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THE ISOLATION OF THE TRACHOMA VIRUS, PRODUCTION OF TRACHOMA VACCINE AND PRELIMINARY RESULTS OF THE CLINICAL TRIALS

(Presented by the Government of the United States of America)

Review of NAMRU-2 Research Studies on Trachoma[1]

World Health Organization experts have estimated that over 400 million people suffer from trachoma today. The largest number of these victims live in Asia and Africa, countries in which agriculture is the main source of income. The damage caused by trachoma results in loss of vision which cannot be corrected either by glasses or by surgical techniques available today. It is quite possible to cultivate a rice paddy in a profitable manner with the loss of a quarter or half of one's vision, but this same individual cannot work in an assembly line. It is thus evident that trachoma may be one of the greatest deterrents to the industrialization which agricultural countries require in order to elevate their standards of living. These considerations were responsible in part for our decision to initiate a trachoma research project.

In 1957 T'ang and his colleagues in Peking isolated three virus strains from patients who had a clinical diagnosis of trachoma. There have been several announcements of the isolation of trachoma virus since the early 1900's, however, the work could not be repeated in other laboratories and it is generally agreed that the establishment of the etiological agent for trachoma is the result of T'ang's work. T'ang's successful isolation was due to the addition of streptomycin to the inoculum placed in the yolk sac of embryonated chicken eggs. The adoption of T'ang's procedure enabled many laboratories throughout the world to isolate viruses from patients with trachoma. It is of some interest that recent studies would indicate that the virus initially isolated by T'ang was probably the virus of inclusion blennorrhea rather than the virus responsible for trachoma.

In 1958 we initiated our studies of trachoma at NAMRU-2 by obtaining cultures from 150 patients. Five viruses were isolated from 87 of these patients who had a clinical diagnosis of trachoma. No viruses were isolated from the 63 patients without eye disease. While we have made no basic change in the procedure of virus isolation as developed by T'ang, minor refinements

/have improved ...

[1] Prepared by Captain R.A. Phillips, Commanding Officer, NAMRU-II.

have improved our ability to isolate the virus and recently 32 viruses were isolated from 67 patients with clinical evidence of trachoma. Three months later, in the same series, 28 isolations were made and a month later, 24 isolations were made for a total isolation of trachoma virus in 42 of the 67 patients in this four-month period. This is portrayed graphically in Table 1. These isolations were made using two egg passages. Attempts at further serial passages did not reveal significant additional information.

After we had several strains of trachoma virus propagating in serial passage, we next decided to ascertain if the virus would in fact reproduce the disease in man. At that time the disease could not be reproduce the disease in man. At that time the disease could not be reproduced in its chronic form with pannus formation in experimental animals and it was necessary to use human volunteers. The Director of the Taipei Blind and Deaf School was approached and one instructor and six students, in all of whom blindness had been caused by traumatic experience and whose eyes were free of infection, volunteered to be human "guinea pigs". Using the double blind procedure, four were given trachoma virus material from egg yolk sac membrane in one eye, two were given uninfected egg yolk sac membrane inoculations in one eye, and the seventh was given adenovirus type 4 in one eye. The adenovirus inoculation was included to demonstrate that our inoculation procedure would produce acute conjunctivitis, which it did. The four individuals who received the viral preparation developed typical acute trachoma and one individual with some remaining corneal tissue developed typical pannus. None of the controls developed the disease until they were subsequently challenged with a viral preparation, following which they too developed trachoma. The virus was isolated repeatedly from the infected eyes.

At the same time these studies were underway, others of our staff were attempting to develop a suitable vaccine. Several preparations were made and it was found that purification of viral material by standard procedures, which included trypsinization and treatment with polymyxin^{B}, resulted in a preparation which produced an appreciable antibody response in monkeys and rabbits. This material was formalized and when given to members of our staff was found to be virtually free of side reactions. As in the animal experiments, antibody levels were elevated as a result of the vaccine.

Further human "guinea pigs" were required and after explaining the requirements, the entire freshman class of the National Defense Medical Center, 142 medical and nursing students volunteered. They were given various amounts of (1) an aqueous vaccine and (2) an aluminum adjuvant vaccine; their increase in antibody titer is shown in Table 2. A further call for human "guinea pigs" from the student group resulted in 17 volunteers and they were challenged with active virus preparations from two of our strains and from a strain obtained from Dr. Snyder at Harvard University.

Of the 17 volunteers, 15 had been vaccinated and 2 had not. Of the 15 vaccinated, 7 did not develop trachoma while 8 of the volunteers did, and the strain of virus inoculated was recovered. Unfortunately, for the purposes of this study, the 2 non-vaccinated volunteers did not develop trachoma; in consequence, these studies were most inconclusive. The 8 volunteers who acquired trachoma were treated with terramycin ointment and all responded with a complete cure.

/Next a field ...

