THE EFFECT OF TRACHOMA VIRUS VACCINE ON THE COURSE OF EXPERIMENTAL TRACHOMA INFECTION IN BLIND HUMAN VOLUNTEERS*

BY J. THOMAS GRAYSTON, M.D., SAN-PIN WANG, M.D., YEN-FEI YANG, M.D., AND ROBERT L. WOOLRIDGE, D.Sc.

(From the United States Naval Medical Research Unit No. 2 (NAMRU-2), and the National Taiwan University College of Medicine, Taipei, Taiwan)

PLATES 114 AND 115

(Received for publication, December 19, 1961)

Trachoma remains the most important cause in the world of blindness and loss of sight. It is a disease that is particularly important in many of the underdeveloped countries where the incidence is high and where the loss of sight in young adults causes a serious economic loss, and may play a role in retarding industrialization. Although trachoma can be cured by several antibiotics and sulfa drugs, attempts to eradicate the disease through mass antibiotic campaigns have shown success only in the more developed countries. Studies of antibiotic treatment are continuing particularly in association with the World Health Organization's campaigns and more effective programs may be developed (1). Research on trachoma has been greatly retarded by the unavailability until recently of the etiologic agent for study in the laboratory. Although trachoma virus has been recognized for many years to be related to the psittacosis-lymphogranuloma venereum (P-LV) group of agents based on the morphology of inclusion bodies seen in conjunctival cell smears, efforts to grow the virus in series and in quantity had been unsuccessful. Although it is likely that the virus was isolated a number of times in the past in embryonated hen egg yolk sac, it had never been maintained in culture until Tang and his colleagues in 1957 (2) succeeded in growing viral agents which were sent to England and confirmed by Collier and Sowa (3). The Chinese workers showed that these viral agents as isolated from trachoma patients' eyes were highly susceptible to penicillin but resistant to streptomycin. By using streptomycin to control bacterial contamination in eggs, similar agents have been isolated from trachomatous patients in a number of laboratories throughout the world. These agents which are related by group antigen to P-LV viruses are now generally accepted as the cause of trachoma. This acceptance is based

* This study was supported in part by funding under Public Law 480, Section 104(c) and in part by the Office of Naval Research, Contract Nour-2121(07). The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

† Dr. Grayston's present address: Department of Preventive Medicine, University of Washington School of Medicine, Seattle 5, Washington. Reprint requests should be addressed to Commanding Officer, NAMRU-2, Box 14, San Francisco, California.
on isolation of these viruses from trachomatous disease, accompanied by development of antibody against the agents and by the reproduction of trachoma following experimental infection of humans.

The six infections reported in this paper represent an experiment which has been carried out in order to establish definitely the etiologic role of these viruses by reproduction of trachoma. In these six infections with a Taiwan strain and in two with an African strain carried out by Collier and coworkers (4, 5), the disease has been allowed to continue long enough to demonstrate the chronic cicatricial changes of trachoma. In addition, a single human infection with a local strain followed for a month with demonstration of pannus formation, has been carried out in Israel (6) and South Africa (7). These human experiments greatly increase the probability that these large viral agents are the cause of trachoma throughout the world. A preliminary report of the first 4 months' course of the Taiwan experimental infections has been published (8).

With availability of the etiologic agent of trachoma for study in the laboratory, there has been considerable interest in the possibility of developing a vaccine for prevention of trachoma. However, a number of serious problems need to be answered before the feasibility of a vaccine in the prevention of trachoma can be judged. The P-LV viruses are considered poor antigens. Frequently, natural infection with these agents does not evoke an adequate antibody response to eradicate completely the organisms. Chronic foci and relapsing disease are a frequent result of infection with this group. Experimental studies with psittacosis vaccines have often been disappointing and have at best shown partial protection. Studies in the past have failed to reveal clear-cut evidence of antibody response in trachoma infection. Trachoma is a superficial disease of the conjunctival cells of the eye and the role of humoral antibodies both in the natural disease and in preventing infection are not known. Although recent studies have suggested that complement-fixing antibodies develop in trachomatous patients (9), these results have not been confirmed.

The results of the studies recorded in this paper provide evidence that humoral antibodies develop during the course of experimental trachoma eye infections and that vaccine made from trachoma virus favorably affects the course of these infections.

