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Trachoma vaccine field trials in The Gambia

By SHIONA SOWA, J. SOWA, L. H. COLLIER and W. A. BLYTH

M.R.C. Trachoma Unit, Lister Institute of Preventive Medicine, London, S.W.1, and Medical Research Council Laboratories, Fajara, The Gambia

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There have been many reports of attempts to induce immunity against ophthalmic infection by the trachoma/inclusion conjunctivitis (TRIC) agents. Vaccines prepared from these members of the genus *Chlamydia* have been tested for their power to prevent infections artificially induced in simian species and in human volunteers, and for their therapeutic and prophylactic efficacy against naturally acquired trachoma in man. Collier (1966) reviewed the problems of producing and testing trachoma vaccines and the results of field trials in various countries. Comparison of these reports, some of which were conflicting, was difficult because of the diversity of vaccines used and of the test conditions; but the general conclusions were that although a measure of protection could be secured, it was usually of short duration, and that no fully effective vaccine then existed. This opinion was reinforced by subsequent articles published in 'Conference on Trachoma and Allied Diseases' (1967) and by our experiments on baboons (Collier & Blyth, 1966*a*, *b*; Collier, Blyth, Larin & Treharne, 1967). Lack of space forbids a detailed review of these papers; but although opinions still differ about the respective merits of live and inactivated vaccines, dosage and the use of various adjuvants, the general experience is that immunity is comparatively short-lived; and that under certain circumstances vaccination may increase both the attack rate in naturally acquired trachoma and the severity of response to artificial challenge. The main exception to these generalizations is the contention of Guerra, Buogo, Marubini & Ghione (1967) that in Ethiopia protective and therapeutic effects were still demonstrable $2\frac{1}{2}$ years after vaccination. This trial was, however, characterized by a high proportion of cures of the infected controls; and like those of other workers, the vaccine used was no more than partially effective.

Our first field trial in The Gambia (Collier, Sowa, Sowa & Blyth, 1963) indicated that a live vaccine given to trachomatous children diminished the severity of the disease in about one-third of those vaccinated, but this effect was of short duration. The present paper describes Trials II and III.

Trial II was a test of the prophylactic efficacy of mineral-oil adjuvant vaccine prepared from a locally isolated strain of trachoma (MRC-187); in Trial III, we tested an aqueous suspension of two strains of trachoma (ASGH and SA-2) isolated respectively in the United States and in Saudi Arabia. Although these vaccines did not induce good immunity, some of the clinical findings and their

modification by vaccination are of interest; for this reason, and because of the expense and difficulty of mounting field trials in developing countries, we report this experimental work in the hope that our methods will be of value to others. The trials were undertaken before the Fourth W.H.O. Scientific Group on Trachoma Research (1966) had recommended standardized methods of clinical examination and scoring for use in field studies of trachoma; our scoring system was, however, similar in principle to that recommended, and our use of the slit-lamp permitted more precise observations than those made by the naked eye or the binocular loupe suggested by the W.H.O. Group for large-scale studies.

MATERIALS AND METHODS

Vaccine for Trial II

TRIC agent

The vaccine was prepared from strain TRIC/WAG/MRC-187/OT (abbreviation: MRC-187) isolated from a Gambian child suffering from early trachoma (Tr I) with micropannus.

Preparation

For the first dose, TRIC agent was purified from yolk sacs infected with the 6th chick embryo passage; sacs were shaken with phosphate-buffered saline (Dulbecco & Vogt, 1954) and strained through gauze to remove the membranes. The crude filtrate was treated with 0·5 % (w/v) trypsin (Difco 1:250) for 30 min. at 37° C., and then subjected to differential centrifugation (Collier, 1961); the elementary bodies were finally deposited at 8000*g* for 20 min., and resuspended in sucrose–potassium glutamate (Bovarnick, Miller & Snyder, 1950) containing streptomycin sulphate 200 μg./ml. The second dose, made some months later, was

Table 1. *Trial II: characteristics of MRC*-187 *vaccines*

	First dose	Second dose
Total elementary bodies ($\log_{10}$/ml.)	9·8	9·4
50 % egg lethal dose ($\log_{10}$/ml.)	5·4	4·2
Titre of group complement-fixing antigen*	1280	Not tested
Total nitrogen (mg./100 ml.)	216·0	31·5

* Reciprocal of dilution giving 50 % fixation with an optimal dilution of antiserum and 2 M.H.D. complement.

prepared from 11th chick embryo passage material. The method was similar except that purification was much improved by slowly adding to the crude yolk sac suspension an equal volume of 2 M-KCl at 0° C. with constant stirring. After centrifugation at 8000 *g* for 20 min. to remove yolk material, the deposit containing the elementary bodies was resuspended as described above. Dummy vaccines were prepared from normal yolk sacs by similar methods. These and the vaccine proper were stored at −70° C. until the day of use. The characteristics of the vaccines before freezing are given in Table 1. Elementary bodies were counted by the dark-ground method of Reeve & Taverne (1962). The *Chlamydia* group antigen content of the first dose vaccine was determined by chess-board titration

of a boiled sample against serum from a sheep infected with enzootic abortion; the use of antibody from this source obviated cross-reactions with antibody to yolk sac. The nitrogen content was determined by the micro-Kjeldahl method.

Safety tests

Vaccines and dummy preparations were tested for the presence of aerobic and anaerobic bacteria (Collier *et al.* 1963) and mycoplasma. They were also tested for extraneous viruses by inoculation into HeLa cell cultures and by intraperitoneal injection into 12 adult mice; and for toxicity by intramuscular injection into three guinea-pigs. No cytopathic effects were observed in the HeLa cells; all the animals remained well for 21 days after inoculation, and no lesions were detected at autopsy.

Oil adjuvant

On the day of use, the vaccines and control preparations were emulsified in an equal volume of a mixture of 9 parts light mineral oil (Drakeol 6 VR, Pennsylvania Refinery Co.) and 1 part of mannide mono-oleate (Arlacel A, Atlas Powder Co.). Arlacel A was proved to be non-toxic by a mouse test (Berlin, 1962). The water-in-oil emulsion of vaccine was prepared and tested as described by Collier & Blyth (1966*b*).