Next a field vaccine trial was initiated with an alum adjuvant vaccine in first grade students and their pre-school siblings in three areas of Taiwan. This study has been underway for almost two years. The annual conversion rate of the pre-school controls in this study has been only 5 per cent, too small a conversion rate to determine the efficacy of the vaccine in the vaccinated siblings at this time. The annual conversion rate in the 5 to 6 year age group is 23 per cent, a rate which is adequate to determine the efficacy of the vaccine in the vaccinated children when they reach this age. Hence, we cannot draw any conclusions until the study has progressed for another year or two.

In order to assess the role of bacterial agents in the development of trachoma, bacteriological cultures were made on eyes of over 400 school children with and without trachoma. There was no difference in the isolation of pathogenic bacteria from the eyes of the trachomatous versus the non-trachomatous eyes. Nor was there any apparent correlation between trachoma and the type of pathogenic organisms isolated. These studies will be continued in an attempt to ascertain if the presence of certain bacteria accelerates or decelerates the development of trachoma in its chronic form.

Utilizing serological techniques, our purified viral preparation has enabled us to distinguish trachoma from the other two members of this group of viruses - psittacosis and lymphogranuloma venereum. Studies we have conducted, using the mouse toxicity test described by Murray of Harvard, has enabled us to compare the antigenic types of trachoma strains isolated in Taiwan with those from other parts of the world. On the basis of the toxicity test, the viral strains we have studied fall into two broad groups.

As we mentioned above, attempts to reproduce trachoma in its chronic form in experimental animals has not been successful. Following acute conjunctivitis, the disease has cleared spontaneously without development of pannus. A year ago we reported the development of pannus in the eye of one monkey before the Asia Pacific Academy Ophthalmological Congress in Manila and the Formosan Medical Association Meeting in November, 1960. Since that time pannus has developed in five other monkeys. The availability of an experimental animal for studies of this disease has enabled us to satisfy Koch's postulates as to the etiology of the disease.

Finally, I should like to make some general comments. For several reasons, it appears to our group of investigators that the classical picture of trachoma with an acute infection of the upper eyelid spreading to a conjunctivitis with the development of pannus followed by scar tissue formation and blindness is not a continuous inevitable process. The disease can be arrested at different stages with appropriate medication and sometimes this arrestment occurs spontaneously. The development of pannus may well be an allergic type of reaction. While our vaccine studies have only been promising in part, we do have studies in which vaccine with Freund's adjuvant has given complete protection in monkey experiments. We are now studying vaccine, with a modified Freund's adjuvant which should be acceptable for human use, in our experiments with monkeys. The vigorous studies on trachoma which are being continued in many institutes throughout the world today leads one to hope that the knowledge which will enable us to control and eradicate this disease will soon be forthcoming.

TABLE 1

REPRODUCIBILITY OF POSITIVE VIRUS ISOLATION AND INCLUSION BODIES IN THREE ISOLATION ATTEMPTS FOR 67 CASES OF CLINICALLY ACTIVE TRACHOMA

		Positive at least - Once	Twice	Three times
Virus Isolation	L-Area	25/38 (66%)	18/37 (47%)	9/33 (27%)
	T-Area	17/29 (58%)	10/28 (36%)	5/28 (18%)
	Total *-	42/67 (63%)	28/65 (43%)	14/61 (22%)
Inclusion Bodies		23/67 (34%)	4/65 (6%)	2/61 (3%)

* NUMBER OF PERSONS POSITIVE/ACTUAL NUMBER OF PERSONS TESTED (PERCENT POSITIVE)

NAMRU-2

TABLE 2

ANTIBODY RESPONSE TO DIFFERENT INOCULATIONS OF THREE TYPES OF TRACHOMA VACCINE IN FRESHMEN MEDICAL AND NURSING STUDENTS

Vaccine, Dose*	No. of Persons	Complement Fixation Antibody Titers + 1 Mo. 0	4	8	16	2 Mo. 0	4	8	16	32	64
A, 2 ml., twice...	12	2	2	4	4	..	..	..	4	3	5
F, 2 ml., twice...	13	3	4	4	2	..	1	7	2	3	..
A, 1 ml., twice...	10	5	3	2	..	2	1	3	3	1	..
F, 1 ml., twice...	14	4	4	4	2	4	2	5	3	..	..
L, 1 ml., twice...	10	3	2	4	1	2	1	5	1	1	..
A, 0.5 ml., twice	11	5	3	2	1	2	1	2	3	2	1
F, 0.5 ml., twice	12	4	2	5	1	2	1	4	4	1	..
F, 0.25 ml., twice	10	4	2	3	1	3	3	3	1	..	..
A, 1 ml., once ..	11	3	2	4	2	3	1	4	3	..	..
F, 1 ml., once...	9	4	1	1	3	5	1	..	3	..	..
S, 1 ml., twice..	10	10	..	..	..	10	..	..	..	..	..
S, 1 ml., once ..	10	10	..	..	..	10	..	..	..	..	..

*A = purified elementary bodies attached to aluminum hydroxide particles; F = purified elementary bodies formalinized; L = live purified elementary bodies; S = formalinized saline.

+ CF antibody titer in initial serum of 132 persons listed in table was <4 (11 persons in this study with initial antibody not shown). Those groups receiving vaccine twice were given the second injection at the same time as the 1-month bleeding.