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Field Studies of Protection from Infection by Experimental Trachoma Virus Vaccine in Preschool-Aged Children on Taiwan.* (28112)

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Following isolation and serial growth in quantity of trachoma virus in a number of places, there has been great interest in the possibility of development of a trachoma vaccine in the hope of hastening eradication of this disease(1). Steps in development and testing of experimental trachoma vaccines on Taiwan have been reported(2-6). These have included preparation of experimental trachoma vaccines, their test in monkeys and then humans for antibody response and protection. Experimental trachoma vaccines have been shown to affect favorably the clinical course and response to antibiotic treatment of experimentally infected human volunteers(7). After 2½ years of observation in a field trial of experimental trachoma vaccine in children on Taiwan, a significant difference between vaccine and control groups has appeared. This report presents the results.

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Materials and methods. Vaccines. Two Taiwan trachoma virus strains were used in preparation of the vaccines. These were TRIC-Taiwan-1 isolated in 1958 (formerly called TW-10) and TRIC-Taiwan-3 isolated in 1959 (formerly called TW-29). Of 29 Taiwan strains tested for cross-protection in the mouse toxicity prevention test, all have fallen into 2 groups of which TW-1 and TW-3 are the prototypes(8). Vaccine tested in the Lungching area was monovalent containing only the TW-3 strain. Vaccine tested in Ta-an was bivalent containing equal amounts of TW-1 and TW-3. In both cases, one ml of vaccine contained virus particles remaining after purification procedure from 0.4 g (40% by weight) of original yolk sac suspension. Details of method of purification of elementary bodies from yolk sac suspensions have been presented(9). Following this purification procedure, which employs trypsin digestion, 3 cycles of high and low speed centrifugation, and polymixin precipitation, the elementary body suspension was killed and stabilized by addition of 0.02% concentration of formaldehyde. Aluminum hydroxide particles were suspended in a 1:200 solution of human gamma globulin. Particles were then sedimented and gamma globulin discarded. When formalized elementary bodies were mixed with coated alum particles, many bodies stuck to the particles. This final aluminum hydroxide vaccine contained about 10

mg of aluminum per milliliter. A suspension of aluminum hydroxide particles in normal saline with 0.02% formalin was used as placebo vaccine. Final suspensions of elementary bodies were tested for sterility, both before and after addition of formalin and aluminum hydroxide particles on blood agar, beef heart infusion broth, thioglycolate broth, and Sabouraud's medium. Sub-cultures were carried out over a total of 15 days.

Serology. Both serum specimens obtained by vena puncture and plasma specimens obtained in a small graduated heparinized pipette by finger puncture were used in serological tests. Capillary blood (0.2 ml) was mixed with 0.9 ml of sterile saline and centrifuged to remove the cells. The remaining 1 ml of 1-10 dilution of plasma was stored at –20°C as were the sera obtained from the venous blood. Capillary specimens were obtained from the smaller children during 1959 and 1960 while venus blood was obtained from those of all ages during 1961 and 1962. The complement fixation test for trachoma as used in this laboratory has been described(9,10).

Trachoma virus isolation and inclusion body demonstration. A sterile eye swab with a cotton tipped applicator stick for virus isolation and a conjunctival scraping with a platinum spatula for inclusion body demonstration were obtained at each examination on each test subject for whom a clinical diagnosis of trachoma dubium or established trachoma was made. In addition, a sampling of children was tested for virus and inclusion bodies at the first examination. Details of technics used for obtaining specimens and for laboratory examinations have been presented along with negative results obtained from children without clinical diagnosis of trachoma(11).

Procedure for field evaluation studies. Field study areas and methods used to choose test subjects have been described(3). The two study areas, Lung-ching and Ta-an, are typical rural townships along the west coast of the Island with average trachoma incidence and severity. Test subjects were preschool-aged children with no clinical evidence of trachoma infection. They were siblings of 1959 first grade children who had active trachoma. The families of these first grade school children with trachoma had a high prevalence of active and healed trachoma(3).

Children 9 months of age or under at the onset of the study received 0.5 ml of vaccine intermuscularly while all other test children received 1 ml. At time of the third and fourth vaccine injection all received 1 ml of vaccine.