Vaccine for Trial III

TRIC agents

The vaccine was made from a mixture of the 'fast-killing' variants (Reeve & Taverne, 1963) of trachoma agents TRIC/2/SAU/HAR-2/OT ('SA-2'; Murray *et al.* 1960) and TRIC/ /USA-Cal/Cal-2/OT ('ASGH'; Hanna, Jawetz, Thygeson & Dawson, 1960).

Table 2. *Trial III: characteristics of the ASGH and SA-2 components of the vaccine*

	ASGH	SA-2
Total elementary bodies (log_{10}/ml.)	9·0	9·3
50% egg lethal dose (log_{10}/ml.)	6·0*	7·4
Infective units in HeLa Cells (log_{10}/ml.)	7·3	7·3
Total nitrogen (mg./100 ml.)	12·5	11·0

* After storage at −70° C. for 4 months.

Preparation

The purified suspensions of ASGH and SA-2 were prepared respectively by Evans Medical Ltd. and Pfizer (Great Britain) Ltd. The methods were identical, and based on those described for Trial II, except that treatment with trypsin was omitted and an extra cycle of centrifugation in molar KCl was introduced. The final volumes were adjusted so that the infective titres in cell cultures (Furness, Graham & Reeve, 1960) were the same. Table 2 gives the characteristics of each component. These tests were made before freezing, except for the egg titration of ASGH; the original egg titration gave an irregular result, and the recorded titre was obtained by a later test on a frozen sample. The dummy preparation was made

in the Evans Medical laboratory by a similar method. Both vaccine proper and control material were frozen at −70° C. after manufacture. They were transported to The Gambia and stored until the day of use in liquid nitrogen.

Safety tests were similar to those for the Trial II vaccine.

Location and size of the trials

From the trachoma prevalence rates observed in the village of Marakissa during 1959 and subsequently (Sowa, Sowa, Collier & Blyth, 1965), Dr I. A. Sutherland (M.R.C. Statistical Unit) calculated the numbers of non-trachomatous children needed to demonstrate a significant degree of protection by vaccines with differing potencies. For example, in a trial limited to 40 vaccinated children and 40 controls, a vaccine would have to be 100 % effective in preventing trachoma for the result to be statistically significant with a 1-year follow-up, and 75 % effective with a 2-year follow-up. With 100 children in each group, the corresponding figures are

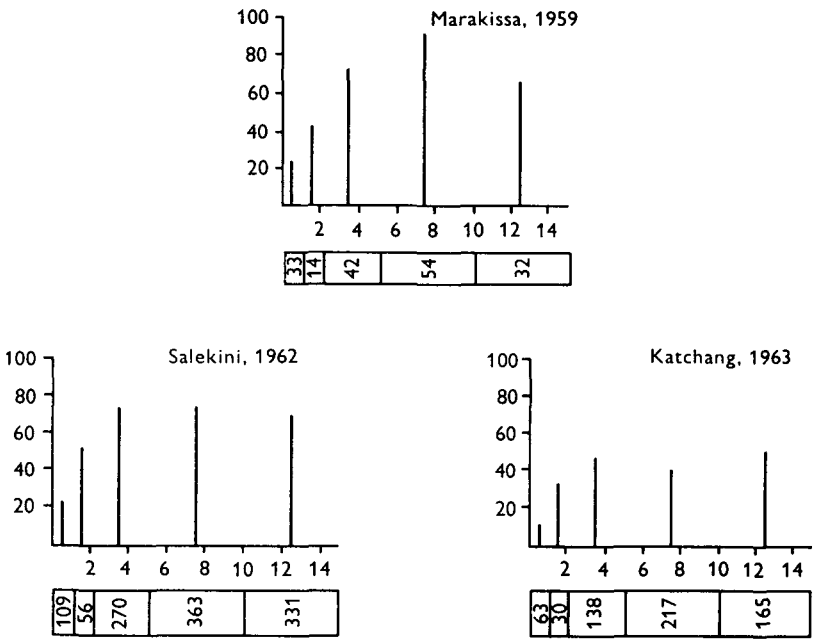

Fig. 1. Prevalence of clinical trachoma (including Tr D) in three Gambian villages. Ordinates: percentage of trachoma. Abscissae: age in years, below which are shown the age groups examined and the total numbers of children in each category; the bars indicating prevalence are placed at the midpoint of each age group.

65 % if observed for 1 year, and 50 % if observed for 2 years. These estimates allow for the high infant mortality rate in The Gambia, which may be as high as 40 % within the first 2 years of life. We hoped to recruit about 300 subjects for Trial II; the Marakissa surveys suggested that this number of children aged from 6 months to 9 years who had not yet acquired trachoma would be provided by a total population of approximately 3000. In case the prevalence of trachoma and losses during the trial proved higher than expected, a somewhat larger population was

desirable, and the villages of Salekini (population 3500) and Katchang (population 1100) were chosen. They are situated on the north bank of the River Gambia about 50 miles (80 km.) from its mouth, and are 15 miles (24 km.) apart. Preliminary surveys showed that the prevalence of trachoma in Salekini was very similar to that in Marakissa, but was substantially lower in Katchang (Fig. 1).

Diagnostic criteria

Trachoma and its various stages were diagnosed in accordance with the main recommendations of the W.H.O. Expert Committee on Trachoma (1962). In brief, *Trachoma dubium* (Tr D) implies clinical signs suggestive of early infection, but follicles and corneal changes are not yet visible or not typical of trachoma, and TRIC agent is not demonstrated in the conjunctiva. Trachoma Stage I (Tr I) means that immature follicles are present on the upper tarsal conjunctiva, including the central area; early corneal changes are usually present, but if not, the diagnosis of Tr I is still permissible if TRIC agent can be isolated. Stage II (Tr II) indicates the presence of well-developed mature soft follicles, papillary hyperplasia and pannus and infiltrates extending from the upper limbus. Stage III (Tr III) is marked by the onset of conjunctival scarring; some or all of the signs of Stage II may be present. A diagnosis of Stage IV (Tr IV) indicates that the follicles and infiltrates have been entirely replaced by scar tissue, and that the disease is healed and no longer infectious (although further changes in cicatrization may follow).