**Materials and Methods**

**Human Volunteers.**—Seven volunteers were recruited from the Provincial Taipei School for Blind and Mute, Taipei, Taiwan. They were selected from the student volunteers examined at the school who showed no evidence of previous trachoma infection. All had normal conjunctivae before inoculation. Volunteer 1 was the only one with a normal cornea. The age, sex, and clinical eye diagnoses were as follows:

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Clinical Description before Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Male, 11 yrs.</td>
<td>Congenital cataract with convergent concomitant strabismus, vision (from 11th International Test Table): 1 m/0.1 non-correct (n.c.) for both eyes. Cornea of both eyes was normal.</td>
</tr>
<tr>
<td>No.</td>
<td>Sex</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2.</td>
<td>Male</td>
</tr>
<tr>
<td>3.</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Female</td>
</tr>
<tr>
<td>7.</td>
<td>Female</td>
</tr>
</tbody>
</table>

These volunteers were admitted to the NAMRU-2 ward on July 15, 1959, at the beginning of the school summer vacation. They were hospitalized for a period of 9 months, except volunteer 7 who was discharged at the end of the first 4 weeks. When the school term began in late September, the volunteers were allowed to attend classes, but they returned to the ward for meals and overnight. After 9 months, when the first period of treatment with oxytetracycline eye ointment was finished, they were discharged from the ward. Close observation was continued by periodic examination.

The clinical illnesses were more acute than would be expected in natural trachoma. Despite the acute nature of the disease, the volunteers remained comfortable throughout the experiment. The lack of discomfort was undoubtedly related to their blindness.

**Virus Inoculums. Trachoma:** The first Taiwan virus isolate, TRIC-1 Taiwan-1-1958, abbreviated TW-1 (formerly called TW-10) (10) was used. Yolk sacs of the seventh egg passage virus were pooled and a 20 per cent yolk sac suspension made in 10 per cent broth saline. After removing gross debris by light centrifugation, the supernatant fluid was distributed in 1 ml quantities to a number of screw-capped vials and stored frozen in an electric freezer at -65°C. The inoculum in this experiment had been stored 4 months. Titration in the yolk sac of eggs of the material used on the day of the first inoculations was ELD<sub>50</sub>: 10<sup>-1.8</sup> and EID<sub>50</sub>: 10<sup>-2.8</sup>. The inoculums used were diluted with a 20 per cent normal yolk sac suspension.

**Adenovirus Type 4:** The prototype adenovirus type 4 harvested from HeLa cell cultures at their complete cytopathogenic stage was used. A 1:2 dilution was made in 20 per cent normal yolk sac suspension.

**Control:** A 20 per cent normal yolk sac suspension derived from 12-day-old embryonated eggs was prepared like the trachoma virus inoculum for a control.

**Inoculation.—**Each of the inoculums had a similar appearance in the gross. They were prepared in sterile coded test tubes in 1 ml amounts for each volunteer. The volunteers were placed on their backs and the upper lid of one eye everted exposing the upper fornix. The conjunctiva of the upper fornix and the palpebral region was gently but firmly rubbed with a sterile cotton swab soaked in the inoculum. An additional drop of the inoculum was placed on the conjunctiva and the lid reverted. The eye was then bandaged with sterile gauze. The

---

1 The designation TRIC for the trachoma-inclusion conjunctivitis virus group has been suggested by Dr. James Gear.
ophthalmologist (YFY) who carried out the inoculation procedure was not informed of the materials used for inoculum.

**Clinical Observations of the Volunteers.**—The eyes of each volunteer were examined by the ophthalmologist at 1 to 3 day intervals during the first 2 months of the experiment. Observations were made at weekly intervals through 8 months and then less frequently up to 1 year. At each examination, findings were recorded by severity (+ to +++) and appropriate

<table>
<thead>
<tr>
<th>No.</th>
<th>Virus</th>
<th>Eye</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>TW-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Chart 1.** The horizontal bars in all of the charts indicate follicular conjunctivitis, and their height its severity. Findings for 1 month after experimental eye inoculation of seven blind human volunteers with trachoma virus, adenovirus, and normal yolk sac.

anatomic location for discharge, congestion, swelling, turbidity, pseudomembrane, petechial hemorrhage, hypertrophy, follicle, papilla, pannus, scar, and lymphadenopathy. Magnifying lenses of 13 and 20 diopters and a slit lamp were used for the clinical examinations.

**Laboratory Examination.**—Laboratory examinations consisted of bacteriologic culture, examination of conjunctival smears for inclusion bodies, embryonated egg yolk sac culture for trachoma virus, and trachoma complement fixation serology. The results were not given to the ophthalmologist to avoid any influence on his clinical observations. The methods used were as follows.

