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Etiologic Studies of Trachoma on Taiwan.* (25607)

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The etiologic agent of trachoma has for many years been considered to be a virus of the psittacosis-lymphogranuloma venereum (psitt.-LGV) group. Until recently strains of elementary bodies probably representing viruses of this group which were isolated in the yolk sac of eggs from trachoma patients were lost or not confirmed(1). Recent improvements in technic, particularly incorporation of streptomycin, have resulted in isolation of viruses thought to be the cause of trachoma (2), which have been confirmed(3). The adenovirus group have been shown to be an important cause of eye infections(4) and they are known to be frequent causes of acute conjunctivitis in Taiwan(5,6). Adenoviruses have been isolated from cases of trachoma in Saudi Arabia and even considered as a possible cause of the trachoma syndrome(7,8). It is also possible that adenoviruses may complicate or cause part of the clinical picture or contribute to the spread of trachoma through seasonal acute conjunctivitis. Utilizing the newly perfected technics for yolk sac isolation of elementary body agents and standard tissue culture technics for adenovirus, a study was undertaken of the viral etiology of trachoma on Taiwan.

Materials and methods. During October and November of 1958, specimens were collected from first grade school children in 15 of the 16 counties of Taiwan, including the Pescadore Islands. A total of 1600 eye swabs, throat swabs and blood specimens were collected from children on whom a diagnosis of the condition of their eyes was made by oph-

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thalmologists attached to the Government of the Republic of China-World Health Organization trachoma control project on Taiwan (9). For the purpose of this control project, 3 categories of diagnosis were used, trachoma, conjunctivitis and nothing pertinent (N.P.). The trachoma diagnosis followed the modified MacCallan classification as recommended by the WHO expert committee on trachoma(10): I = trachoma at onset, II = established trachoma, III = cicatrizing trachoma and IV = cicatrized or healed trachoma. In addition, trachoma d was used for suspected but not definite trachoma. Almost all those children with the diagnosis of conjunctivitis had chronic follicular or papillary conjunctivitis which was thought to be non-trachomatous usually on the basis of lack of corneal lesions visible with a monocular lens. A few conjunctivitis cases appeared to be acute. The N.P. diagnosis included normal appearing eyes and pathology not due to infections. All children, except those with the diagnosis of N.P. or trachoma IV, were treated with antibiotic eye ointment under the control project. The eye swab and throat swab cultures were taken with dry cotton tipped applicator sticks, then immersed in individual screw capped vials containing 2.5 ml of brain heart infusion broth pH 7.3, without antibiotics for eye specimens, and containing 1000 units of penicillin and 1 mg of streptomycin per ml for throat specimens. The specimens were frozen in dry ice until returned to the laboratory, where refrigeration was continued at –65°C in a mechanical freezer. *Adenovirus isolations*: The technic for isolation and identification of adenoviruses used in this laboratory has been reported(11). HeLa cell tissue cultures were employed and each specimen observed for a minimum period of 30 days prior to being declared negative. *Trachoma virus isolation*: Eye specimens were unfrozen in a 37°C water bath and streptomycin added. The specimen was then allowed to stand at 4°C for 4 hours after which 0.2 ml containing 4 mg of streptomycin was inoculated into the yolk sac of each of 5 embryonated eggs, 6 to 8 days of age. The eggs were incubated at 35°C and candled daily. Upon death, or survival for 12-13 days, the yolk sac of the egg was harvested and an impression smear was stained by a modified Macchiavello method(12) for demonstration of the presence of elementary bodies. Each egg was cultured on blood agar for bacterial contamination. Two yolk sac subcultures were made of negative material. Eggs positive for elementary bodies were passed separately. Positive isolations were associated on passage with increasing numbers of elementary bodies and with death of embryos.

Results. Adenovirus isolations: A total of 922 eye specimens and 878 throat specimens from 6-7 year old children representing all areas of Taiwan were cultured for presence of adenovirus. One adenovirus was isolated from the eye specimens of 468 children with trachoma, 4 were isolated from 374 in the conjunctivitis category and none were isolated from 80 N.P. children, for an isolation rate of 0.5% from the 922 eye specimens. A total of 27 adenoviruses were isolated from the 878 throat specimens examined, for an isolation rate of 3.1%. The isolations were apparently unrelated to the diagnosis of eye condition, since the percent of specimens positive for adenovirus by diagnosis was 4.0 for trachoma, 1.8 for conjunctivitis and 4.6 for N.P. The adenovirus types isolated from eyes included 2 type 8, 2 type 4 and one type 3. A wide variety of adenovirus types was isolated from the throat specimens, including all types from 1 to 10, except types 4 and 9. The most frequently found types were type 10 isolated from 10 specimens and type 5 isolated from 5. One child with type 8 isolated from the eye specimen also had type 8 in his throat specimen. The throat specimen of one child with type 4 in the eye yielded type 5 adenovirus.

Trachoma virus isolations. A total of 4 elementary body virus strains was isolated from 100 eye specimens cultured through 3 passes in the yolk sac of embryonated eggs. A total of 121 specimens was cultured, but 21 were lost by contamination. Isolation methods detailed above are now used, but some of the earlier specimens in this study were cultured without being frozen and with as much as 8-fold less streptomycin. These earlier specimens were more frequently contami-

TABLE I. Trachoma Virus Isolations from Eyes of First Grade School Children on Taiwan in Relation to Diagnosis of Eye Disease.