Children without clinical evidence of trachoma are divided into two categories. Those with completely normal eyes are referred to as normal (N); those with minor signs, such as papillae, with or without scanty follicles restricted to the outer angles are given the non-committal diagnosis of 'Abnormal' (Ab).

Selection of children

In Trial II children aged from 6 months to 2 years were examined with an illuminated $\times 10$ loupe; all older children were examined with a slit-lamp. Those with signs suggestive of trachoma were dismissed forthwith, and those with completely normal eyes were admitted; conjunctival scrapings from children diagnosed as Ab were examined for inclusions and were inoculated into chick embryos. When TRIC agent was demonstrated by either method, the child was regarded as trachomatous and dismissed from the trial.

In Trial III the children were selected by slit-lamp examination and, when appropriate, by tests for TRIC agent, from those born in the 2 years since the start of Trial II, and from some older children who were not available at the time of the previous trial. The proportion of children under 2 years of age was thus greater than in Trial II.

Scoring system

The clinical findings in Trial II are analysed only in terms of diagnostic categories; the physical signs were scored according to severity, but the results are not given here because the slit-lamp was not used for all examinations and the

scores are thus not strictly comparable. For Trial III, in which the slit-lamp was used throughout, the system of Collier *et al.* (1963) was used to assess the severity of trachoma acquired after the start of the trial (Table 3). When the physical signs varied in the two eyes, the score for the eye with the most advanced lesions was used in the analysis of results.

Table 3. *Scoring system for physical signs of trachoma*

Physical sign	Degree of involvement	Score
Conjunctival follicles	Scattered follicles over whole of upper tarsal conjunctiva or a few confined to circumscribed area	1
	Follicles over whole of upper tarsal conjunctiva, but not confluent	2
	Confluent follicles over whole of upper tarsal conjunctiva.	3
Papillary hyperplasia	Slight, without cellular infiltration	1
	*Moderate; normal vessels appear hazy	2
	*Pronounced; conjunctiva thickened and opaque, normal vessels obscured	3
Limbal follicles	*One to three typical follicles	1
	*More than three but entire upper lunula not involved	2
	Follicles involving one half or more of the corneal circumference	3
Superficial punctate keratitis	Small localized subepithelial punctate lesions	1
	Punctate lesions affecting approximately half the corneal area	2
	Numerous punctate lesions scattered over entire cornea	3
Pannus	Extension of vessels from upper limbus 0·5 to < 2·0 mm	1
	2·0 to < 4·0 mm	2
	⩾ 4·0 mm	3
Herbert's pits	As limbal follicles, but scores given negative values	−1 to −3
Conjunctival scars	Fine scarring of upper tarsal conjunctiva	−1
	*Moderate readily recognizable scarring with no shortening or distortion of the upper tarsus	−2
	Dense scarring of upper tarsus with or without distortion	−3

N.B. Signs recorded as very slight but definite (i.e. less than 1) are scored + or −0·5.
* Criteria and scoring identical with those of W.H.O. Scientific Group on Trachoma Research (1966).

Tests for TRIC agent

The methods for detecting the agent in conjunctival scrapings by staining with iodine and by inoculation into chick embryos were those of Sowa *et al.* (1965). These tests were made on all children with signs suggestive of active trachoma at the follow-up examinations indicated in Table 4.

Complement-fixation tests

In Trial II only, serum samples from children aged 2 years and over were held at 4° C. until tested for antibody to group antigen; titrations were done on Perspex plates similar to those described by Fulton & Dumbell (1949). The heated antigen was prepared from infected yolk sacs; the end-point was taken as the dilution of serum giving 50% fixation with an optimum concentration of antigen and 2 M.H.D. of complement (Collier & Blyth, 1966*a*).

Table 4. *Trials II and III: summary of vaccination and follow-up procedures*

	Trial II		Trial III	
Type of vaccine	Oil adjuvant		Aqueous suspension	
Strain of TRIC agent	MRC-187		SA-2*f* ASGH*f*	
First dose	0·5 ml. intramuscular in buttock, May 1963		0·5 ml. deep subcutaneous in arm, January 1965	
Second dose	0·5 ml. intramuscular in opposite buttock 6 months later		0·5 ml. deep subcutaneous in opposite arm 3 weeks later	
Follow-up examinations (months after 1st dose)	6	12	12	24
Clinical examination	× 10 loupe	× 10 loupe or slit-lamp	Slit-lamp	Slit-lamp
Tests for inclusions	No	Yes	Yes	Yes
TRIC agent isolation attempted	No	Yes	No	No

Randomization and 'double-blind' procedures

A punch card was prepared for each child in the trial. The cards were sorted into age groups and those in each group were thoroughly shuffled and allocated alternately to vaccine and control sets. The vaccine and control preparations were identified only by colour code, and the cards were marked correspondingly. For follow-up examinations fresh unmarked cards were prepared showing only the child's name and number; the examiner was therefore unaware of the preparation given and the previous clinical findings.

Methods of vaccination

In Trial II two intramuscular doses each of 0·5 ml. were given at an interval of 6 months, the first into the right buttock and the second into the left.

In Trial III two deep subcutaneous doses each of 0·5 ml. were given at an interval of 3 weeks, the first into the deltoid area of the right arm and the second into the left.

Reaction to vaccination

In Trial II the vaccinated and control children in the first batch were inspected at 24 and 48 hr. In Trial III about 20 children in each group were examined at 24, 36 and 48 hr.

The vaccination and follow-up procedures used in both trials are summarized in Table 4.

RESULTS

Trial II

Composition of control and vaccinated groups. Table 5 gives the age distribution of the children selected for this trial, and the clinical diagnoses before vaccination.

