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Serological studies on group- and species-specific antigens of trachoma and inclusion conjunctivitis (TRIC) agents*†

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INTRODUCTION

It was shown by Bedson (1936) that psittacosis virus possesses both heat labile and heat stable C.F. antigens. The heat stable antigen was found to be shared by other viruses of the same group (Bedson, Barwell, King & Bishop, 1949; Monsur & Barwell, 1951; Hilleman, 1955; Ross & Gogolak, 1957).

A whole series of procedures have been described for the preparation of ether soluble, alkali soluble and other materials revealing in C.F. reactions group antigen activity of indistinguishable specificity. Both the group- and the species-specific antigenic activity of elementary body suspensions, first observed by Bedson in 1936 in his studies on psittacosis, seem to be confirmed also by recent studies on trachoma. Collier & Sowa (1958) tested, by complement fixation, sera from LGV patients and Frei-negative trachoma patients against the psittacosis–lymphogranuloma group antigen and against three antigens prepared from various trachoma strains. Their 'results revealed a very close relationship between all four antigens'.

Woolridge, Jackson & Grayston (1960) reported that group antigens prepared from trachoma, psittacosis or LGV, all reacted similarly, and they concluded 'that trachoma virus contained the group antigen of the P-LV group and in addition a specific antigen'.

Murray (1962) reported on tests with group antigens prepared from over 40 different trachoma strains as well as from about 20 strains of psittacosis, LGV, meningopneumonitis, feline pneumonitis and bovine abortion. These 60 group antigen preparations were indistinguishable from each other when tested by C.F. with the sera of patients diagnosed as trachoma, psittacosis, or LGV.

Reeve & Taverne (1962) tested boiled antigens prepared from different strains of trachoma and inclusion conjunctivitis with immune sera against different strains of trachoma, inclusion conjunctivitis and with LGV convalescent serum. The results showed that 'the viruses of trachoma and inclusion blennorrhoea are anti-

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genically similar to other members of the group'. Terzin, Fornazarić & Birtašević (1963) prepared group-reactive soluble antigens from trachoma and psittacosis infected yolk membranes. About 100 serum specimens were tested with these antigens; these included normal sera, sera of men and pigeons recovered from ornithosis infections, sera of rabbits and guinea-pigs immunized with ornithosis virus and sera of Bedsonia-positive sheep, cattle, and fowl. All sera showed comparable C.F. titres with group antigens, prepared from trachoma and psittacosis organisms. Both the specificity and the sensitivity (as illustrated by results of checkerboard titration) of the two antigens was found to be indistinguishable by C.F.-test. In the sera of trachoma patients which were free of group antibodies, they were able to demonstrate the presence of species-specific C.F. antibodies, as detected by semipurified elementary body suspensions of trachoma.

The bibliographical data just reviewed briefly, supplemented with the results presented in this report, seem to fit a definite pattern of notions which will be discussed at the end of this paper.

MATERIALS AND METHODS

Diluents

Ca-Mg buffered saline (*pH* 7·2). This was used as diluent for ingredients of the complement fixation test and was that described by Mayer, Osler, Bier & Heidelberger (1946).

Sucrose phosphate saline (*SPS*). Normal saline solution containing 0·2 M sucrose and 0·02 M potassium phosphate buffer pH 7·2. Sterilized by autoclaving at 15 lb. for 20 min.

Sucrose phosphate albumin (*SPA*). SPS-solution with 0·6 % crystalline bovine albumin. The albumin was first dissolved in distilled water to make a 6 % concentration, sterilized by filtration and added to the autoclaved SPS solution in proportions to make a final concentration of 0·6 %.

Bedsonia strains

Inclusion conjunctivitis strain MRC-1/G (formerly LB-1), trachoma strains TE-55 and Cal-1 (formerly Bourassa), psittacosis strain 6BC and mouse pneumonitis strain Nigg (MoPn) were all maintained in yolk sac passages in this laboratory. Also, lines of MRC-1/G and TE-55 strains maintained in cultures of McCoy cells were used.

Complement fixation (C.F.) *tests*

The technique used in performing the C.F. tests was that described earlier (Terzin *et al.* 1963).

Antigens

Suspensions of purified elementary bodies (E.B. suspension) were prepared from agents grown both in yolk membranes of embryonated eggs and in cultures of McCoy cells.

Yolk grown E.B. suspensions. Heavily infected yolk membranes (with strains MRC-1/G, TE-55, Cal-1 or psittacosis 6BC) were homogenized three times for

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10 sec. in a Waring blender. In the subsequent description, the volume of the homogenate is designated as X ml. Equal volumes of homogenate and precooled SPS were mixed and centrifuged at 35,000g for 20 min. The supernatant fluid with a thick fatty layer was discarded and the sediment resuspended in $2X$ ml. of SPS. Armour Tryptar (trypsin) diluted in SPS to 500 units/ml. was added in a volume of $2X$ ml. and the resulting $4X$ ml. suspension (with a final trypsin concentration of 250 units/ml.) was kept at room temperature for 1 hr. under constant stirring.