Clinical eye diagnosis*	No. examined	Positive
Trachoma d	17	0
I	11	0
II	32	4
III	15	0
Conjunctivitis	20	0
N.P.	5	0
Total	100	4

* Modified MacCallan classification of trachoma except d = suspected or questionable trachoma.
N.P. = nothing pertinent.

nated. Table I shows that the 4 isolations came from among a group of 32 children with clinical diagnosis of stage II trachoma. There were no isolations from 43 children with other stages of trachoma, 20 with conjunctivitis or 5 with N.P. It is of further interest that 3 of the 4 isolations came from among 10 patients especially selected by the ophthalmologists as having particularly active disease. Elementary bodies were seen on first passage of 2 of the strains isolated and on second passage of the other 2. All strains killed embryos on second passage. Two strains have been through 19 and 16 passes respectively without any gross changes except some increase in egg pathogenicity. On yolk sac smear elementary bodies resembling those of the psitt.-LGV group are regularly seen.

The 4 Taiwan elementary body strains were shown to be related serologically to the psitt.-LGV group by complement fixation test. Table II presents the results of testing the 4 trachoma strains as antigen with a LGV, a psittacosis and a trachoma antiserum. The LGV serum was from a patient with the disease, the psittacosis serum was from an immunized guinea pig and the trachoma serum from an immunized rabbit. The 4 viruses fixed complement with each of the 3 serums. With the LGV and psittacosis serums, the trachoma strains produced a lower antibody titer than the homologous antigens.

Discussion. Isolation of adenovirus from the eyes of only 0.2% of children with trachoma and 1.1% with conjunctivitis, mostly chronic, would suggest that adenoviruses do not cause either syndrome on Taiwan. Concurrent isolation of 3% adenoviruses from throat specimens of the same children would indicate that the low isolation rate from eyes was not due to inadequate technics of specimen collection, storage, or culture. The adenovirus types isolated from the eye specimens, types 3, 4 and 8 have been frequently isolated from acute conjunctivitis in Taiwan (5.6), while type 3 and 8 have been associated with acute eye disease in other parts of the world(4). The adenovirus types isolated from the throat specimens have been less consistently associated with disease and the significance of these isolations will be considered in a separate report, in conjunction with studies of isolations of adenoviruses from various clinical syndromes on Taiwan. The frequency of adenovirus isolation from eyes of 6-7 year old children on Taiwan agrees closely with that reported from Saudi Arabia(7) for children over 3 years of age. If adenovirus eye infections are usually acute and the viruses present for only several days, a prevalence of 0.5% at one time could represent a considerable incidence during a year.

It is noteworthy that isolation of elementary body virus strains in the yolk sac of eggs

TABLE II. Serological Relation of Taiwan Trachoma Viruses (TW) to the Psittacosis-lymphogranuloma Venereum Group in Complement Fixation Test.

	Serum antibody titers						
	Antigens						
Antiserum	TW-10	TW-21	TW-89	TW-97	LGV	Psitt.	NYS
LGV patient	16	16	8	16	128		4
Psitt. guinea pig	32	64	32	64		256	4
TW-10 rabbit	256	256	256	256			8

Titers expressed as reciprocal of original serum dilution, +++ end point, NYS = normal yolk sac. Trachoma antigens are phenolized, heated 2½% yolk sac suspensions. LGV and psitt. antigens are commercial preparations (Markham). Psittacosis guinea pig serum prepared by immunization with the Markham antigen, human pneumonitis strain. Rabbit immunized with 20% yolk sac suspension of TW-10.

from patients with trachoma is associated with the most active cases. The failure to isolate these viruses from normal appearing eyes, from children diagnosed as having non-trachomatous conjunctivitis and from less active cases of trachoma adds one type of evidence to support the contention that these viruses are the etiologic agent of trachoma. This finding suggests that the clinical diagnostic problems of trachoma, which are usually the less typical and less active cases, would not be helped by virus isolation attempts at this stage of development of isolation technics. Collier (13) has reported isolation of 14 strains of elementary bodies from 14 persons with inclusion body positive trachoma. Snyder *et al.* (14) have isolated 14 trachoma strains, all from persons whose conjunctival cells showed inclusions. In this laboratory trachoma virus has been reisolated 22 times out of 23 attempts beginning 2 weeks after infection, from 6 human volunteers who have been infected with one of the Taiwan trachoma strains in a study to be reported. In these volunteers typical inclusions have been present on conjunctival cell smears at all periods when virus has been isolated. Conjunctival cell smears were not obtained on the first grade school children in this study. However, studies subsequently made in similar groups suggest that the proportion of trachoma that is inclusion body positive in first grade school children in most areas of Taiwan is only about 1%. This is probably the reason for the small number of isolations in this study. One additional Taiwan strain (TW29) has been isolated from an adult male with an active gelatinous stage III trachoma which was inclusion body positive.

In addition to the viruses from China(2) and Africa(3), similar elementary body strains of the psitt.-LGV group have been isolated from trachoma patients in Saudi Arabia (14) and Israel(15). On the basis of morphology and serology the Taiwan strains belong to the psitt.-LGV group and they are probably similar to those being isolated in egg yolk sac in other parts of the world. We believe the Taiwan elementary body strains are trachoma viruses because of the controlled type of isolation studies used and because the disease has been reproduced in 6 human volunteers with one of these strains.

Summary. Eye specimens from first grade school children on Taiwan were cultured in yolk sac of embryonated chicken eggs and in HeLa cell tissue cultures. Four viruses, morphologically and serologically related to psittacosis-lymphogranuloma venereum group, were isolated in yolk sac from 32 children with stage II trachoma, while negative results were obtained with similar cultures of 68 specimens from other stages of trachoma, conjunctivitis and uninfected eyes. These viruses are thought to be etiologic agents of trachoma. One adenovirus was isolated from 468 children with trachoma and 4 adenoviruses were isolated from 375 children with chronic conjunctivitis. Within limitation of age group studied, it is concluded that adenoviruses do not play an important role in trachoma syndrome on Taiwan.

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