Reactions to vaccination. No local or general reactions were observed in the samples of children examined. We received only one report of an untoward reaction; some time after the second dose, the mother of a vaccinated child said that an abcess had developed at the injection site. It had healed without treatment by the time we were notified.

Table 5. *Trial II: age distribution of control and vaccinated children*

Clinical diagnosis at start*	Nos. of children in age groups				
	0–11 months	12–23 months	2–4 years	5–9 years	Totals
Controls					
N	33	14	36	11	94
Ab	19	6	13	1	39
Totals	52	20	49	12	133
% of group total	39·1	15·0	36·8	9·0	—
Vaccinated					
N	36	11	36	11	94
Ab	16	9	12	2	39
Totals	52	20	48	13	133
% of group total	39·1	15·0	36·0	9·8	—

* For explanation of the abbreviations used in this and subsequent tables see 'Diagnostic criteria', page 703.

First follow-up examination at 6 months. No microbiological tests were made on this occasion. In interpreting the results of this and all other follow-up examinations, *Trachoma dubium* (Tr D) is counted as trachoma. On this basis, 17 (14·4 %) of the control group had acquired trachoma compared with 7 (5·9 %) of the vaccinated group (Table 6). Fisher's exact test for 2×2 tables (1958) shows that this difference borders on significance at the 5 % level of probability ($P = 0{\cdot}053$).

Second follow-up examination at 12 months. Table 7 shows that 25·9 % of the controls had acquired trachoma, compared with 34·3 % in the vaccinated group; the difference is not significant. This result is reflected in the tests for TRIC agent (Table 8). In Salekini, one-third of those examined were positive in both vaccinated

and control groups. By contrast, in Katchang only one conjunctival scraping (from a control subject) yielded TRIC agent; this point will be discussed later.

Irrespective of whether they belonged to the vaccinated or control group, children originally diagnosed as 'Abnormal' acquired trachoma more frequently than those with completely normal eyes at the outset (Tables 6 and 7); by Fisher's exact test this difference was significant at the 6-month follow-up ($P = 0{\cdot}028$) and even more so at 1 year ($P = 0{\cdot}0003$). However, an analysis of variance in which

Table 6. *Trial II: conversions to clinical trachoma at first follow-up examination*

		Clinical diagnosis at Six months after first dose				Percentage conversions to trachoma (including Tr D)
Start		N	Ab	Tr D	Tr I	
Controls						
N	86	44	33	9	—	10·5
Ab	32	4	20	7	1	25·0
Totals	118	48	53	16	1	14·4
Vaccinated						
N	83	32	48	2	1	3·6
Ab	34	7	23	4	—	11·8
Totals	117	39	71	6	1	5·9

Table 7. *Trial II: conversions to clinical trachoma at second follow-up examination*

		Clinical diagnosis at Twelve months after first dose						Percentage conversions to trachoma (including Tr D)
Start		N	Ab	Tr D	I	II	III	
Controls								
N	78	43	22	6	4	1	2	16·7
Ab	30	4	11	10	4	1	—	50·0
Totals	108	47	33	16	8	2	2	25·9
Vaccinated								
N	74	32	21	13	2	3	3	28·4
Ab	31	5	11	10	3	1	1	48·4
Totals	105	37	32	23	5	4	4	34·3

all children who acquired trachoma (including Tr D) were scored 1, and those who did not (including subjects converting to or remaining Ab) were scored 0, showed that the prophylactic effect of vaccination on children with an initial diagnosis of Ab did not differ from that in children with normal eyes.

Antibody response to vaccination. If serum titres of less than 1/20 are disregarded, a positive antibody response to vaccination was observed in only two of 24 subjects tested 6 months after the first dose, and in four of 19 tested 6 months after the second dose of vaccine (Table 9).

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Table 8. *Trial II: tests for TRIC agent in conjunctiva at second follow-up examination*

Clinical diagnosis	Controls		Vaccinated	
	No. tested	TRIC positive*	No. tested	TRIC positive*
		Salekini village		
Ab	14	3	8	1
Tr D	6	2	10	3
Tr I	7	3	4	2
Tr II	2	1	3	3
Tr III	1	1	3	—
Totals	30	10	28	9
		Katchang village		
Ab	12	—	10	—
Tr D	11	1	13	—
Tr I	2	—	2	—
Tr II	—	—	3	—
Tr III	—	—	1	—
Totals	25	1	28	—

* Inclusions demonstrated in conjunctiva, and TRIC agent isolated in chick embryos, except for three subjects in whom only one of these tests was positive.

Table 9. *Trial II: results of complement-fixation tests on serum samples*

Months after vaccinations ...	Nos. of children with titre*				Not tested
	<10	10	20	40	
Controls					
0	29	0	0	0	0
6	25	0	0	0	4
12	21	1	0	0	7
Vaccinated					
0	29	0	0	0	0
6	24	2	2	0	1
12	19	4	3	1	2

* Reciprocal of serum dilution giving 50% fixation with 2 MHD complement and an optimal concentration of *Chlamydia* group antigen.

Trial III

Composition of control and vaccinated groups. The age distribution (Table 10) may be compared with that in Trial II (Table 5). In Trial III, a somewhat higher proportion of children was aged 2 years and under.

Reactions to vaccination. There were no reactions in the 19 control subjects examined, but in 12 of 21 children receiving vaccine proper, mild or moderate local swelling and inflammation appeared within 24 hr. and subsided within the next day or so, without lymphadenitis.

First follow-up examination at 1 year. The overall rates of conversion to clinical trachoma (Table 11) were virtually identical for both vaccinated and control

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subjects. The attack rate of over 60% was about twice that in Trial II (Fig. 2). Isolation of TRIC agent was not attempted in this trial; but the incidence of conjunctival inclusions in Salekini was not affected by vaccination. In Katchang, only two subjects, both in the control group, were inclusion-positive (Table 12).

