*J. Hyg., Camb.* (1972), **70**, 727
*Printed in Great Britain*



# The serum and conjunctival antibody response to trachoma in Gambian children

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(*Received* 4 *May* 1972)

SUMMARY

Ninety-nine young Gambian children were studied for 61 weeks. About half of them had trachoma at the outset, and 80% of the remainder acquired the disease while under observation. IgG trachoma antibody in the serum and IgG and IgA antibodies in the conjunctival secretions (CS) were titrated by an indirect immunofluorescence method. In serum samples obtained in capillary tubes the mean titre was slightly higher than in samples collected on filter paper. Serum antibody at titres $\geqslant 1/10$ was invariably associated with a clinical diagnosis of trachoma; it increased both in frequency and titre as the disease progressed, and was present in about half of those with Tr II. In CS, IgG antibody was present less often and at lower titres than in serum, and IgA antibody was detected even less frequently. There was some evidence of correlation between the titres of IgG and IgA antibodies in CS, but none for a relationship between the titres of the antibodies in serum and those in CS. Antibodies were almost never present in the absence of conjunctival follicles, but their titres were unrelated to the degree of follicular hyperplasia; there was no obvious relationship between the serological findings and corneal lesions. In children diagnosed clinically as trachoma, serum antibody was present in almost all those with conjunctival inclusions, and in a proportion of inclusion-negative subjects; the mean titre was much higher in the inclusion-positive group.

These findings do not settle whether CS antibodies are made locally, or are derived partly or wholly from the blood. They suggest that the indirect immunofluorescence test may be a useful diagnostic aid in trachoma, particularly in view of the rarity of false positive reactions; but there is at present little to choose between it and complement-fixation tests in terms of sensitivity.

## INTRODUCTION

Many workers have studied the serological response to naturally acquired trachoma; most of their reports deal with serum antibody that fixes complement with the heat-stable *Chlamydia* group antigen, but the consensus of opinion seems to be that the appearance of such antibody is insufficiently constant to be of much use in diagnosis (see, for example, Tarizzo, Nabli & Labonne, 1968). Little is

Table 1. *Classification of children by age and clinical diagnosis at start of the investigation* (*Survey* 1)

| Age group (months) | No. of children | Clinical diagnosis | | |
|---|---|---|---|---|
| | | N | Ab | Early active trachoma |
| 0–6 | 33 | 25 | 2 | 6 |
| 7–12 | 20 | 3 | 5 | 12 |
| 13–18 | 14 | 3 | 3 | 8 |
| 19–24 | 9 | 2 | 0 | 7 |
| 25–36 | 23 | 4 | 1 | 18 |
| Totals | 99 | 37 | 11 | 51 |

N = normal eyes. Ab = minor abnormalities (e.g. papillae, scanty follicles at angles) not suggestive of TRIC infection.

known about the time-course of the antibody response to natural infection, and the role of antibody in immunity, pathogenesis and recovery is even more obscure.

In 1966, Bernkopf, Orfila & Maythar first reported the presence of antibody in the conjunctival secretions of people with active trachoma. Anti-TRIC antibody that stained with anti-human gamma globulin conjugated with fluorescein isothiocyanate was found in the conjunctival fluids of 10 of 21 patients; the titres ranged from 1/10 to 1/120, and in some instances were higher than those in the blood sera. Stimulated by these findings, we began in 1968 an investigation to elucidate the following points:

(1) Whether IgA as well as IgG trachoma antibody is present in conjunctival secretions.

(2) The relationship between conjunctival and serum antibody.

(3) The relationship of the presence of antibody to (*a*) clinical diagnosis and (*b*) presence of conjunctival inclusion bodies.

(4) The diagnostic value of trachoma antibody detectable by immunofluorescence.

(5) The relationship between presence of antibody and physical signs such as follicles and corneal lesions.

In addition, the opportunity was taken to compare the merits of the immunofluorescence and iodine techniques for detecting conjunctival inclusions; this study has already been reported (Sowa, Collier & Sowa, 1971).