**Bacteriology:** A sterile platinum loop was rubbed against the lower fornix of each eye using great care to avoid lid or hairs. The loop was immediately streaked on sheep blood agar plates. Bacterial colonies appearing on the plates after 48 hours' incubation at 37°C were identified by appropriate techniques.

**Inclusion bodies:** Scrapings from the conjunctiva of the upper lid were taken with a sterile
platinum spatula and streaked on a clean slide. This was quickly dried in air and fixed with anhydrous methyl alcohol for 5 minutes and again air-dried. The slides were stained with Giemsa solution type B class 2 obtained from Hartman Leddon Co., Philadelphia. The detailed instructions on use of the stain which accompanied the product were followed. Each slide was examined microscopically for 30 minutes and the following gradings for the presence of inclusion bodies recorded: 0, No typical inclusion bodies found; +, Typical inclusions found only in 1 or 2 places on the slide; ++, Typical inclusions found in several areas of the slide or 2 to 3 inclusions in a single field; ++++, Typical inclusions easily found in many areas of the slide or 3 to 5 inclusions in a single field.

Virus Isolation.—Our method for isolation of trachoma virus has been published (10). When swabbing the conjunctiva great care was exercised to avoid touching other areas and contaminating the specimen. Two egg passages were carried out for each eye specimen before declaring it negative. The isolation of adenovirus was accomplished in HeLa cell tissue culture.

Serology.—Trachoma antibody was measured by a complement fixation (CF) test using a purified elementary body antigen which has been shown to give a specific reaction for trachoma (11). Neutralizing antibody against adenovirus type 4 was measured in HeLa cells (12). Results of both tests are presented as reciprocals of original serum dilutions.

Trachoma Vaccine.—The vaccine used was prepared from both TW-1 and TRIC-Taiwan-3-1959 (formerly TW-29) strains of trachoma virus. The method of preparation has been presented in a previous report (8). Partially purified elementary bodies inactivated with formalin and attached to aluminum hydroxide particles were used as vaccine. This vaccine contained a 20 per cent concentration of original yolk sac by weight and was similar to that used in an antibody response study in humans (8, 13) and half the concentration employed in field trials (14). The placebo was prepared from normal yolk sacs in a similar fashion.

Treatment.—Oxytetracycline ophthalmic ointment with polymyxin B sulfate (Pfizer Laboratories, Brooklyn, New York) was used for local application and sulfamethoxypyridazine (MIDICEL: Parke Davis & Co., Detroit) was used for oral therapy.

RESULTS

Differentiation Between Inoculums

Seven blind eyes, one from each of the seven volunteers, were subjected to the first inoculation on July 17, 1959. The results following inoculation during the first 4 weeks are presented in Chart 1.

The two eyes which received normal yolk sac did not show an inflammatory process except for slight congestion and swelling of the conjunctiva, probably due to manipulation. The four eyes inoculated with TW-1 and the one inoculated with adenovirus type 4 showed acute follicular conjunctivitis within a week. The inflammatory process was milder and of shorter duration in volunteer 6 who received adenovirus type 4. In the adenovirus infection there was not as much congestion or edema, fewer follicles appeared and they were more transparent. After 2 weeks the follicular conjunctivitis began to decrease and by 1 month after inoculation, the eye appeared normal. Infection with adenovirus type 4 was substantiated by the reisolation of the virus and by an increase of neutralizing antibody titer. The adenovirus type 4 antibody titer on 0, 12, 38, and 52 days after inoculation was <4, 4, 4, and 8. In the volunteers who received TW-1 virus, irrespective of dilution, the signs showed progression over the 4 week period.
The second inoculation was carried out in three volunteers on August 15, 1959, with greater dilutions of the TW-1 virus pool which had been used in the first inoculation.

One eye (No. 5-L) which had been inoculated with normal yolk sac before and two uninoculated eyes (No. 6-R and No. 2-R) received either $10^{-2}$, $10^{-3}$, or $10^{-4}$ dilutions of virus. The results of the second inoculation are shown at the bottom of Chart 1. All three eyes developed follicular conjunctivitis. The $10^{-3}$ dilution of the virus, which represented the limit of dilution of yolk sac infectivity ($EID_{50}: 10^{-3.6}$) caused infection in the eye inoculated, with an acute onset similar to that seen in the eyes inoculated with more concentrated virus.

