TRACHOMA STUDIES : TRANSMISSION OF JANE FURSE VIRUS TO A HUMAN VOLUNTEER

J. GRAHAM SCOTT, *Ophthalmologist, Johannesburg*, and JAMES GEAR, E. CUTHBERTSON, and D. M. SMITH,

Poliomyelitis Research Foundation, South African Institute for Medical Research, Johannesburg

As described in the paper* by Whitney and Gear,[1] a virus resembling the virus of trachoma was isolated from a patient Dorothea Phogole, an African girl aged 7 years, seen at the Jane Furse Hospital, Sekukuniland, North-Eastern Transvaal, and presenting the clinical picture of trachoma. Inclusion bodies resembling those of trachoma were detected in smears taken from her eyes. Eggs were inoculated with a suspension prepared from swabs taken at the same time and a virus established in yolk-sac serial passage.

Smears made from the yolk sacs of eggs of the 4th passage showed numerous granules staining red with Machiavello's stain and resembling the elementary bodies of trachoma virus. Profuse growths have occurred in all subsequent passages. The properties of this virus have been described in detail in a paper† by Cuthbertson *et al.*[2] Attempts to establish it in tissue cultures of a variety of human, monkey, rodent and chick-embryo tissues, have so far yielded negative results. Its pathogenicity for experimental animals has been studied in detail and it has been shown to be relatively non-pathogenic to both adult and one-day-old white mice, to guinea-pigs and to gerbils. The inoculation of virus suspensions directly onto the conjunctivas of guinea pigs, rabbits and monkeys have so far failed to produce clinical signs of infection, and the attempts to isolate virus from the eyes of the inoculated animals have given negative results.

The virus was successfully transmitted to the eye of a human volunteer. The volunteer, Mr. vW., a European male aged 45 years, was in good health and had not previously suffered from chronic or subacute eye inflammation, and before being inoculated his eyes were carefully examined and found to be normal.

A cotton-wool swab soaked in infected yolk-sac suspension was rubbed on the tarsal conjunctiva of the left eye. The right eye was then rubbed with an uninfected swab soaked in sterile nutrient broth.

The volunteer was then examined twice weekly. Material for virus culture and smears for microscopic examination were taken twice during the 1st week and then at weekly intervals. His right eye remained normal until towards the the end of the 1 month's period of observation, when he complained of slight irritation and showed slight congestion of the conjunctiva. His left eye developed the typical picture of trachoma. The condition progressively evolved and showed no signs of improvement during the period of observation until he was treated.

As the volunteer was a sighted man, treatment was begun on the 31st day after infection, when laboratory studies had repeatedly confirmed the diagnosis. Ilotycin ophthalmic ointment (0·5% erythromycin Eli Lilly) was instilled into his eye 4 times a day for 1 week. This treatment resulted in considerable improvement. It was continued 3 times a day for a further 2 weeks, by which time he was clinically cured. Treatment was stopped after another week and there was no recurrence in the subsequent month. The infected eye recovered without visible signs of damage. The slight congestion of the uninfected eye responded rapidly to the treatment and the eye remained normal thereafter. The clinical findings during this period are summarized in Table I.

TABLE I. CLINICAL FINDINGS AFTER INFECTION

D Day	Discomfort	Discharge	Infection	Oedema	Diffuse infiltration	Papillary hypertrophy	Follicles	Pannus
3	++	+++	++++	+++	+++	—	—	—
8	+	++	+++	+++	+++	+	—	—
10	+	++	+++	++	+++	++	—	—
17	—	++	+++	++	+++	++	+	—
24	—	+	+++	+	+++	++	+	+
31	—	+	+++	+	+++	++	++	+
Treatment 38/7	—	—	++	—	++	+	+	+
45/14	—	—	+	—	+	+	—	?
52/21	—	—	—	—	? +	? +	—	?
59/28	—	—	—	—	—	—	—	—
Treatment stopped 87	—	—	—	—	—	—	—	—

LABORATORY FINDINGS

Methods

On each occasion two or more films were prepared on the spot from conjunctival scrapings. One was stained with Machiavello stain and the other, after fixation in methyl alcohol, with Giemsa stain 1 : 20 overnight.

Machiavello's stain colours the trachoma virus pink to red and the pink or red granules of the virus show up very clearly against the blue background of the cells. With Giemsa's stain, the granules or elementary bodies in the cells or lying free stain purple. The characteristic cytoplasmic inclusions also stain dark purple, and usually the elementary bodies composing the mass can be clearly defined.