Examinations and inoculations were carried out in 6 primary schools, 4 in Lung-ching and 2 in Ta-an. Two ophthalmologists (KHC and IHC) were each assigned to 3 schools—one in Ta-an and 2 in Lung-ching. They examined the same children throughout the study. Ophthalmological examination and eye swab and scraping specimens were obtained in a room separated from that in which children received vaccine inoculations and had blood specimens drawn. Upon examination the ophthalmologists had no knowledge of their prior diagnoses on a child nor whether the child was in the vaccine or placebo group. Randomization of test subjects between the placebo and vaccine groups was accomplished through assignment of a family number to the first grade school children. Numbers were assigned consecutively at time of examination of each of the first grade school children prior to examination of this child's younger siblings. If the final digit in this number was even, the first preschool child in that family who became eligible for the study received placebo vaccine. If the family number was odd, the first child received trachoma vaccine. If more than one child in the family was found suitable for study, the second and subsequent children received placebo or trachoma vaccines depending upon whether the next to the last digit in the family number was odd or even.

Results. Complement fixation antibody response. In the Lung-ching area all subjects were bled before first injection of vaccine and again one month later at the time of second vaccine injection. Thereafter, blood was obtained on ¼ to ½ the test subjects at each examination. Children who were bled were rotated so that the same child was not bled more often than on every third examination. Four blood specimens were obtained from most of the children during the 10 examina-

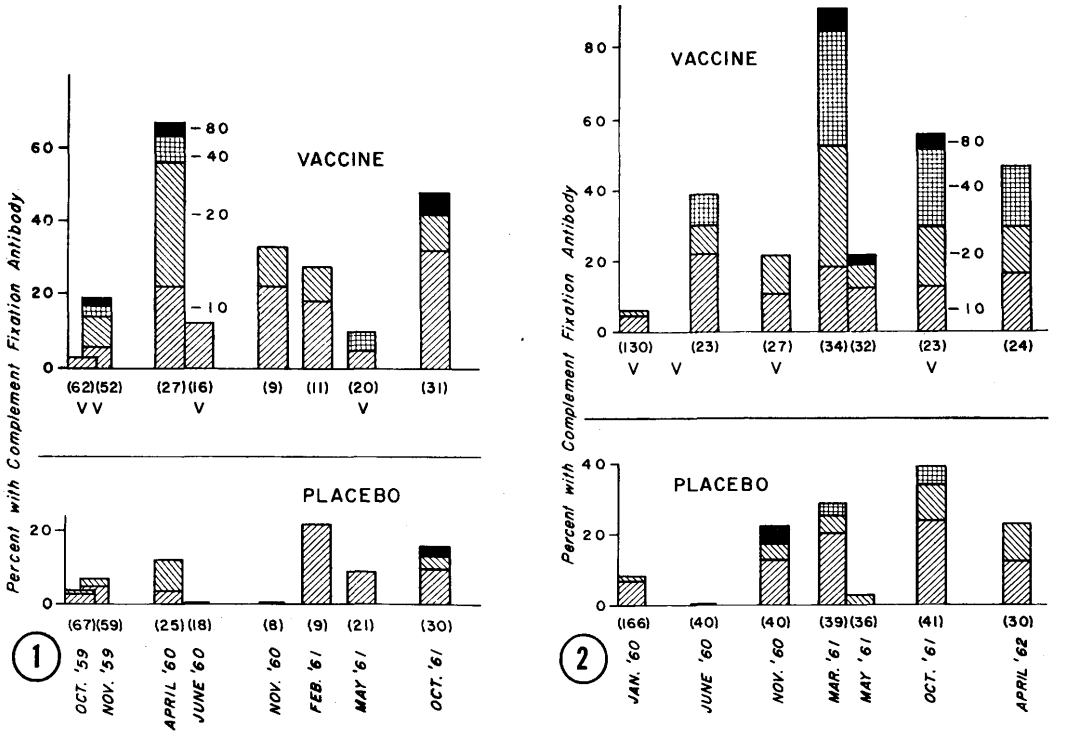

FIG. 1. Results in Lung-ching area of trachoma complement fixation antibody measurement during field protection studies. Per cent of test subjects with antibody and the antibody titer is shown for placebo and vaccine groups at each examination. Number tested appears in parentheses and V indicates time of vaccine injection.