Typical inclusion bodies of Halberstaedter-Prowazek were demonstrated in each eye inoculated with TW-1, starting 5 to 6 days after inoculation. Trachoma virus was also isolated from each of these eyes on one or more occasions during the first 4 weeks.

---

**Chart 2.** Findings for 1 year after experimental trachoma eye infection of a blind human volunteer with normal corneas.
Clinical Findings in Experimental Trachoma

The clinical illnesses in the six volunteers who received TW-1 virus inoculation were observed for a year.

A serosuppurative discharge was found in each inoculated eye the 2nd day after inoculation and persisted for 3 to 8 months. The conjunctivas became congested, turbid, and edematous in a few days. Follicles developed by 6 days, first in the fornixes and then in the palpebral conjunctivas of both upper and lower lids. Petechial hemorrhages of the conjunctivas and pseudomembranes were also present. Preauricular lymph nodes were tender in all cases. The follicular conjunctivitis increased in intensity and progressed in severity for 3 to 4 months. Follicles in the bulbar conjunctiva was found in each case 4 to 5 months after infection. Decrease in acute changes thereafter was accompanied by appearance of chronic changes. Trachomatous scars developed in the infected eyes of each volunteer 5 to 10 months after inoculation.

Figs. 1 to 6 show different stages of the clinical course including the acute onset and the development of pannus and scar. Figs. 7 and 8 show typical inclusion bodies.

A sample of the clinical course is illustrated in Chart 2 where clinical changes and some laboratory findings for volunteer 1 are presented.

The approximate grades of each main finding are graphically expressed in the chart. Since No. 1 was the only volunteer with undamaged corneas, the development of pannus was carefully observed. Initial pannus was first found by slit lamp during the 4th week after inoculation. Pannus formation progressed for 3 months with the maximum extent of vascularization being 1 mm from the limbus. The pannus remained stationary until treatment was completed. Epithelial keratitis was seen during the formation of pannus but disappeared during the 4th month. Papillary hypertrophy was found after development of follicles and also persisted until completion of treatment. The acute symptoms in the left eye continued for 4 months and then gradually decreased. The follicles became gelatinous after 5 months and conjunctival scars, typical of trachoma, developed during the 8th month.

The right eye of volunteer 1 remained normal for 4½ months, but then a trachomatous follicular conjunctivitis developed. Preauricular lymphadenopathy, papillary hypertrophy, pannus, and later scar formation were observed. The right eye was treated with oxytetracycline eye ointment during the 8th and 9th months and the left eye during the 9th month. Local treatment of both eyes was discontinued at the end of the 9th month. Both eyes had an acute reactivation of disease by the end of the 10th month. A course of sulfamethoxypyridazine for 1 month cleared up the recurrent disease. There remained some flat and transparent follicles and linear scars in the conjunctiva and fine papillae and scars on the right at the end of 1 year's observation.

The Effect of Trachoma Vaccine

Toward the end of the 2nd month of the experiment when the trachomatous changes had become well developed in each inoculated eye, the volunteers were
divided into two groups in order to study the effect of vaccine on the course of their illness. Three of the volunteers (Nos. 2, 4, 5) were given a series of inoculations with trachoma vaccine while the other three (Nos. 1, 3, 6) received placebo injections. Again the identity of the volunteers receiving vaccine was withheld from the ophthalmologist making the clinical evaluation. A total of six 2 ml intramuscular injections were given. Except for local pain and a 24 hr temperature rise experienced by some volunteers following the alum vaccine, there were no reactions.

In Chart 3, findings in the six volunteers are shown over the 1 year course of the experiment. Natural spread of infection to the uninoculated eye occurred in three of the volunteers. First, No. 6 to the left eye within 1 month after inoculation, then No. 1 to the right eye at 4.5 months, and No. 3 to the left eye after 5 to 6 months. These volunteers all received the placebo. There was no spread of infection in volunteers 4 and 5 of the vaccine group. Volunteer 2 had been inoculated in both eyes.

**CHART 3.** The results of egg yolk sac cultures for virus and conjunctival cell smears for inclusion bodies in six blind human volunteers infected with trachoma virus. The dotted areas show the periods of antibiotic and sulfa drug treatment.
Local treatment with oxytetracycline ointment (four times daily) was applied to the eyes of No. 1-R and No. 3-L (spread infections) during the 8th month; then both eyes of the six volunteers were treated during the 9th month. All trachomatous changes showed great improvement and local treatment was discontinued at the end of the 9th month. The volunteers were discharged from the ward at this time.