Table 10. *Trial III: age distribution of control and vaccinated children*

Clinical diagnosis at start	Nos. of children in age groups: 0–11 months	12–23 months	2–4 years	Totals
Controls				
N	40	12	10	62
Ab	16	11	5	32
Totals	56	23	15	94
% of group total	59·6	24·5	15·9	—
Vaccinated				
N	36	25	9	70
Ab	17	9	3	29
Totals	53	34	12	99
% of group total	53·5	34·3	12·1	—

Table 11. *Trial III: conversions to clinical trachoma at first follow-up examination*

		Clinical diagnosis at: Twelve months after first dose							Percentage conversions to trachoma (including Tr D)
Start		N	Ab	Tr D	I	II	III	IV	
Controls									
N	45	10	7	3	3	16	3	3	62·2
Ab	26	4	5	1	1	11	4	—	65·4
Totals	71	14	12	4	4	27	7	3	63·4
Vaccinated									
N	52	12	11	5	1	16	5	2	55·8
Ab	27	1	4	1	6	14	1	—	81·5
Totals	79	13	15	6	7	30	6	2	64·6

Second follow-up examination at 2 years. Again, the trachoma conversion rate in the vaccinated group was similar to that in the controls (Table 13). Although in Salekini there was a higher proportion of inclusion-positive subjects among vaccinated children, the difference between them and the controls was not statistically significant. As before, there were fewer inclusion-positive subjects in Katchang (Table 14).

At 2 years, but not at 1 year, the conversion rate was significantly higher in children initially diagnosed as Ab than in those with normal eyes ($P = 0{\cdot}020$ by Fisher's exact test); but, as in Trial II, analysis of variance revealed no significant difference in the prophylactic effect of vaccination on these two categories.

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Table 12. *Trial III: tests for conjunctival inclusions at first follow-up examination*

Clinical diagnosis	Controls		Vaccinated	
	No. tested	Inclusion-positive	No. tested	Inclusion-positive
Salekini village				
Ab	1	—	—	—
Tr D	1	—	2	—
Tr I	3	—	4	2
Tr II	16	8	23	6
Tr III	5	—	4	—
Totals	26	8	33	8
Katchang village				
Ab	4	—	6	—
Tr D	3	1	4	—
Tr I	1	—	3	—
Tr II	11	1	7	—
Tr III	—	—	—	—
Totals	19	2	20	—

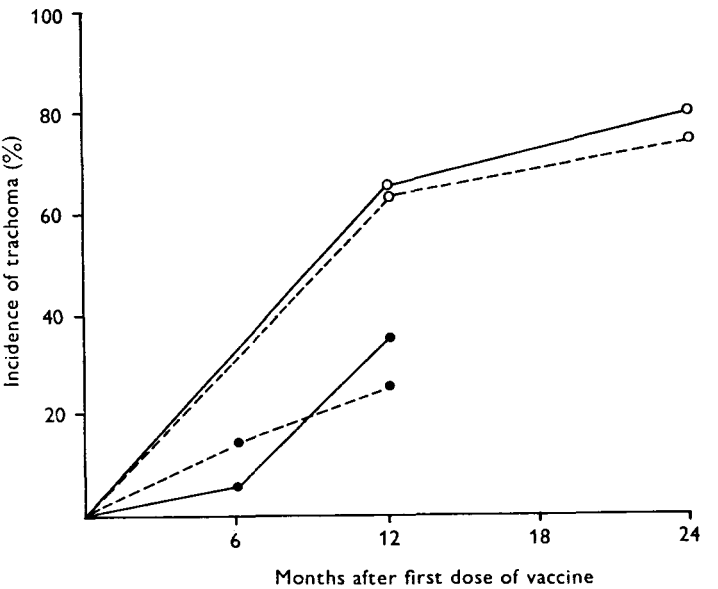

Fig. 2. Trials II and III: trachoma attack rates in vaccinated and control children. Closed circles = trial II; open circles = trial III; continuous lines = vaccinated children; broken lines = control children.

Influence of vaccination on course and severity of trachoma. Table 11 shows that at 1 year there was little difference between the numbers of vaccinated and control children progressing to cicatricial trachoma (Tr III and Tr IV). Two years after vaccination the proportion of subjects with scarring was higher in the controls; of 47 with trachoma, 18 were Tr III or Tr IV, compared with nine of 55 vaccinated children who acquired trachoma (Table 13). By Fisher's exact test the difference is significant ($P = 0{\cdot}034$).

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The mean clinical score of severity did not differ in the vaccinated and control groups at 1 year. At 2 years after vaccination it was significantly higher in the vaccinated group (Table 15).

Table 13. *Trial III: conversions to clinical trachoma at second follow-up examination*

		Clinical diagnosis at Twenty-four months after first dose						Percentage conversions to trachoma (including Tr D)
Start	N	Ab	Tr D	I	II	III	IV	
Controls								
N	38	6	6	—	—	17	5	4	68·5
Ab	25	4	—	—	—	12	8	1	84·0
Totals	63	10	6	—	—	29	13	5	74·6
Vaccinated								
N	47	6	7	1	1	23	6	3	72·4
Ab	22	—	1	—	—	21	—	—	95·5
Totals	69	6	8	1	1	44	6	3	79·7

Table 14. *Trial III: tests for conjunctival inclusions at second follow-up examination*

	Controls		Vaccinated	
Clinical diagnosis	No. tested	Inclusion-positive	No. tested	Inclusion-positive
Salekini village				
Ab	1	—	—	—
Tr D	—	—	—	—
Tr I	—	—	—	—
Tr II	15	8	21	17
Tr III	3	1	3	—
Totals	19	9	24	17
Katchang village				
Ab	3	—	—	—
Tr D	—	—	—	—
Tr I	—	—	1	—
Tr II	6	—	13	1
Tr III	4	1	—	—
Totals	13	1	14	1

Table 15. *Trial III: comparison of clinical scores in control and vaccinated children who acquired trachoma*

Months after vaccination	Group	No. of children	Mean score	Student's *t*	Degrees of freedom	*P*
12	Control	45	4·100	−0·8749	94	> 0·100
	Vaccinated	51	4·569			
24	Control	47	3·085	−2·3529	100	< 0·025 and > 0·020
	Vaccinated	55	4·255			

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Relation between clinical score and presence of conjunctival inclusions. It is generally accepted that inclusions are more likely to be found in severe than in mild trachoma (see, for example, Nichols, Bobb, Haddad & McComb, 1967; Tarizzo, Nabli & Labonne, 1968), and our findings support this contention. Table 16 relates the clinical scores at the two follow-up examinations to presence or absence of inclusions; no distinction is made here between vaccinated and control subjects. At both examinations the mean score in inclusion-positive subjects was about twice that in inclusion-negative children—a highly significant difference.