FIG. 2. Results in Ta-an area of trachoma complement fixation antibody measurement during field protection studies. Per cent of test subjects with antibody and the antibody titer is shown for placebo and vaccine groups by time interval. Number tested appears in parentheses and V indicates time of vaccine injection.

tions. In the Ta-an area blood was obtained from all children at the first examination, from none at the second, and from ¼ of the subjects at each subsequent examination with rotation of the children. Three blood specimens were obtained from about ½ the children and 2 from the others. These sera were tested in CF tests with trachoma antigen. Results are shown in Fig. 1 and 2. Only a small number of the test children showed antibody on initial examination. In area 1 the proportion of these showing antibody rose following each vaccine injection. The highest proportion exhibiting antibody was found in April 1960, 6 months after beginning of the study. The proportion of subjects with CF antibody tended to fall gradually with passage of time and to rise again after booster vaccine inoculations. The placebo group showed a slowly increasing number with CF antibody corresponding to the gradual appearance of active trachoma infections. In Ta-an where the first 2 vaccine injections were more widely spaced, highest incidence of CF antibody response (92% of those tested) was found in March 1961, 14 months after the start of the study. Serological tests on a different ¼ of the children 2 months later showed only 22% positive. Again, a rising proportion of the placebo group showed CF antibody as the study progressed. Since the same children were not bled at each interval, these CF results can be interpreted only as indicating a general trend of antibody response.

When results were examined for each individual test subject who had no antibody on first examination, it was found that 53% of those receiving vaccine and 20% of those receiving placebo had a rise in CF antibodies during the study. These percentages were the same in both areas. In both areas and irrespective of whether they received vaccine

TABLE I. Results of Field Trials of Trachoma Vaccine. Cumulative number of children converting* to trachoma by date of examination.

No. in group	Oct. '59	Nov. '59	Jan. '60	Apr. '60	June '60	Nov. '60	Mar. '61	May '61	Oct. '61	Apr. '62
					Lung-ching area study					
	V	V			V			V		
Placebo–62	0	2	6	7	8	9	10	10	10	13
Vaccine–67	0	0	1	2	2	2	3	3	3	3
					Ta-an area study					
			V	V		V			V	
Placebo–155			0	2	5	8	12	13	22	26
Vaccine–121			0	0	0	3	3	4	10	12

V = vaccine given.

* Conversion depends on 2 consecutive diagnoses of active trachoma (MacCallan I, II, or III), or laboratory proof by virus isolation or inclusion body demonstration.

or placebo, children 4-6 years of age at onset of the study showed CF antibody much more frequently than children 1-3 years of age. In those receiving placebo, 3 times as many of the older children developed antibody, while among those given vaccine, older children developed antibody 1½ times as often as the younger. Since only a portion of the children were bled on each examination, it is likely that some developed CF antibodies following booster vaccine injections which were not demonstrated in an earlier or later serum. This finding in individuals taken in association with the results shown in Fig. 1 and 2 would suggest that perhaps 50% of the children had a CF antibody response to vaccine independent of infection.

Protection from clinical trachoma. In the 2 study areas, from 8-10 clinical examinations of the eye were carried out over 2½ years. In Table I the cumulative number of children in both study areas developing trachoma by date of examination is given. The number receiving placebo or vaccine in each area shown in Table I represents the number of test subjects observed throughout the period. There was a loss of subjects from the study group of 13% in area 1 and 7% in area 2 over the 2½ years of observation. Clinical conversion to trachoma was accepted following 2 consecutive diagnoses of active trachoma (excluding trachoma dubium). Date of conversion shown in Table I was that of the first clinical diagnosis. If laboratory evidence of trachoma, either by virus isolation or demonstration of typical inclusion bodies was obtained, conversion was accepted without 2 consecutive active trachoma diagnoses. Diagnosis of trachoma only by laboratory evidence occurred 4 times in each area. In each instance it consisted of laboratory proof of infection in a case diagnosed as trachoma dubium. Table I demonstrates that there were a smaller number of cases of trachoma and a delay in time of the occurrence of cases in those receiving vaccine compared to the placebo group.

In Table II the number of test subjects converting to trachoma by their age at initiation of the study is shown. The total test group is broken down into 3 age groups. It can be seen that the highest frequency of conversion to trachoma occurred in the older

TABLE II. Conversion to Trachoma by Age Group in Trachoma Vaccine Field Trials.