## MATERIALS AND METHODS

### *General plan*

After preliminary work on immunofluorescence methods, a group of 99 infants was selected in Salekini, a large Gambian village with 3500 inhabitants (Sowa, Sowa, Collier & Blyth, 1969; Sowa *et al.* 1971). Thirty-seven of these children had normal (N) eyes (Table 1); the remainder had early active trachoma with or without corneal lesions, or minor conjunctival abnormalities not suggestive of TRIC infection (Ab). The intention was to make observations not only on well-established trachoma, but also on the appearance of physical signs and antibody in

Table 2. *Comparison of antibody titres in blood samples obtained in parallel by the filter-paper and capillary methods from* 46 *subjects* (*Survey* 4)

| Anti-TRIC IgG antibody titre* | No. of samples | |
|---|---|---|
| | Filter paper | Capillary |
| < 5 | 33 | 31 |
| 5 | 1 | 2 |
| 10 | 1 | 0 |
| 20 | 3 | 4 |
| 40 | 6 | 4 |
| 80 | 2 | 5 |
| No. with titre ⩾ 1/5 | 13 | 15 |

* Reciprocal of end-point serum dilution by indirect FA method: dilutions of samples eluted from filter paper are notional (see text).

infants acquiring the disease during the period of study. The initial survey (S 1) was made in January 1969, and the children were examined on five subsequent occasions (S 2–6) during the next 61 weeks at the intervals shown in Fig. 1. At each survey, the eyes of every infant were examined by slit-lamp, and conjunctival scrapings were examined for inclusions. At S 1, 2, 4 and 5 the blood sera and conjunctival secretions were tested for antibodies to TRIC agent.

*Clinical examination*

The eyes were examined with a Haag–Streit slit-lamp and physical signs were scored by the method of Sowa *et al.* (1969). All observations were made by one ophthalmologist (S. S.); the clinical diagnoses and scores were recorded without reference to the results of previous examinations or to the laboratory findings.

*Tests for inclusion bodies*

Conjunctival scrapings were taken in duplicate and stained at random either with iodine or by an indirect fluorescent antibody (FA) method (Sowa *et al.* 1971). For the purpose of the present investigation, the result was taken as positive if inclusions were found by either method.

*Blood samples*

Blood was taken by finger prick, or, from very young babies, by heel prick. At S 1 and S 2 it was collected on 6 × 24 mm. strips of Whatman no. 1 filter paper, each of which required 0·075 ml. fluid for saturation. At S 4, the method was compared with collection in 2·6 × 75 mm. glass capillary tubes. Table 2 compares the antibody titres in blood samples collected by the two methods; the geometric means for the filter paper and capillary methods are 1/29 and 1/32 respectively; they do not differ significantly (Student's $t$ = 0·271 with 26 D.F.), but since the capillary method appeared to hold a slight advantage it was used exclusively for S 5. Strips and capillaries were transported from the village to the laboratory on wet ice. Thereafter, strips were stored in screw-capped bottles at −60° C. pending

tests for antibody. Capillaries were left for 24 hr. at 4° C., and then centrifuged at 800 *g* for 10 min.; the serum was removed, and kept at −60° C. until needed.

*Conjunctival secretions*

At S1 and S2, conjunctival secretions (CS) were collected by exploiting the technique originally described by Schirmer (1903) for measuring tear flow. The end of a filter-paper strip similar to those used for blood samples was inserted into the lower fornix of each eye; after saturation was complete, each pair of strips was placed in a screw-capped bottle. At S5, a capillary method was used: one drop (0·075 ml.) of sterile 0·85% (w/v) NaCl solution was instilled into the eye; 5–10 sec. later it was collected at the outer canthus into a capillary tube. The fluids from both eyes were pooled. The conditions of transport and storage were like those for blood.

*Tests for antibody by indirect immunofluoresence method*

At each survey, blood samples were tested for specific anti-TRIC IgG antibody, and CS for both IgG and IgA antibodies.

*Phosphate-buffered saline* (*PBS*) (Fothergill, 1964) was used as diluent throughout, and for all washing procedures.

*TRIC agent slide antigen.* BHK-21 cells were grown as monolayers on 9 × 35 mm. cover-slips in Leighton tubes. When just confluent they were seeded with the 'fast-killing' variant (Reeve & Taverne, 1963) of TRIC/2/GB/MRC-4/ON (formerly LB4) at a concentration that infected most of the cells. After a further 36–38 hr. incubation at 37° C., cover-slips bearing cells containing mature inclusions were fixed in acetone for 10 min. at room temperature; preliminary washing was omitted because it impaired fluorescence staining, probably by leaching out soluble antigen. Cover-slips were stored at −60° C. until required.