Table 16. *Trial III: relation of clinical score to presence or absence of conjunctival inclusions*

Months after vaccination	Inclusion-positive (+) or negative (−)	No. of children	Mean score	Student's *t*	Degrees of freedom	*P*
12	+	18	7·000	5·7287	95	< 0·001
	−	79	3·696			
24	+	28	6·910	9·5190	68	< 0·001
	−	42	3·083			

Table 17. *Trial III: differences between the Salekini and Katchang populations in terms of clinical scores for children who acquired trachoma*

Months after vaccination	Population		No. of children	Mean score	Student's *t*	Degrees of freedom	*P*
12	Salekini	control	26	4·461	1·2247	43	> 0·100
	Katchang		19	3·605			
	Salekini	vaccinated	34	5·147	2·1206	49	< 0·050 > 0·025
	Katchang		17	3·411			
24	Salekini	control	29	3·690	2·8302	45	< 0·010 > 0·005
	Katchang		18	2·111			
	Salekini	vaccinated	33	5·258	3·4960	53	< 0·001
	Katchang		22	2·750			

Table 18. *Trials II and III: numbers of children dead or absent at follow-up examination*

		Months after first dose of vaccine					
		Trial II			Trial III		
		0	6	12	0	12	24
Controls	Dead	—	12	2	—	16	6
	Absent	—	3	8	—	7	2
	Remaining	133	118	108	94	71	63
Vaccinated	Dead	—	13	2	—	15	7
	Absent	—	3	10	—	5	3
	Remaining	133	117	105	99	79	69

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Difference in severity of trachoma in Salekini and Katchang. The average clinical severity of trachoma acquired during the trial period was greater in Salekini than in Katchang (Table 17). This difference was not pronounced in the control children examined at the first follow-up examination, but was statistically significant in the vaccinated children; and 2 years after vaccination, the average scores in both control and vaccinated children in Salekini were nearly twice those in the corresponding children in Katchang. These findings probably account for the difficulty of detecting TRIC agent in the Katchang population. To confirm that our failure to demonstrate TRIC agent was not the result of sampling under adverse field conditions, 14 children with active trachoma were brought from Katchang to the laboratory and carefully examined for TRIC agent; inclusions were found in one child only.

Efficiency of follow-up. Table 18 shows the losses of children due to death and absenteeism in both trials. The villagers attributed most of the deaths to measles and malaria.

DISCUSSION

Live vaccines were used in these trials because in our laboratory their immunogenicity in baboons was consistently better than that of inactivated preparations (Collier, 1961; Collier & Blyth, 1966*a*, *b*; Collier *et al.* 1967). This may in part be accounted for by our observation that 'fast-killing' TRIC agents multiplied in a primate host after parenteral injection, with a presumed increase in antigenic stimulation (Collier & Smith, 1967). Nevertheless, good short-term immunity was also induced in baboons by live vaccines made from 'slow-killing' strains which may multiply to a much lesser extent *in vivo* (Collier & Mogg, 1969).

A mineral oil vaccine prepared from strain MRC-187 did not perform well in baboons (Collier & Blyth, 1966*b*), but was nevertheless used in Trial II in the hope that the immunity induced would be sufficient to overcome the small infectious dose received in naturally acquired infection, although it was inadequate against the more severe challenge given under laboratory conditions. A barely significant measure of protection was demonstrable 6 months after the first dose, but not after 12 months, despite a second dose given 6 months after the first. Our findings thus agree with those in a field trial in Saudi Arabia reported by Snyder *et al.* (1964), but contrast with those of Wang, Grayston & Alexander (1967), the efficacy of whose mineral oil vaccine they attributed to its content of more than 10^8 elementary bodies per ml.—a condition that was adequately met by our vaccine (Table 1). The serological findings in Trial II (Table 9) confirm our observation in baboons that mineral oil adjuvant vaccine does not induce a prolonged rise in complement-fixing antibody.

In Trial III, the mineral oil adjuvant was abandoned and a live vaccine of greater purity and higher infective titre was used. The ASGH and SA-2 strains of trachoma were selected as representatives of the two main serotypes defined by the mouse toxicity protection test (Bell & Theobald, 1962). The 'fast-killing' (*f*) variants of each strain (Reeve & Taverne, 1967) were used, since yolk sacs can be

harvested comparatively soon after inoculation and the elementary bodies can be purified much more easily than from chick embryos dying late after inoculation with 'slow-killing' (*s*) strains. In one respect, this decision was unfortunate because Graham (1967) later showed that the $s \rightarrow f$ transformation tends to be associated with loss of antigenic specificity, so that the Trial II vaccine cannot be considered as truly bivalent. However, Graham also pointed out that in mice *f* mutants are more immunogenic than the parent strains, perhaps because they are more invasive; and we ourselves obtained good evidence of the immunogenicity of one such strain in our baboon experiments.