Area		Age in yr 1 & 2		3 & 4		5 & 6		Total	%
Lung-ching	Placebo	5/28		0/20		8/14		13/62	21
	Vaccine	0/32		1/23		2/12		3/67	5
Ta-an	Placebo	4/52		8/58		14/45		26/155	17
	Vaccine	1/42		7/41		4/38		12/121	10
Total	Placebo	9/80	11%	8/78	10%	22/59	37%	39/217	18
	Vaccine	1/74	1%	8/64	13%	6/50	12%	15/188	8

TABLE III. Evaluation of Effectiveness of Trachoma Vaccine in Field Trials.

		Converters to trachoma by April 1962		t-test, P value less than	No. cases with positive laboratory diagnosis	Months of trachoma for all cases		t-test, P value less than
		No.	%			Total	Avg	
Lung-ching	Placebo–62	13	21.0	.01	6	226	3.65	.03
	Vaccine–67	3	4.5		1	72	1.07	
	Effectiveness	79%				71%		
Ta-an	Placebo–155	26	16.8	.10	12	420	2.77	.04
	Vaccine–121	12	9.9		6	157	1.30	
	Effectiveness	41%				52%		
Total	Placebo–217	39	18.0	.005	18	646	2.98	.005
	Vaccine–188	15	8.0		7	229	1.22	
	Effectiveness	56%			62%	59%		

children. Thirty-seven per cent of the 5-6 year old children in the placebo group developed trachoma during the study compared to 10% of younger children in the placebo group.

Because conversion to trachoma occurred later in the study in some subjects than in others and because there was also occasional reversion to normal after trachoma diagnosis was established, the differences between the 2 groups in respect to total number of months of trachoma was compared as another means of measuring effectiveness of the vaccine. In Table III significance of the differences between placebo and vaccine groups in the trachoma conversion rates and in the total months of trachoma disease, are examined for both study areas. When the significance of the differences observed were examined by the t-test, it was found that the difference in proportions of converters was significant for the Lung-ching study and for the combined studies while the difference in months of trachoma was significant in both the Lung-ching and Ta-an studies individually. Utilizing the measurement of conversion to trachoma, the percentage reduction in conversion rates was calculated for each of the areas separately and for the 2 areas combined and called the effectiveness (in per cent). Similar effectiveness percentages were calculated based on measurement of average months of trachoma in each area. Effectiveness was somewhat higher in Lung-ching than Ta-an but averaged about 60% in the combined studies irrespective of which measurement was used. The 95% confidence interval on the 60% reduction in trachoma was 26 to 83% by the method by Noether(12). This provides additional evidence that the observed effect of the vaccine was significant.

Also shown in Table III is the total number of cases of conversion which were proven either by virus isolation, demonstration of inclusion bodies, or both. Laboratory evidence of infection only by demonstration of inclusion bodies occurred 3 times and only by virus isolation 11 times.

One other similar study has been carried out on Taiwan using a bivalent vaccine prepared in the same manner as that used in the Ta-an study except that it was inactivated with ultra-violet light instead of formalin. This study was undertaken in a primary school in Taipei City, again utilizing younger siblings of first grade children with trachoma. This study was carried out with Professor Y. F. Yang of National Taiwan University College of Medicine. A total of 89 children who did not have trachomatous eye disease at beginning of the study were randomly divided with 36 receiving vaccine and 53 placebo. Three injections of vaccine and 6 clinical examinations were performed over 2 years of observation. By January 1962, 28% of the control children and 11% of those receiving vaccine had converted to trachoma. If an effectiveness is calculated for the vaccine in this small group in the same manner as shown in Table III, it is found that the effectiveness

percentage for reduction of conversion to trachoma was 61% and for reduction in total months of trachoma was 72%, figures similar to those obtained in the Lung-ching area. The difference in average months of trachoma between the placebo and vaccine groups was significant in the t-test.