The inclusions in these monolayers were stained by the indirect FA method with both cross-absorbed (type-specific) and unabsorbed antisera prepared in donkeys against TRIC agents grown in yolk sac, and with an anti-donkey FITC conjugate; these reagents were obtained through the courtesy of Dr Roger Nichols, Harvard School of Public Health. The fast-killing variant MRC-4*f* used in these experiments stained well with unabsorbed sera directed against serotypes 1, 1b and 2 and with anti-type 2 serum absorbed with type 1 antigen; but not at all with low dilutions ($\leqslant$ 1/3) of anti-type 1 or 1b sera cross-absorbed respectively with type 1b and 1 antigens. This variant thus types like its parent strain as type 2 (Sowa *et al.* 1971) but reacts with unabsorbed antisera to types 1 and 1b.

*Anti-human IgA conjugate* was kindly made by Dr W. D. Brighton (National Institute for Medical Research). The serum was from a sheep which had been immunized with human serum IgA according to the schedule recommended by a Medical Research Council subcommittee (1966). The serum was fractionated first by precipitation with neutralized 1·7 M-$(NH_4)_2SO_4$, pH 7·2, then by elution from DEAE Sephadex to produce a globulin fraction. Half of this globulin fraction was conjugated with fluorescein isothiocyanate (FITC) by the method of Brighton (1966).

The original serum was tested for freedom from cross-reactivity with IgG and IgM by double diffusion in agarose gel. The globulin fraction was tested for freedom from other serum proteins by immunoelectrophoresis and the conjugate for freedom from unconjugated fluorochrome by thin layer chromatography on Sephadex G 200 gel. The contaminants were below the maximal levels recommended by Brighton & Lampard (1970).

*Anti-human IgG conjugated with FITC* prepared in goats was obtained from Microbiological Associates, Bethesda, Md, U.S.A. and used at an optimum dilution of 1/20 determined by chess-board titration with a known positive serum.

*Antibody titrations.* At each survey, samples were stored until observations were completed, and then tested together within a period of a few days. CS and blood sera collected in capillaries were serially diluted in PBS. Samples obtained on filter paper were eluted by adding four drops of PBS to each vial, so that the strips were immersed, and holding at 4° C. for 24 hr. The eluate was regarded as a starting 1/5 dilution for further twofold steps. Cover-slips were cut into four equal sections, each of which was used to test one dilution; for staining, the sections were lightly attached with adhesive, cells uppermost, to microscope slides. Cover-slips were treated with dilutions of CS or blood sera, washed in PBS, and then stained with the appropriate dilution of anti-IgG or anti-IgA conjugate. Incubation and washing procedures were as described for yolk sac slide antigens (Sowa *et al.* 1971), except that counterstaining with Evans's blue was omitted. For microscopy, the cover-slips were mounted cell-side down on fresh slides in glycerol-PBS, 9:1. They were examined under dark field illumination with a Zeiss Photomicroscope equipped with an HB 200 ultraviolet lamp; exciter filter BG 12/4 was used in combination with barrier filters 47 and −65. The intensity of fluorescence was read with a X 40 apochromat objective, and scored with the aid of a set of graded density filters (Collier, 1968). The titration end-point was taken as the dilution giving a score of 2.

## RESULTS

### *Diagnostic criteria for trachoma*

In our earlier descriptions of the group of children participating in this study, we stated that '37 had normal (N) eyes, 34 had active trachoma (Tr I or II), 10 had physical signs suggestive of early trachoma (Tr D), and 18 had minor conjunctival abnormalities not suggestive of trachoma (Ab)' (Sowa *et al.* 1971). In describing the criteria on which this classification was based (Sowa *et al.* 1969) we mentioned that the diagnosis of trachoma was made in accordance with the recommendations of the WHO Expert Committee on Trachoma (1962); we also noted that children with a diagnosis of Ab were more likely to acquire classical trachoma than N children. This tendency was pronounced in the study described here; furthermore, all children diagnosed clinically as 'trachoma dubium' (Tr D) (i.e. who had physical signs suggestive of TRIC infection, but who failed to meet the WHO criterion of at least two of the cardinal signs) sooner or later developed the classical disease. We therefore considered that a more realistic classification might be arrived at by considering the clinical history of each child individually