We should have preferred an interval longer than 3 weeks between the first and second doses in Trial III, but difficulties of organization made this impossible. The mild local reactions to subcutaneous vaccine were probably due to multiplication of TRIC agent; similar lesions were observed in baboons (Collier & Smith, 1967). At the first follow-up 1 year after vaccination, there was no difference between vaccinated and control children in the incidence or severity of trachoma; the attack rate was double that in Trial II (Fig. 2), probably because the average age of the Trial III population was lower (cf. Tables 5 and 10). Two years after vaccination, the trachoma conversion rates were still similar in both vaccinated and control groups; but in vaccinated children the proportion progressing to cicatricial trachoma was smaller, and the clinical severity greater. Deleterious effects of trachoma vaccination have now been reported by a number of workers. In a field trial in Taiwan, Woolridge *et al.* (1967) observed over a 2-year period a significantly higher attack rate in children receiving a monovalent oil adjuvant vaccine; but a more commonly reported phenomenon is an increased severity of infection induced by conjunctival challenge of monkeys or baboons previously given vaccines of low potency, vaccines made from TRIC agents differing antigenically from the challenge inoculum, or vaccines with mineral oil adjuvant (for example, Wang *et al.* 1967; Mordhorst, 1967; Collier & Blyth, 1966*b*). These more severe responses are usually ascribed to delayed hypersensitivity induced by vaccination; Wang and his colleagues provide evidence that the allergen is the TRIC agent itself rather than egg material contained in both vaccine and challenge inoculum. Although delayed hypersensitivity is the most immediately obvious explanation of our findings, it is hard to understand why it was not apparent 1 year after vaccination; perhaps the physical signs in vaccinated children who acquire trachoma are the resultant of two forces: a resistance to infection that decreases comparatively quickly and a delayed-type hypersensitivity that may be maintained for long periods. In Trial III, the increased severity on the one hand and failure to heal by cicatrization on the other are probably related, since trachomatous scarring is normally preceded by a diminution of inflammation and follicular hyperplasia.

Another interesting feature of these trials is the increased likelihood of acquiring trachoma in children with an initial diagnosis of 'Abnormal' (Ab), compared with those having completely normal eyes at the outset. Some Ab children may have been suffering from early trachoma, and TRIC agent was in fact isolated from 3 of 14 in Trial II; nevertheless, the physical signs were not at all consistent with

this diagnosis, and it may be that the minor abnormalities observed predispose to TRIC infection.

The pronounced difference between the populations of Salekini and Katchang in terms of severity of trachoma and presence of TRIC agent in the conjunctiva might be explained by environmental factors. In both villages the tribal pattern, general living standard and way of life are similar; but Salekini, with 3500 inhabitants, occupies about the same area as Katchang, whose population is only a third of this. The differences observed may thus be due to overcrowding. In their masterly report on the epidemiology of trachoma in Saudi Arabia, Nichols and his colleagues (1967) draw attention to the paramount importance of poor living conditions as a factor in prolonged infection, which in turn is closely related to severity of disease, persistence of TRIC agent in the conjunctiva, and disabling sequelae.

In reiterating the hope that the methods used in these trials will be useful to others we draw attention particularly to three points. First, the establishment of an efficient follow-up organization permitted considerable economy in the size of the trials; second, examination by slit-lamp, which, given adequate immobilization, is possible with very young babies and greatly improves the accuracy of observation; and third, the use of a scoring system to facilitate the application of statistical tests to the analysis of results. Although the value of scoring systems is generally agreed, the method of application is still open to argument. For example, an important difference between our method and that proposed by the W.H.O. Scientific Group on Trachoma Research (1966) is that we assign negative values to scores for regressed limbal follicles ('Herbert's pits') and cicatrization, regarding them as signs of healing, and in a different category from signs such as follicles and pannus. We think that this procedure is justifiable when assessing the influence of a vaccine or drug on the course of trachoma; in such instances the onset of scarring might be held to represent a beneficial effect of the treatment. By contrast, an epidemiological study of the 'relative gravity' of trachoma in a given population (W.H.O. Expert Committee on Trachoma, 1962) entails evaluation of the prevalence of disabling sequelae; in this case, addition of scores for cicatrization to the total would give a truer picture of the situation. In fact, cicatricial lesions were not a prominent feature of the disease in the very young children in our trials; and our conclusions are unaffected even when scores for Herbert's pits and scarring are added to those for other lesions.

In conclusion, our findings support the view that the prophylactic efficacy of trachoma vaccines prepared by conventional methods falls well short of what is desirable; for much of the argument in favour of vaccination depends on the assumption that a high degree of protection can be maintained for long periods with a minimum of reinforcing doses. Production methods will have to be reappraised, perhaps along the lines of making much more highly concentrated vaccines at an economic cost. It is also clear that the complex mechanism of specific immunity to trachoma needs further study; and the possibility that vaccination decreases rather than enhances resistance to infection must be eliminated.

SUMMARY

The ability of two live trachoma vaccines to protect against naturally acquired infection was tested in young Gambian children. With a mineral oil adjuvant vaccine prepared from a Gambian strain of trachoma (MRC-187) a barely significant measure of protection was demonstrable 6 months after the first dose, but not at 1 year, despite a reinforcing dose given 6 months after the first. In a later trial an aqueous vaccine prepared from the 'fast-killing' variants of strains 'SA-2' and 'ASGH' failed to induce immunity. Two years after vaccination, the proportion of vaccinated children progressing to cicatricial trachoma was less than in the controls, and the average severity of the disease in terms of clinical score was greater; vaccine-induced hypersensitivity may have contributed to this result.

Irrespective of whether they had received trachoma vaccine, children with completely normal eyes at the outset were less likely to acquire trachoma than those with slight conjunctival folliculosis or papillary hyperplasia. In children acquiring trachoma, there was a highly significant positive correlation between severity of the disease and the presence of conjunctival inclusions. The pattern of trachoma differed significantly in the two villages used in both trials; the prevalence, severity and proportion of inclusion-positive subjects were all higher in the village with the greater population density.

An efficient follow-up organization, use of a slit-lamp for clinical observations, and a scoring system for recording physical signs are all desirable for trachoma vaccine field trials.

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REFERENCES

BELL, S. D. & THEOBALD, B. (1962). Differentiation of trachoma strains on the basis of immunization against toxic death of mice. *Ann. N.Y. Acad. Sci.* **98**, 337.

BERLIN, B. S. (1962). Tests for biologic safety of Arlacel A. *Ann. Allergy* **20**, 472.

BOVARNICK, M. R., MILLER, J. C. & SNYDER, J. C. (1950). The influence of certain salts, amino acids, sugars and proteins on the stability of rickettsiae. *J. Bact.* **59**, 509.

