalp infection. No other local or systemic treatment had been given.

Case 2, a boy aged 4 years, had Trachoma Stage I. There were scanty follicles and papillae, but no pannus. Repeated scrapings from 27.2.57 showed inclusions constantly present, associated with a trachomatous type of cytology.

6.5.57: He was admitted to hospital. Culture showed a scanty growth of Koch-Weeks bacilli and *Corynebacterium*. Scrapings showed inclusions ++. Penicillin treatment was commenced.

9.6.57: Scrapings showed fewer inclusions, which were large, vacuolated, and degenerate in appearance.

11.6.57: Culture was sterile. Scrapings showed no inclusions, but a few inflammatory cells seen.

13.6.57: Culture was sterile. Scrapings showed no inclusions. The patient almost clinically normal.

5.7.57: Culture was sterile. Scrapings showed normal epithelial cells only. Patient clinically normal.

Case 3 a girl aged 12 years, had Trachoma Stage II with many follicles, few papillae, and no pannus. Repeated scrapings from 6.3.57 had shown inclusions associated with a trachomatous type of cytology.

6.5.57: She was admitted to hospital. Culture showed a heavy growth of *Staph. pyogenes* and a scanty growth of *Corynebacterium*. Scraping showed many inclusions. Penicillin treatment was commenced.

8.6.57: Scrapings showed few inclusions, which were large, vacuolated and degenerate.

12.6.57: Culture was sterile. Scrapings showed no inclusions, and nearly normal epithelial cells containing iodine-staining granules.

14.6.57: Culture was sterile. Scrapings showed no inclusions. The patient was clinically nearly normal.

5.7.57: Culture showed a heavy growth of pneumococci and a fair growth of *Staph. albus*. Scrapings showed normal epithelial cells only. Patient clinically normal.

Case 4, a girl aged 5 yrs, had Trachoma Stage II, with few follicles, many papillae, and 1.5 mm. pannus tenuis. Scrapings from 19.12.56 constantly showed inclusions to be present.

18.6.57: She was admitted to hospital. Culture showed a fair growth of *Corynebacterium*. Scrapings showed many inclusions. Penicillin treatment was commenced.

20.6.57: Culture showed a scanty growth of *Staph. pyogenes*. Scrapings showed no inclusions, but a few inflammatory cells.

22.6.57: Culture showed a heavy growth of Koch-Weeks bacilli. Scrapings showed no inclusions. Clinically the patient was much improved.

24.6.57: Culture showed a scanty growth of *Staph. albus*. Scrapings showed no inclusions.

5.7.57: Culture showed *Staph. albus*. Scrapings showed normal epithelial cells only. Patient clinically normal.

Case 5, a boy aged 12 yrs, had Trachoma Stage II, of the proliferative type, with gross papillae, a few follicles, 1.5 mm. pannus, and Herbert's pits. Scrapings from 27.2.57 showed inclusions constantly present, sometimes in vast numbers (up to thirteen in one 1/12" objective field).

6.6.57: He was admitted to hospital. Culture showed a scanty growth of *Corynebacterium* and *Staph. albus*. Scrapings showed many inclusions present. Penicillin treatment was commenced.

8.6.57: Scrapings showed fewer inclusions, which were large, vacuolated, and degenerate in appearance.

11.6.57: Culture showed a scanty growth of *Corynebacterium*. Scrapings showed no inclusions, but some epithelial cells with iodine-staining granules. Clinically the patient was much improved.

13.6.57: Culture was sterile. Scrapings showed no inclusions, but mainly normal epithelial cells.

5.7.57: Culture showed a scanty growth of *Corynebacterium*. Scrapings showed no inclusions, but some inflammatory cells were present. Clinically the patient was much improved. There were some residual papillae.

In conjunction with this series, other cases of Trachoma Stage II and III, in which inclusions had been consistently present for 3 or more months, were treated as outpatients with local applications of penicillin and streptomycin ointment (Mystrepton. Penicillin 2,000 units/g.: Dihydrostreptomycin sulphate 10,000 units/g.) twice daily. In three of these, all siblings, vacuolated degenerative changes were observed in the inclusions, which later disappeared and had not recurred up to one month later. In the remaining three cases, after transient changes, the inclusions persisted. The cultures became sterile in the former three but remained positive in the latter.

Discussion

It has not been possible to prolong the follow-up of these cases or to enlarge the series to a more satisfactory size, but substantially our findings confirm those of Bietti (1948, 1949), who reported degenerative changes in the inclusion bodies of trachoma after the local application of penicillin, using Giemsa stain.

The changes were specific and constant in character, occurring on the second or third day of treatment, associated with a definite reduction in the number of the inclusions, and preceding their total disappearance. Such changes have not been observed in the absence of therapy.

The present series, however, differs from that of Bietti in two respects:

(1) The use of the iodine-staining method, which showed the morphological changes in the inclusions more clearly than when Giemsa or Wright's stains are used. With these latter stains the later degenerative changes become increasingly difficult to differentiate from other appearances not connected with the inclusions, which may be present in the cytoplasm of the epithelial cells.

(2) The use of a very large short-term systemic dosage unaccompanied by local therapy.

Bietti (1951) stated that in trachoma local application is the most efficacious form of therapy, and this view was supported and quoted by Nataf (1952). More recently, Bietti (1955) has acknowledged the possibility that a considerably larger systemic dosage might be effective and favourable results with a dosage smaller than ours have been reported by several authors, including Siggia and Mamola (1950), Scuderi and Bellomio (1950), and Zeppa (1951). Bietti and Pannarale (1955) have also reported encouraging results, with the occurrence of degenerative inclusion changes, following repository injections of N-N diethylenediaminedibenzylpenicillin G (DEBP), again with a smaller effective daily dose than in our series.

In view of the small size of this series, no attempt is made to present the reported regime of dosage as curative of trachoma. None the less the definite clinical response and the changes in the inclusions indicate that further investigation of the therapeutic possibilities of heavy systemic dosage merits consideration.

It is rather our purpose to suggest that the use of penicillin in connexion with any procedures directed to the cultivation of the trachoma virus should be avoided. Degenerative changes in the inclusion bodies have been reported following the clinical use of the newer antibiotics, Terramycin, chloramphenicol, and Aureomycin, and similar considerations may apply in connexion with these substances.

Summary

The degenerative changes and the disappearance of inclusion bodies in a small series of longstanding inclusion-positive cases of trachoma after short courses of massive systemic daily dosage with penicillin are described.

The significance of these findings in relation to the use of antibiotics in the attempted tissue culture of the trachoma virus is discussed.

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