By the end of the 10th month when the volunteers were called back for examination, reactivation of illness was found in both eyes of volunteers 1, 3, and 6. These were the volunteers who had been given the placebo. There was no reactivation of illness in the three volunteers who had received vaccine.

A course of oral administration of sulfamethoxypyridazine was commenced immediately in all volunteers. Each was given 1 gm of the drug on the 1st day, followed by a daily dose (6 days per week) of 0.25 to 0.5 gm (depending on body size) for a month. The reactivated disease improved rapidly during the course of this sulfa drug administration, following which all cases appeared cured. Residual clinical findings included conjunctival scars and a few flat transparent follicles and/or fine papillae.
The laboratory findings and clinical picture in volunteers 4 and 5 improved somewhat faster than in the others during the 4th to 9th month. The impression of more rapid healing in these volunteers who received vaccine was supported by the lack of spread infection and by their more favorable response to eye ointment treatment.

CHART 5. Results of bacteriologic cultures of conjunctiva during the course of experimental trachoma infections in six human volunteers.

**Laboratory Findings**

A summary of the results of examinations for inclusion bodies and of attempts at reisolation of trachoma virus are also presented in Chart 3. The typical inclusion bodies of Halberstaedter-Prowazek (also substantiated by the demonstration of positive glycogen reaction of the matrix with iodine stain) were found in the epithelial cells of the conjunctiva of all infected eyes. There was some correlation between numbers of inclusion bodies and clinical activity of the disease. Typical inclusion bodies were also found in the corneal scrapings of the right eye of volunteer 3 on several occasions (see Fig. 7).
Virus isolations were obtained on numerous occasions from each inoculated eye with the exception of volunteer 5 from which there was only one isolation at the beginning of infection. Several early eye swab specimens were contaminated. In volunteer 5, inclusion bodies were found up to the beginning of the 7th month. In the other volunteers, virus isolations and/or inclusions were obtained up to the time oxytetracycline ointment treatment was begun. The spread infections and the reactivation diseases after oxytetracycline ointment were also substantiated by demonstration of inclusions and/or virus isolations. There were no positive laboratory findings after sulfa drug treatment.

Results of CF tests for trachoma, performed with serums obtained throughout the course of the experiment, are presented in Chart 4.

The three volunteers (Nos. 2, 4, 5) receiving vaccine showed higher and more persistent titers than those receiving placebo. Serum antibody titers due to infection alone fluctuated between 8 and 16 with one 32 in the three volunteers (Nos. 1, 3, 6) not receiving vaccine. The pattern of antibody titer increase and decline during the course of infection varied among the volunteers, but there was a definite tendency for the antibody titers to fall while active disease was still present.

The results of bacteriologic cultures of the six volunteers during the experiment are given in Chart 5. Generally, the conjunctivas were free from bacteria. Staphylococcus pyogenes was the only pathogenic bacteria found during the experiment. There was no correlation between isolated bacteria and the disease in the eyes, nor were there any particular organisms associated with severity of illness.

DISCUSSION

These six human volunteer infections, in addition to providing important evidence that the large virus agents isolated from trachomatous eyes are the etiologic agent of trachoma, have shown that vaccine prepared from egg yolk sac–grown virus exerts a favorable influence on the course of experimental trachoma infections. These findings are encouraging to an effort to develop a practical and effective preventive vaccine. We have employed experimental trachoma vaccines in humans, both in antibody response studies and in small scale field protection studies. The preliminary results of these studies have been encouraging (14, 15). Snyder et al (16) have reported an experiment in which one person given vaccine had a milder experimental trachoma infection than a control not given vaccine.

Monkeys and other primates are the only laboratory animals in which trachomatous eye disease can be reproduced. As experience has been gained with monkey eye infections, disease more like that observed in humans has been obtained including the production of pannus (17). However, only some of the trachoma virus isolates have proven pathogenic for the monkey eye and only one strain has produced acute disease in high dilution (17, 18). Using monkeys
for studies of vaccine protection, we have obtained evidence that under certain circumstances, vaccines may prevent or modify experimental disease (8, 14). Collier (19) has obtained protection from infections with the closely related inclusion blennorrhea virus in baboons with homologous vaccine, and Dawson et al. (20) have modified monkey trachoma infection with vaccine.