COLLIER, L. H. (1961). Experiments with trachoma vaccines: experimental system using inclusion blennorrhoea virus. *Lancet* i, 795.

COLLIER, L. H. (1966). The present status of trachoma vaccination studies. *Bull. Wld Hlth Org.* **34**, 233.

COLLIER, L. H. & BLYTH, W. A. (1966*a*). Immunogenicity of experimental trachoma vaccines in baboons. I. Experimental methods, and preliminary tests with vaccines prepared in chick embryos and in HeLa cells. *J. Hyg., Camb.* **64**, 513.

COLLIER, L. H. & BLYTH, W. A. (1966*b*). Immunogenicity of experimental trachoma vaccines in baboons. II. Experiments with adjuvants, and tests of cross-protection. *J. Hyg., Camb.* **64**, 529.

COLLIER, L. H., BLYTH, W. A., LARIN, N. M. & TREHARNE, J. (1967). Immunogenicity of experimental trachoma vaccines in baboons. III. Experiments with inactivated vaccines. *J. Hyg., Camb.* **65**, 97.

COLLIER, L. H. & MOGG, A. (1969). Dissemination and immunogenicity of live TRIC agents in baboons after parenteral injection. II. Experiments with a 'slow-killing' strain. *J. Hyg., Camb.* **67**, 449.

COLLIER, L. H. & SMITH, A. (1967). Dissemination and immunogenicity of live TRIC agents in baboons after parenteral injection. *Am. J. Ophthal.* **63**, 1589.

COLLIER, L. H., SOWA, S., SOWA, J. & BLYTH, W. (1963). Experiments with trachoma vaccines: therapeutic effect on established trachoma. *Orient. Archs Ophthal.* **1**, 67.

CONFERENCE ON TRACHOMA AND ALLIED DISEASES, SAN FRANCISCO. (1967). *Am. J. Ophthal.* **63**, No. 5, part II.

DULBECCO, R. & VOGT, M. (1954). Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. exp. Med.* **99**, 167.

FISHER, R. A. (1958). In *Statistical Methods for Research Workers*, 13th ed., p. 96. Edited by R. A. E. Crew. Edinburgh: Oliver and Boyd Ltd.

FULTON, F. & DUMBELL, K. R. (1949). The serological comparison of strains of influenza virus. *J. gen. Microbiol.* **3**, 97.

FURNESS, G., GRAHAM, D. M. & REEVE, P. (1960). The titration of trachoma and inclusion blennorrhoea viruses in cell cultures. *J. gen. Microbiol.* **23**, 613.

GRAHAM, D. M. (1967). Growth and immunogenicity of TRIC agents in mice. *Am. J. Ophthal.* **63**, 1173.

GUERRA, P., BUOGO, A., MARUBINI, E. & GHIONE, M. (1967). Analysis of clinical and laboratory data of an experiment with trachoma vaccine in Ethiopia. *Am. J. Ophthal.* **63**, 1631.

HANNA, L., JAWETZ, E., THYGESON, P. & DAWSON, C. (1960). Trachoma viruses isolated in United States. I. Growth in embryonated eggs. *Proc. Soc. exp. Biol. Med.* **104**, 142.

MORDHORST, C. H. (1967). Experimental infections and immunogenicity of TRIC agents in monkeys. *Am. J. Ophthal.* **63**, 1603.

MURRAY, E. S., BELL, S. D. JR, HANNA, A. T., NICHOLS, R. L. & SNYDER, J. C. (1960). Studies on trachoma. 1. Isolation and identification of strains of elementary bodies from Saudi Arabia and Egypt. *Am. J. trop. Med. Hyg.* **9**, 116.

NICHOLS, R. L., BOBB, A. A., HADDAD, N. A. & MCCOMB, D. E. (1967). Immunofluorescent studies of the microbiologic epidemiology of trachoma in Saudi Arabia. *Am. J. Ophthal.* **63**, 1372.

REEVE, P. & TAVERNE, J. (1962). A simple method for total particle counts of trachoma and inclusion blennorrhoea viruses. *Nature, Lond.* **195**, 923.

REEVE, P. & TAVERNE, J. (1963). Observations on the growth of trachoma and inclusion blennorrhoea virus in embryonate eggs. *J. Hyg., Camb.* **61**, 67.

REEVE, P. & TAVERNE, J. (1967). An assessment of the evidence for regarding the T'ang strain as an authentic TRIC agent. *Am. J. Ophthal.* **63**, 1437.

SNYDER, J. C., NICHOLS, R. L., BELL, S. D. JR, HADDAD, N. A., MURRAY, E. S. & MCCOMB, D. E. (1964). Vaccination against trachoma in Saudi Arabia: design of field trials and initial results. *Indian trop. Hlth* **5**, 65.

SOWA, S., SOWA, J., COLLIER, L. H. & BLYTH, W. (1965). Trachoma and allied infections in a Gambian village. *Spec. Rep. Ser. med. Res. Coun.* no. 308.

TARIZZO, M. L., NABLI, B. & LABONNE, J. (1968). Studies on trachoma. II. Evaluation of laboratory diagnostic methods under field conditions. *Bull. Wld Hlth Org.* **38**, 897.

WANG, S. P., GRAYSTON, J. T. & ALEXANDER, E. R. (1967). Trachoma vaccine studies in monkeys. *Am. J. Ophthal.* **63**, 1615.

WOOLRIDGE, R. L., GRAYSTON, J. T., CHANG, I. H., CHENG, K. H., YANG, C. Y. & NEAVE, C. (1967). Field trial of a monovalent and of a bivalent mineral oil adjuvant trachoma vaccine in Taiwan school children. *Am. J. Ophthal.* **63**, 1645.

WORLD HEALTH ORGANIZATION (1962). Expert Committee on Trachoma: Third Report. *Tech. Rep. Ser. Wld Hlth Org.* no. 234.

WORLD HEALTH ORGANIZATION (1966). Fourth Scientific Group on Trachoma Research. *Tech. Rep. Ser. Wld Hlth Org.* no. 330.