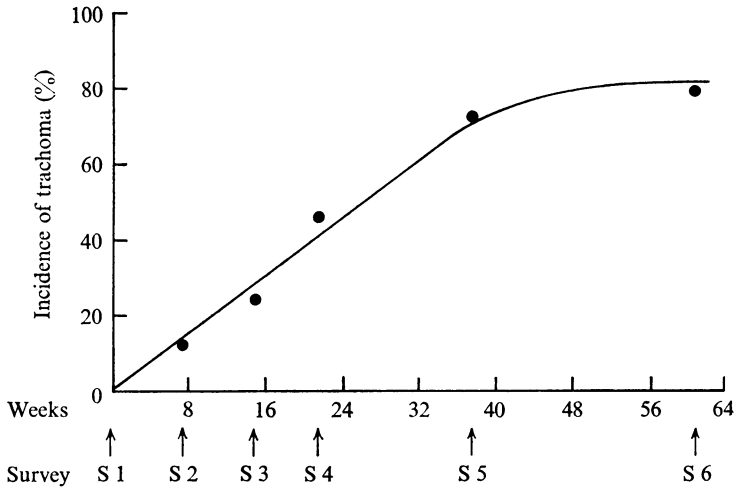


Fig. 1. Cumulative incidence of trachoma in 37 children diagnosed as normal at Survey 1.

at the end of the investigation, taking into account the results of tests for inclusions but without any reference to the results of the antibody studies. At each survey, children were diagnosed as trachoma if they fell into one or more of the following categories:

(*a*) Those with an unequivocal clinical diagnosis of trachoma with at least two of the cardinal signs (follicular hyperplasia, keratitis, pannus, cicatrization).

(*b*) Those with conjunctival inclusions.

(*c*) All those, originally diagnosed as Ab and Tr D, who developed classical trachoma at later surveys; but not children in whom the diagnosis of classical trachoma was immediately preceded by a diagnosis of N (normal eyes).

The reasoning underlying (*c*) is that Ab and Tr D children converting without interruption to classical trachoma were probably infected with TRIC agent from the outset; but this is less likely in children who reverted to N in the interim. An example may help: at six successive examinations, child A was diagnosed as N, N, Ab, Ab, Tr I, Tr II; child B was diagnosed as N, Ab, N, N, Tr I, Tr II. For the purposes of this paper, A is regarded as having acquired trachoma at the 3rd examination, and child B at the 5th.

Table 1 shows the composition of the group at S 1; the 51 children with definite or putative TRIC infections are now classified together as 'early active trachoma'; 34 of them had pannus. Fig. 1 shows the trachoma attack rate in children diagnosed as normal at the outset. Use of the criteria described above for the clinical diagnosis of trachoma gave a plot of the cumulative incidence that conformed better with the expected curve than one based on rigid adherence to the WHO recommendations. The incidence increased regularly until about the 40th week, after which flattening of the curve suggested that almost all the susceptible children had become infected.

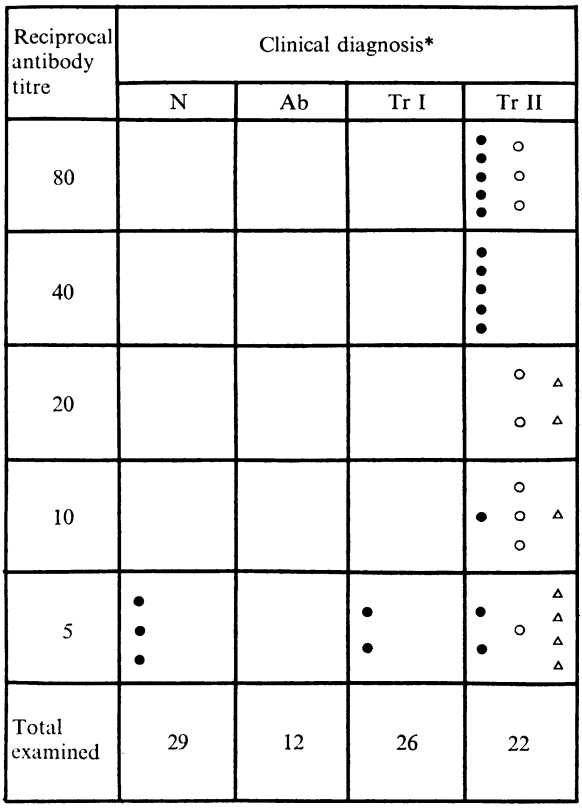


Fig. 2. Relationship of antibodies in sera and conjunctival secretions to clinical diagnosis (Survey 2), ●, IgG antibody in serum; ○, IgG antibody in CS; △, IgA antibody in CS. * See text for abbreviations.