Discussion. Results of these field trials, along with the favorable effect of vaccine on the course of experimental human infections (7), show that first generation experimental trachoma vaccines from this laboratory with relatively low antigen mass have favorably influenced trachoma infection in humans. These studies have emphasized that natural infection under field conditions is a less rigorous challenge than experimental eye infections in both humans and monkeys where acute disease is the inevitable result. These first generation vaccines when tested in monkeys and human volunteers for protection against experimental challenge, although giving partial protection in some experiments, often produced hypersensitivity which caused a more severe infection than that seen in controls (3,5). Although he has presented no experimental results, Cox mentioned several times recently a similar phenomenon in studies he carried out with killed Japanese encephalitis, lymphocytic choriomeningitis, Rocky Mountain Spotted Fever, and epidemic typhus vaccines. He found that preparations which did not contain enough antigen to produce a good immunogenic vaccine actually sensitized the vaccinated animals and made them more susceptible to challenge than the non-vaccinated controls(13).

In these studies in preschool-aged children, a much poorer CF antibody response to vaccine was observed than that found in young adult volunteers who showed 100% antibody response(4). Even in these studies the older children showed a better antibody response to vaccine than the younger, suggesting that some of the responses were secondary or recall phenomenon in individuals previously infected with the trachoma virus but showing no residual eye disease.

Efforts are underway to increase the antigenic mass in trachoma vaccine. Vaccines containing 25 times the antigenic mass of the first generation vaccines have been prepared in these laboratories and studied in monkeys. Complete protection of monkeys, not just modification of disease, has been obtained in a majority of monkeys immunized with these preparations. Human field trials will follow.

Three other laboratories have studied immunization with TRIC viruses. Collier(14) has given live inclusion blennorhea virus subcutaneously and intravenously to baboons. He found that two subcutaneous doses plus one intravenous dose of live virus gave protection against subsequent eye challenge with the same virus strain. Three subcutaneous doses gave partial immunity. He reported that when formalin killed inclusion blennorhea vaccine was given to baboons, it failed to protect against infection and a more severe disease resulted than that seen in controls(15). Snyder and his colleagues(16) gave a formalin-inactivated trachoma virus vaccine to one of 2 human volunteers who were subsequently challenged with the same trachoma virus in the eye. Although both volunteers became infected, a reduced severity was observed in the vaccinated volunteer. Dawson *et al.*(17) immunized monkeys parenterally with live crude yolk sac suspensions of trachoma virus. All immunized monkeys and all controls became infected but a reduced severity was reported in the immunized monkeys.

Summary. Field trials of experimental egg-grown trachoma virus vaccine have been carried out in preschool-aged children in 2 rural areas on Taiwan. Four hundred and five children randomly divided between a placebo and trachoma vaccine groups have been followed for 2½ years during which 4 injections of vaccine have been given. Eighteen per cent of the placebo group have converted to trachoma compared to 8% of those receiving vaccine. An average of 2.98 months of trachoma have been observed in the placebo group compared to 1.22 months in the vaccine group. Both these measures suggested that the vaccine was approximately 60% effective in reducing trachoma. A third study in an urban area with 89 children showed similar vaccine effectiveness.

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Detoxification of Snake Venoms and Venom Fractions by Formaldehyde.* (28113)

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Previous reports from this institution have described the separation of Near Eastern snake venoms into fractions with various toxic, biochemical and antigenic properties (1,2,3). The venom of *Vipera palestinae* was shown to be composed of 2 main toxic fractions, a neurotoxic and a hemorrhagic, whereas *Echis colorata* venom which contains two different hemorrhagins, was found to be devoid of neurotoxic activity. Recent investigation of the venom of two other snakes, *Walterinnesia aegyptia* and *Pseudocerastes fieldii*, showed them to be mainly neurotoxic (4).

Production of potent antivenins is an elaborate and time-consuming procedure and often the antisera obtained are not satisfactory as in the case of *Vipera palestinae* in which antivenin was devoid of antineurotoxic activity (5). Detoxification of snake venoms by formaldehyde treatment has been attempted in the past(6,7). In the search for more effective methods of preparation of antisera against the venoms of Near Eastern snakes we have investigated the effect of formaldehyde treatment on their toxicity and immunogenic activity.

Materials and methods. Venoms and venom fractions. Venoms of *Vipera palestinae* and *Echis colorata* were obtained according to methods described previously(2,3), dried from the frozen state and stored at −20°C until use. Fractionation was carried out by chromatography on DEAE cellulose. Venom fractions consisted of pools of tubes at the peaks of toxic activity. LD_{50}'s of venoms and venom fractions were determined

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