The cotton-wool swab after rubbing the conjunctiva was placed in nutrient broth containing 10,000 μg of streptomycin This suspension was inoculated through a hole in the blunt end of the shell into the yolk sac of 7th-day embryonated eggs. With each suspension 6 or 12 eggs were inoculated and were candled each day. Moribund eggs were opened and impression smears made from the yolk-sac membrane. These were stained with Machiavello's stain and examined microscopically for the presence of pink-red granules.

Five passages were carried out from each primarily inoculated batch of eggs.

Results

Smears. Particles staining red or pink with Machiavello's stain and purple with Giemsa's stain were detected in the smears taken from the eye 3 days after infection. Similar particles in increasing numbers were detected in the smears taken on the 7th and 9th day, and on subsequent occasions.

Cytoplasmic inclusion bodies consisting of a compact mass of granules resembling those of trachoma were not detected until the 16th day after infection. They were again found in moderate numbers on the 23rd and 30th day.

* See page 451 of this issue.
† See page 453 of this issue.

Culture

A virus in appearance resembling the virus of trachoma was successfully established in yolk-sac culture from each swab taken after infection and before the institution of treatment. Profuse growths occurred in the primary cultures, suggesting that the virus had retained its egg-adapted properties in spite of its one intervening human passage.

After treatment, particles resembling the elementary bodies of trachoma virus were detected in the smears taken on the 3rd day, but were not detected in the smears taken on the 11th day after the beginning of treatment.

No virus particles were detected in the smears made from the yolk sacs of the eggs inoculated with the suspension prepared from the swabs taken on the 5th day after beginning treatment.

None were detected in the smears made from the yolk sacs of the eggs in the 5 subsequent passages.

The results of the laboratory studies are summarized in Table II.

TABLE II. LABORATORY STUDIES AFTER INFECTION

Date	Day after infection	Smears		Culture in embryonated eggs
		Virus particles	Cytoplasmic inclusions	
29.7.59	0	—	—	—
1.8.59	3	+	—	+
5.8.59	7	+	—	+
7.8.59	9	+	—	+
14.8.59	16	+	+	+
21.8.59	23	+	+	+
28.8.59	30	+	+	+
30.8.59	Treatment			
	Day after treatment			
2.9.59	3	+	+	0
4.9.59	5	0	0	—
11.9.59	12	—		—

Re-infection

The volunteer remained well until 6 months later, when the same eye was infected again by rubbing a swab dipped in a suspension of virus prepared from infected yolk-sac membranes onto the tarsal conjunctiva. Three days later he developed acute conjunctivitis similar to that seen in the primary infection. After taking smears and swabs to confirm the diagnosis of trachoma-virus infection, treatment was prescribed as before. The response was again prompt and satisfactory.

It was significant that the primary infection had not conferred immunity of sufficient degree to prevent re-infection of the same eye. As the infection is a surface infection of a tissue not directly exposed to the action of serum antibodies, this lack of immunity is perhaps not surprising, but it does suggest the possibility that vaccines against this disease may have little or no value. However their value can only be determined by clinical trial.

SUMMARY

A volunteer, an adult man 45 years old, who had not previously suffered from a sub-acute or chronic eye infection, was inoculated in the left eye by rubbing it with a cotton-wool swab soaked in yolk-sac suspension of trachoma virus. This virus had been isolated from a clinical case of trachoma seen at the Jane Furse Hospital in Sekukuniland in the North Eastern Transvaal.

The volunteer developed acute conjunctivitis 3 days after inoculation. His condition showed no improvement but continued to evolve until the 31st day, when treatment was commenced. Response to treatment was rapid and satisfactory.

In laboratory studies, the virus particles were observed in each of the smears taken at weekly intervals from the 3rd to the 31st day. The virus was also established readily in egg cultures from swabs taken at the same time. Typical cytoplasmic inclusion bodies were not seen until the 16th day after inoculation, but were detected on each subsequent occasion until treatment. Virus particles were seen on the 3rd day after treatment but were not detected thereafter. Cultures gave negative results on the 5th day and subsequently. The volunteer remained well for 6 months, when he was re-infected in the same manner, again developed acute conjunctivitis after an incubation period of 3 days. This attack also responded promptly to treatment.

REFERENCES

1. Whitney, E. and Gear, J. (1960): S. Afr. Med. J., 34, 451.
2. Cuthbertson, E., Smith, D. M. and Gear, J. (1960): *Ibid.*, 34, 453.