*Relationship of antibodies to clinical diagnosis*

Figs. 2 and 3 are examples of the results obtained as the investigation proceeded; they show the titres of antibodies recorded at S 2 and S 5, 8 and 38 weeks respectively after the start of the investigation.

*IgG antibody in serum.* A titre of 1/5 was occasionally recorded in non-trachomatous children. Titres of 1/10 and over were invariably associated with a clinical diagnosis of trachoma, and this figure will henceforth be regarded as the limiting value in statements about the presence or absence of antibody in the serum. As the disease progressed from Stage I to Stage II, antibody increased both in frequency and in average titre. In S 5, for example, it was present in 29 % of 24 patients with Tr I, with a geometric mean titre of 1/16; in 35 children with Tr II, the corresponding figures were 46 % and 1/28. Antibody appeared to be diminishing in children who had progressed to Tr III, but there were not enough of them to permit a definite conclusion on this point.

*IgG antibody in CS* was present less frequently and at lower titre than the corresponding serum antibody. At S 5, it was present at a titre $\geqslant$ 1/10 in only 6 (17 %) of the 35 children with Tr II. Almost invariably, children with IgG antibody in their CS also had a significant titre of serum antibody.

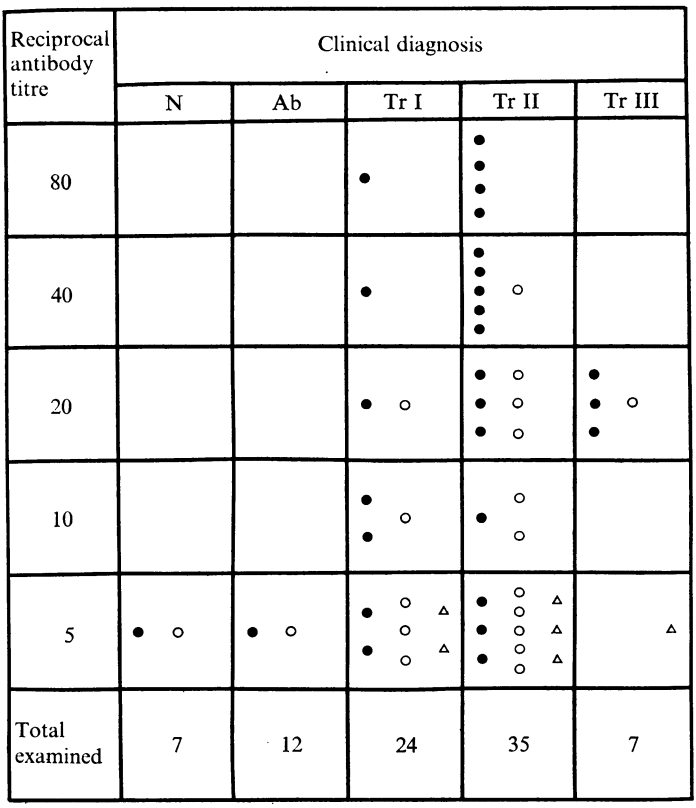


Fig. 3. Relationship of antibodies in sera and conjunctival secretions to clinical diagnosis (Survey 5). Abbreviations and symbols as in Fig. 2.

*IgA antibody in CS* was detected even less frequently than IgG. It was never detected in non-trachomatous children, even at the lowest dilution tested (1/5). In children with trachoma, the titre never exceeded 1/20.

*Relationship between antibody titres in serum and CS*

Fig. 4 is an example, taken from Survey 4, of the interrelationships of the various titres in 15 children with antibodies both in blood and CS. The titre of IgG antibody in the blood was not related to those of the IgG or IgA antibodies in CS. In this survey there was a significant positive correlation between the titres of the two sorts of antibody in the conjunctival secretions; but in other surveys the correlation coefficient for these two variables did not always attain a statistically significant value.

*Relationship of antibodies to clinical and microbiological signs of trachoma*

*Follicular hyperplasia.* There was no evidence that in terms of prevalence or titre the antibodies in the sera or CS were related to the degree of conjunctival follicular hyperplasia; it may, however, be significant that although follicles were often present without detectable antibody, in only one instance was antibody found – in the serum – in the absence of follicles.