The demonstration of a favorable effect of trachoma vaccine on the course of experimental infection suggests that humoral antibodies can play a role in trachoma and serves to disprove partially theoretical objections to vaccine. Many problems remain in the development of an effective trachoma vaccine. The use of infected yolk sacs to produce vaccine poses difficult problems of purification. However, we have so far been unable to adapt any of our trachoma virus strains either to other chambers of the egg or to tissue culture. An additional problem concerns the number of different antigenic strains of trachoma virus. Studies with the mouse toxin prevention test have suggested that there are at least two separate trachoma strains (21) and there may be more (22). The significance of the strain differences in the mouse protection test in relationship to human protection against trachoma remains to be determined. The possibility of significant strain differences complicates the development of vaccine.

SUMMARY

The TRIC-Taiwan-1-1958 strain of elementary body virus isolated from a trachoma patient on Taiwan has been proven capable of reproducing trachoma by experimental inoculation of six human volunteers. Virus material derived from the seventh passage in embryonated hen eggs caused the clinical picture of trachoma in every inoculation, even at the dilution of $10^{-4}$ of infected yolk sacs (approximately 1 EID$_{50}$). There was a similar clinical picture with each inoculation beginning with an acute follicular conjunctivitis which progressed for 4 months and then persisted with chronic changes until 9 months when treatment was begun. The illness was generally more acute than would be expected in natural trachoma. That trachoma was reproduced was shown by the involvement of the cornea with epithelial keratitis and pannus, and by the occurrence of gelatinous follicles and eventual cicatrization of the conjunctiva. These clinical findings were supported by repeated demonstrations of typical inclusion bodies of Halberstaedter-Prowazek from conjunctival and even corneal cells, by repeated reisolation of elementary body virus in egg yolk sacs, and by the development of complement-fixing antibody with a "specific" trachoma antigen in each volunteer. Control inoculations with adenovirus type 4 and normal yolk sac showed different clinical and laboratory findings.

Experimental trachoma vaccine was given to three of the volunteers to study its effect on the course of illness. An antibody response to the vaccine was demonstrated and there was a modification of disease in the volunteers receiving vaccine. While the three volunteers who received placebo each developed cross-
infection of their uninoculated eye and had an acute reactivation of the bilateral disease after 1 to 2 months of antibiotic eye ointment therapy, the vaccinated volunteers remained free of infection in uninoculated eyes and showed no relapse after ointment therapy.

Treatment with sulfamethoxypyridazine, a sulfa drug with prolonged action, proved to be an effective and relatively simple method of therapy for experimental trachoma.

**BIBLIOGRAPHY**


**EXPLANATION OF PLATES**

**PLATE 114**

**FIG. 1.** Volunteer 5 (left eye). Normal conjunctiva 15 days after inoculation with normal yolk sac. × 4.

**FIG. 2.** Volunteer 5 (left eye). Acute follicular conjunctivitis 18 days after inoculation of TW-1. The conjunctiva is congested, swollen, and turbid. A number of large follicles are present and residual patches of pseudomembrane (arrows) can be seen. × 4.

**FIG. 3.** Volunteer 3 (right eye). Conjunctiva 35 days after inoculation of TW-1 shows distortion of vessel pattern by edema and follicular and papillary hypertrophy (arrow). × 4.

**FIG. 4.** Volunteer 1 (left eye). Enlargement of upper cornea 54 days after infection with TW-1 showing pannus formation with vessel infiltration of about 1 mm (arrow). × 16.
(Grayston et al.: Trachoma infection)
Fig. 5. Volunteer 2 (left eye). Conjunctiva 240 days after inoculation with TW-1. “Cobble-stone” appearance due to numerous large gelatinous follicles which have coalesced to form folds on the fornix conjunctiva. × 4.

Fig. 6. Volunteer 2 (left eye). Conjunctiva 344 days after infection with TW-1 and 2 weeks after sulfa drug treatment. The inflammatory process has subsided leaving some papillae, transparent follicles, and scars (arrow) on the upper fornix. × 4.

Fig. 7. Giemsa-stained smear of corneal scraping from volunteer 3, 196 days after infection showing a corneal cell with an inclusion body in an intermediate stage of development × 950.

Fig. 8. Giemsa-stained conjunctival cell smear of left eye of volunteer 2, 186 days after infection showing inclusion bodies in the late vesicle stage prior to rupture and release of elementary bodies × 950.
(Grayston et al.: Trachoma infection)