LABORATORY STUDIES OF EYE INFECTIONS IN SOUTH AFRICA WITH SPECIAL REFERENCE TO THE VIRUS OF TRACHOMA*

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It is clear from surveys carried out by Murray,[1] Amies, Murray, Scott and Warren,[2] and Amies, Loewenthal, Murray and Scott,[3] and from the surveys organised by the South African National Council for the Blind, that eye diseases are extremely prevalent in the population of some regions in South Africa. Their incidence is an outstanding challenge to preventive medicine, which was accepted with good results. A challenge was also posed to laboratory workers.

LABORATORY STUDIES IN SOUTH AFRICA

For the last decade, studies into the aetiology of eye diseases so prevalent in South Africa have been carried out at the South African Institute for Medical Research. In one of the first of these, Dr. C. R. Amies, working in collaboration with Dr. Neil Murray, Dr. Graham Scott and Dr. R. Warren, demonstrated that a proportion of the cases examined showed the inclusions similar to those of trachoma. Of 109 cases, 23% showed these inclusions. In addition it was noted that bacterial infections were frequent. These included Koch-Weeks bacilli and other bacteria resembling *H. influenzae*, which were detected in 75% of the cases examined. These findings thus confirmed that some at least of the cases which on clinical grounds had been diagnosed as trachoma were in fact examples of this disease, and also emphasized the importance of bacterial infections in the condition. It remained and remains uncertain whether the bacterial infection renders the conjunctival epithelium more susceptible to the virus of trachoma or whether they occur as secondary

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invaders in an eye already damaged by this virus. Only further detailed study is needed to determine the relative roles of virus and bacteria and also to determine the importance of other factors. To gain more knowledge of the virus, it was essential that it should be cultured in the laboratory. Only when this had been done would it be possible to determine its physical, chemical and immunological characteristics, and only then could a systematic study of its response to drugs and antibiotics be undertaken.

In 1955, after discussion with Dr. Graham Scott, it was decided that an attempt should be made to culture the virus of trachoma in tissue culture. Facilities for the application of the most modern methods were available at the newly established laboratories of the Poliomyelitis Research Foundation.

Arrangements were made for the collection of suitable specimens in the Potgietersrust district, and in the region of the Jane Furse Hospital in Sekukuniland. In addition, an infant with clinical evidence of early disease was brought to Johannesburg, so that material could be collected from the patient in the laboratory. Suspensions were prepared from these materials and inoculated into a variety of tissue culture and into chick embryos. Several passages were made from each culture, but in no instance was there evidence of the growth of the virus. However, examination of the films taken from the patients chosen for culture had failed to reveal the typical inclusions. Conjunctival snips were then taken by Dr. Neil Murray from typical cases and immediately placed in tissue-culture fluid for an attempt to grow out the conjunctival cells and with it the virus. This experiment also gave negative results.

A large number of films collected by ophthalmologists in various parts of the Transvaal and in South West Africa after suitable staining were examined in the laboratory. In a small percentage only were inclusions resembling those of trachoma seen. A large proportion showed bacteria similar to Koch-Weeks bacilli. It seemed wise to concentrate the attempts to culture the virus on patients in whom virus inclusions had been detected.

Material and Methods

Four one-day expeditions in all were undertaken in an attempt to isolate trachoma virus from early clinical cases of untreated trachoma:

Expedition I	Jane Furse Hospital	29 9 58
Expedition II	Skilpadfontein	17 11 58
Expedition III	Jane Furse Hospital	11 3 59
Expedition IV	Orlando	5 5 59

Conjunctival smears and cultures for virus studies were collected. For the procedure the eyelid was everted. A cotton swab was rolled over the surface and this was immediately immersed in one ml. of broth containing 2,000–5,000 μg. of streptomycin, after which the excess broth was pressed out of the swab and the swab was then used to inoculate a chocolate agar slant and a tube of nutrient broth. The virus broth cultures were immediately frozen in dry ice (CO_2). Certain cultures, however, were inoculated in the field into yolk sacs of 7-day-old embryonated hens' eggs. The inoculum per egg was 0·25 ml. The eggs after sealing with scotch tape were placed in a specially insulated wooden box and transported to the laboratory, when the same evening they were incubated at a temperature of 35°C. The frozen specimens were rapidly thawed a day or so after return and inoculated into yolk sac of 7-day eggs as described. All infected eggs were candled daily for 9 days. Those dying in the first 3 days were discarded after bacterial cultures from the yolk sac had been made on blood agar and nutrient broth. Yolk sacs were harvested aseptically from eggs dying on the 4th day or later, or still alive on the 9th day. Suspensions were prepared by grinding the tissue to a smooth paste and adding 1·5 ml. of nutrient broth per yolk sac. The suspension was clarified by centrifuging at 1,500 – 1,800 r.p.m. for 5 minutes. The supernatant fluid was removed. A portion was stored frozen at –20°C, and a portion to which 5,000 μg of streptomycin had been added was used to inoculate another group of 7-day-old eggs. From each specimen 4-5 blind passages were made before it was considered to be negative for trachoma.

Impression films as well were prepared from each harvested yolk sac. After the excess yolk was rinsed in normal saline from the tissue, this tissue was dried on filter paper and then used for impression smears. The slides were heat-fixed and were stained according to Macchiavello's method. Elementary bodies stained as fine red granules.

The original conjunctival smears were numbered on return to the laboratory. One slide was stained overnight at a temperature of 35°C in a 1 : 40 dilution of Giemsa stain prepared with buffered water of pH 7·2. The other slide, unfixed, was stained with Lugol's solution (5% iodine in 10% aqueous potassium iodide).[4] The inclusions stain a deep orange-brown colour. The iodine-stained slides after examination were re-stained with Giemsa when the oil had been removed with xylol and iodine decolorized with methyl-alcohol. On the iodine-stained slide the inclusion body was noted, and the same cell was then studied when re-stained with Giemsa.

Storage virus. Suspensions, 20% by weight, were prepared from these yolk sacs that showed elementary bodies on passage. The yolk sacs were ground to a smooth paste and nutrient broth containing no streptomycin was added. The suspension was clarified as described for passage of virus. The supernatant fluid was removed and dispensed in 1-ml. amounts into ujophile tubes. Some of these were immediately sealed in a flame and then frozen in dry ice and alcohol, after which they were stored in a CO_2 box. The remaining tubes were shell-frozen in dry ice and alcohol and dried under vacuum for 5 hours, at which time nitrogen was introduced in the system and the vials sealed and then stored at a temperature of –20°C.

Results

The attempt to culture virus from a material collected on the first two expeditions were negative. However, in March, Dr. Graham Scott collected slides from likely cases in the neighbourhood of the Jane Furse Hospital, to which several of them were admitted. Typical virus inclusion-bodies were detected in the smears of one of these cases. An intensive effort was then made to culture the virus from this case, as well as 5 others. Smears made from the 4th egg passage of this show numerous elementary bodies similar to those of trachoma virus.

It appears that the efforts to culture this virus have at last been successful. It will now be possible to develop diagnostic tests to define exactly the distribution and incidence of this

disease in Southern Africa and to determine the relative importance of virus and bacterial infections and to carry out comparative tests with virus isolated elsewhere. It will be possible to produce a vaccine, but its value could only be determined by extensive and prolonged field trials.

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