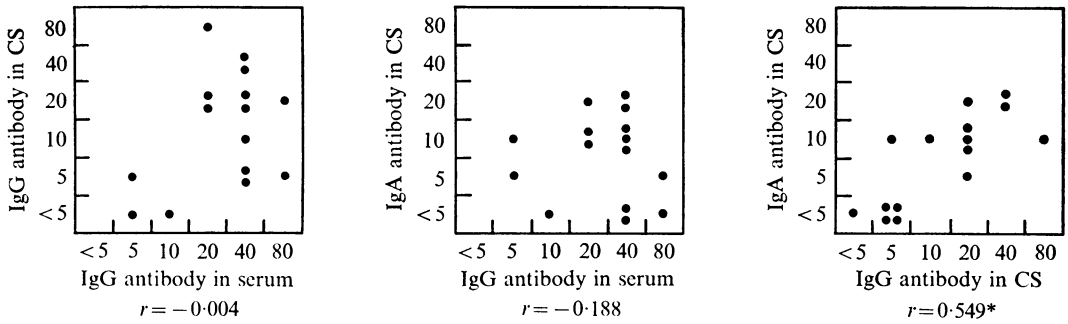


Fig. 4. Relationships of anti-TRIC antibody titres in sera and conjunctival secretions (CS) (Survey 4). Titres are given in terms of reciprocals of end-point dilutions by indirect FA method. *Correlation coefficient significant at 5% level.

Table 3. *Relationship of titre of IgG antibody in serum to presence of conjunctival inclusions in children with clinical trachoma*

| | Inclusion positive | | Inclusion negative | |
|---|---|---|---|---|
| Survey | No. of children | Mean titre* | No. of children | Mean titre* |
| S1 | 10 | 54 | 41 | 2 |
| S2 | 2 | 41 | 46 | 2 |
| S4 | 8 | 22 | 56 | 2 |
| S5 | 16 | 22 | 50 | 2 |

* Geometric means of reciprocal titres.

Table 4. *Relationship of presence of IgG antibody in serum to that of conjunctival inclusion bodies in children with clinical trachoma*

| | | No. of children with antibody titre | |
|---|---|---|---|
| Survey | Inclusions | ⩾ 1/10 | < 1/10 |
| S1 | + | 10 | 0 |
| | − | 4 | 37 |
| S2 | + | 2 | 0 |
| | − | 9 | 37 |
| S4 | + | 7 | 1 |
| | − | 6 | 50 |
| S5 | + | 13 | 3 |
| | − | 8 | 42 |

*Corneal lesions.* Both keratitis and pannus were often present before antibody appeared, but there was no constant relationship between these physical signs and the serological findings.

*Inclusions.* Table 3 shows that in children with trachoma the geometric mean titre of IgG antibody in the serum was much higher in inclusion-positive than in inclusion-negative subjects; but although serum antibody was present in almost all those with inclusions, it was also detectable in a significant proportion of inclusion-negative subjects diagnosed clinically as trachomatous (Table 4). Since

serum antibody was not present in children without clinical trachoma (Figs. 2, 3) its presence at a titre $\geqslant 1/10$ may be a more sensitive laboratory test than the finding of inclusions.

## DISCUSSION

Since this investigation was started, other reports have appeared on the use of immunofluorescence for detecting trachoma antibodies in man and in animals. McComb & Nichols (1969) examined Saudi Arab children. With a system employing yolk-sac slide antigens and rabbit anti-human globulin, they found, like us, that the prevalence and mean titre of antibody in CS collected on filter-paper strips were similar to those in a series of samples collected by eye dropper. No antibody was found in 30 non-Arab control subjects; of 81 trachomatous Arab children, 50 had antibody in CS at titres ranging from 1/2 to 1/320, with a geometric mean of 1/48. These figures are of the same order as ours; a further point of agreement was the finding by McComb and Nichols of a highly significant correlation between the presence of antibody and the finding of TRIC agent in the conjunctiva. These workers detected gamma globulin antibody in the CS of 89% of children with Tr II, compared with our figures of 45–60% for IgG globulin (Figs. 2, 3); However, the lowest dilution tested by them was 1/2, compared with 1/5 in our experiments.

