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# OBSERVATIONS ON THREE VIRUS STRAINS ISOLATED FROM TRACHOMA CASES

by

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## INTRODUCTION

Recent reports by Wang et al (1957), Collier et al (1958) and Snyder et al (1958) have indicated that the virus of trachoma could be isolated and cultivated in the yolk sac of the chicken embryo. Previous attempts by other workers had given only partial or controversial results - with the possible exception of those by Macchiavello (1944) and Stewart and Badir (1950) - and up to very recent times none of these claims had been considered as conclusive (Thygeson and Nataf, 1958).

The present report summarizes the observations made on three strains of an agent isolated from trachoma cases in June 1959 and which appear to be identical to those isolated by the previously mentioned authors from material from China, Gambia and Saudi Arabia.

## MATERIALS AND METHODS

The material used for inoculation had been obtained by conjunctival scraping from cases of clinical trachoma positive on microscopic examination for typical inclusion bodies. These cases were children of two to nine years from the neighbourhood of Tunis who had not received specific treatment prior to the collection of material.

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The material had been collected by light scraping of the upper tarsal conjunctiva. Immediately after scraping, part of the material was put into a vial containing 1 ml of balanced salt solution (Hanks) to which twenty per cent horse serum and 1 mg/ml of dihydrostreptomycin had been added. The remaining part of the scraping was smeared on a slide for microscopic examination. While this examination was performed, the vials with the material were kept at +4°C for a period of four to six hours.

The inoculations were made into the yolk sac of six to eight days old chicken embryos, according to the technique described by Cox (1938). Each egg received 0.10 ml of the suspension.

After inoculation the eggs were incubated at +35°C. The mortality occurring within forty-eight hours after inoculation was considered as traumatic and these eggs were not routinely controlled.

All eggs dying between the third and the twelfth day after inoculation were opened and the yolk sacs harvested, ground and suspended in equal volume of Hanks solution. This suspension of ground yolk sac was utilized, after centrifugation, for serial passages, for microscopic examination and for routine bacteriological controls. When no mortality was present in a series of eggs, blind passages were made on the seventh day after inoculation.

Control series included non-inoculated eggs and eggs inoculated with Hanks solution, horse serum and streptomycin and with suspension of normal yolk sac.

RESULTS

A total of six attempts have been carried out so far following the technique described above, three strains have been isolated and are now maintained through serial passages in eggs.

The yolk sacs of the eggs infected with these strains have a constant hyperaemic appearance and numerous elementary bodies are present in the microscopic preparations of all infected eggs; these elementary bodies stain red with Macchiavello's, dark red to purple with Giemsa's (pH 7.2) and blue with Castaneda's method. The series of infected eggs show a regular mortality pattern, occurring in general from five to eight days after inoculation.

In the other three attempts the presence of a few elementary bodies had been observed in a few eggs up to the second passage, but it had not been possible to maintain them through further passages, the mortality which was also present had disappeared.

No elementary bodies are observed in the controls and the mortality in the control series is practically absent.

The three strains are now at their twenty-first to twenty-third serial passage. After the first passages the mortality pattern has become more regular and the number of elementary bodies present in the yolk sac preparations has increased.

Under certain conditions the three strains can withstand freezing at -60°C and thawing without losing their virulence for the eggs; they can also be preserved at +4°C for up to seventy-two hours with only a partial loss of virulence. They survive for thirty minutes at +45°C but are inactivated after sixty minutes; they are also inactivated after fifteen minutes at +50°C.

The sensitivity of these strains to antibiotics is being investigated; the results obtained so far are reported in a separate note.

Preliminary observations indicate that these strains are not pathogenic for mice by intra-cerebral and intravenous inoculation and for guinea pigs by intra-peritoneal inoculation.

Attempts to neutralize these strains by putting them in contact with their homologous serums have given no results.

DISCUSSION

This is a preliminary report which summarizes some of the observations made; further work is under way and will be reported in detail elsewhere.

We are not yet in a position to draw any definite conclusion as to the nature of this agent. The fact that three strains, apparently identical in their characteristics, have been isolated under similar conditions from three individuals with clinical trachoma and positive for inclusion bodies, and that these strains are - as far as we can say - similar to those proved by T'ang and by Collier to be capable of producing an experimental trachoma, strongly suggest a close relationship to trachoma.

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