Hathaway & Peters (1971) reported, without quoting titres, that in 25 Saudi Arab children with trachoma antibody in their CS, it was of the IgG type in all and of the IgA type in 20; IgE antibody was also present in 20 (not necessarily the same subjects). By contrast, none of 20 samples from Navajo Indians (presumably trachomatous, although this is not specifically stated) contained IgG or IgE antibody; this group was not tested for IgA antibody.

The findings of Jawetz *et al.* (1971) also varied according to the population studied. In 143 trachomatous Tunisian children IgG antibody was present in the CS of 16 at titres ranging from 1/4 to 1/64. Nine of these children also had IgA antibody in CS with titres of 1/4 to 1/16, but IgM antibody to trachoma was not detectable; complement-fixing antibodies were present in the sera of seven children. Of the 16 children with antibody in CS, five were inclusion-positive; but another 31 children with inclusions had no detectable antibody in their CS. By contrast, the prevalence of antibody in sera and CS was higher in patients diagnosed in San Francisco as having acute TRIC ocular infection; but antibody was found in only 1 of 47 American Indians with very mild trachoma. In the San Francisco series IgG antibody in CS was again present more frequently and at higher titres than was IgA antibody. Jawetz and his colleagues suggested that, since in some patients the antibody titre in the blood is considerably higher than in CS, there is a possibility of transudation from serum to CS through the inflamed conjunctiva; they also put forward the speculation that the presence of antibody in tears may be related to the amount of viable TRIC agent in the conjunctiva.

Some of the findings in man have their counterparts in the results of animal experiments. Wang & Grayston (1971) demonstrated strain-specific antibody in the eyes of Taiwan monkeys infected with TRIC agents; the titre often reflected the

severity of infection. These authors' observations led them to opposing, but not necessarily mutually exclusive explanations of the source of CS antibody. Its titre appeared to be related to that in the serum, and a preliminary experiment indicated that the IgG antibodies in sera and CS were of the same serotype; these results suggested to Wang & Grayston that 'the most important source of eye secretion trachoma antibody is the serum'. On the other hand, the finding of IgA but not IgM antibody in CS also led them to suppose that 'the eye antibody is not directly from the serum, and may to some extent be locally produced'.

Murray *et al.* (1971) used a 4-layer indirect FA technique to study antibodies in owl monkeys; the classes of specific immunoglobulin induced were not defined. Antibodies apparently specific for *Chlamydia* were detected in the sera of some monkeys on receipt in the laboratory. Ophthalmic infection with either of two serotypes of TRIC agent induced antibody both in CS and serum, and protection against challenge with the homologous or heterologous strains; it was suggested that resistance to re-infection might be related to the presence of CS antibody. These observations were confirmed and extended by McComb *et al.* (1971), using similar methods. They demonstrated that owl monkeys with antibody in their sera before exposure to trachoma antigens sometimes had it in their CS as well, but CS antibody was not detectable when it was absent from the serum. There was a positive correlation between the titre of serum antibody and resistance to ophthalmic infection. Killed trachoma antigens instilled into the conjunctival sac induced small rises in CS antibody titres and much greater rises in serum titres; the degree of antibody response was related to resistance to challenge, measured in terms of the numbers of inclusions in conjunctival scrapings. McComb and co-workers concluded that 'the relationship in primates between circulating antibody and response to challenge in the eye with trachoma organisms must be considered established'.

Murray & Charbonnet (1971) adduced further evidence that CS antibody may be related to immunity to *Chlamydia*. In guinea-pigs both conjunctival inoculation of live guinea-pig inclusion conjunctivitis agent (Gp-ic) and intraperitoneal injection of formalin-inactivated organisms induced serum antibody that fixed complement; the conjunctival inoculation also induced IgA antibody in the CS and resistance to challenge, whereas the injection of killed organisms evoked neither.

Turning now to our own findings, we must first refer to the necessity for defining clearly the criteria on which the diagnosis of ophthalmic TRIC infection is based; unless this is done the validity of statements about the relation between antibody and clinical syndromes is open to question. We recognize that the criteria described in this paper depend in part on sequential studies and are thus unsuitable for general use; but we believe that they give a truer picture of the incidence of trachoma in the population investigated than do those of the WHO Expert Committee (1962).

We have previously mentioned (Sowa *et al.* 1971) that the only serotypes of TRIC agent isolated in Salekini, and in another Gambian village, were types 1 and 2 (Bell, Snyder & Murray 1959; Bell & McComb, 1967). Neither type 1b of Bell & McComb nor types D, E and F (Alexander, Wang & Grayston, 1967) have ever

been isolated by us in this area. Since the MRC-4*f* slide antigen used in our tests reacted adequately with unabsorbed antisera prepared against types 1 and 2, it is unlikely that it failed to detect any serum antibodies prevalent in this population.

The preceding summary of other workers' findings indicates that differing views may be held about the origin of antibody in the serum and conjunctival secretions; but these interpretations are as yet based on circumstantial evidence only. Our observations agree well with those of others, but do not settle the question of where these antibodies are formed. Anti-TRIC IgG antibody in the serum seems always to be present at higher titre than the corresponding antibody in CS, and thus may be formed elsewhere than in the eye; although the possibility that it is formed locally but passes more readily into the blood than into the CS cannot be discounted. By analogy with other infections of mucous membranes it seems reasonable to suppose that the IgA antibody in CS, which was shown by Hathaway & Peters (1971) to be of the secretory type, is produced locally. Our finding of a rather tenuous relationship between the titres of IgA and IgG antibody in the CS may be evidence that some at least of the IgG antibody is produced locally; on the other hand, the observation that IgG antibody in CS is detectable only when it is also present in the serum argues for the possibility, mentioned by various authors, of transudation through the inflamed conjunctiva. If antibody is produced locally the obvious site is the conjunctival follicle, which was shown by Jones (1971) to produce antibodies to adenovirus and to the causal agents of molluscum contagiosum and cat scratch disease; in our children conjunctival antibody was never found in the absence of follicular hyperplasia, although the reverse was by no means always true.

We found, like others, that antibody is more likely to be present both in serum and CS if TRIC agent can be demonstrated in the conjunctiva. This relationship may however be indirect since the detection of both antibody and TRIC agent appears to depend on the severity of infection. A consideration of this sort might also apply to inferences about the relation of antibody to immunity; although there is a strong *prima facie* case for supposing that it does play a part, there is at this stage an obvious danger in making *post hoc* inferences about the concomitant appearance of antibody and resistance to infection; cellular factors may be of equal or even greater importance.

One of us (Collier, 1967) suggested that trachomatous pannus might be due to an antigen/antibody reaction at the limbus, but the finding that there was no constant relationship between the appearance of corneal lesions and that of conjunctival antibody does not sustain this supposition.

One important result of these researches is that the presence of IgG antibody in the serum at a titre of 1/10 or greater appeared to be a more sensitive diagnostic indication of trachoma than the finding of conjunctival inclusions, either by the iodine or FA methods. It should, however, be noted that in this investigation the proportion of inclusion-positive subjects was lower than in some of our previous studies in The Gambia (see, for example, Sowa, Sowa, Collier & Blyth, 1965). Tests for trachoma antibody by the indirect immunofluorescence technique may be most useful when the physical signs are atypical or not fully developed, and

when it is difficult to demonstrate TRIC agent in the conjunctiva; the rarity of false positive reactions is a particular advantage. Since the antibody titres reported by those who used complement fixation (CF) methods are of the same order as those measured by FA techniques, it might be inferred that these tests do not differ greatly in sensitivity. However, Hanna *et al.* (1972) recently published the results of parallel CF and FA tests on '150 random sera from Tunisia'. Nearly 20% were positive for antibody to TRIC agent (at titres $\geqslant$ 1/8) by both methods; 48·7% were positive by FA and negative by CF, whereas only 3·3% were positive by CF and negative by FA. Nevertheless, all workers seem to agree that every method so far used in field studies fails to detect antibody in a significant proportion of people diagnosed clinically as trachoma, especially when the disease is mild. It may well be that these relatively crude techniques will be complemented, or even superseded, by more sensitive methods such as the radioisotope precipitation test first used for psittacosis antibody by Gerloff & Watson (1967), or the binding assay with $^{125}$I-labelled Fab' molecules recently described for TRIC agents by Macdonald & Barenfanger (1971); if so, inferences from the results of immunofluorescence tests may have to be modified.

We are grateful to Professor R. Nichols (Harvard School of Public Health, Boston, U.S.A.) and Dr W. D. Brighton (National Institute for Medical Research, London, England) for providing serological reagents; to Mr Malcolm Race for his competent technical help; and to Dr I. A. McGregor and his staff at the M.R.C. Laboratories, Fajara, for assistance with various facilities.

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