The trypsinized suspension was centrifuged for 15 min. at 1500 r.p.m. in a horizontal centrifuge, the sediment was discarded and the supernatant centrifuged for 20 min. at about 27,000g. The separated pellet was resuspended in X ml. of SPA diluent and mixed with celite in the proportion of 1 g. of celite per 10 g. of yolk sac. After stirring for 5 min. at room temperature, the mixture was spun for 15 min. at 1500 r.p.m. The supernatant was decanted and designated as Supernatant I and the sediment was mixed with $X/2$ ml. of SPA for 3 min. at room temperature. By centrifugation for 15 min. at 1500 r.p.m., the sediment was separated and discarded, and the supernatant pooled with 'Supernatant I'. A total volume of approximately $1{\cdot}5X$ ml. of the pooled supernatants was centrifuged for 20 min. at 27,000 g to obtain a supernatant which was discarded and a dark yellow pellet of homogeneous appearance. At this stage of the preparation some of the sediments were kept overnight at 4° C., in well-protected containers. The pellet was resuspended and washed three times in SPA diluted 1/4 with saline (using centrifugation in a Spinco centrifuge at 27,000g for 25 min.). The washed pellet was resuspended and well homogenized in $X/20$ ml. of SPA, spun for 5 min. at 1500 r.p.m. in a horizontal centrifuge to remove clumps and the supernatant decanted was designated as 'E.B. susp.' of the respective strain. The LD 50 for chick embryos of the different E.B. suspensions was found to be about $10^{-6} \pm 1$ log unit.

Tissue culture grown E.B. suspensions. Strains of TE-55 and MRC-1/G were grown in McCoy cells by methods previously developed by Gordon, Quan & Dressler (1963). Harvests of cells and fluids from twelve chronically infected T-30 flask cultures, that had been maintained for weeks or months on Eagle's medium supplemented with vitamins, glutamine and 10% horse serum, were pooled and processed as follows. After disruption of the cells in a 10 KC Raytheon sonicator at full power for 5 min., the suspension (about 100 ml.) was centrifuged for 40 min. at 8000g in a Spinco rotor. The sediment was resuspended in 20 ml. of phosphate buffer (0·15 M, pH 7·2) in saline and trypsinized for 1 hr. at room temperature (final concentration of Armour Tryptar 250 units/ml.). After centrifugation at 26,000g for 30 min., the sedimented particles were resuspended either in 10 ml. phosphate buffer (pH 7·4) which contained a final concentration of 0·2 M sucrose or in 10 ml. of SPA. The suspensions without albumin were used for inoculation of rabbits, while the E.B. suspended in SPA were used as antigens in C.F. tests. The E.B. *suspensions were stored for 3 months at* $-70°$ C., *or at* 4° C. *for at least* 1 month, with no detectable change in potency.

Boiled E.B. susp. of mouse pneumonitis. A yolk grown purified E.B. suspension was suspended in phosphate-saline (potassium phosphate buffer pH 7·2) at a final

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concentration 0·02 M instead of diluents containing sucrose and albumin. It was heated for 10 min. in a boiling water-bath.

Samples taken from the same suspension before and after boiling when titrated with psittacosis antisera showed a four- to eightfold increase of the group-specific antigen titre due to steaming of the suspensions.

Ether soluble psittacosis antigen. This was prepared from yolk sacs infected with 6BC or P-4 strains, by a procedure described elsewhere (Terzin, Matuka, Fornazarić & Hlača, 1961). In contrast to the E.B. suspension antigens preserved by sucrose-albumin, both the boiled E.B. suspension prepared from MoPn, and the ether soluble psittacosis antigen represent monospecific Bedsonia-group reagents.

Human sera

Fifty-three serum specimens from young adult trachoma convalescents (from 1 to 16 years after the onset of the disease) were collected in Yugoslavia by Dr B. V. Birtašević; three serum specimens from trachoma patients were obtained from Dr E. S. Murray; fourteen Bedsonia negative serum specimens from apparently healthy children of age varying from 3 to 6 months were obtained from Dr A. J. Vargosko; and eight specimens of sera were drawn from the personnel of this laboratory.

Animal sera

Seven serum samples were collected at different time intervals in this laboratory from two monkeys infected experimentally with trachoma.

Anti-sera were prepared by immunization of rabbits with MRC-1/G and TE-55 strains grown in tissue culture.

The preparation of inocula used for the immunization was described under 'Tissue culture grown E.B. suspensions'. Each of four rabbits received two courses of injections separated by a month; one consisted of six and the other one of four injections. The first injection in each course was given intraperitoneally and the rest intravenously. In each of the immunization courses the animals were given single doses of the suspension increasing from 0·5 to 1·5 ml. per animal. The total amount given to each of the four animals amounted to 9 ml. of the respective suspensions. Eight days after the last injection of the second course the animals were bled.

The antisera prepared by immunization with tissue culture grown purified elementary bodies gave no reaction with the crude control antigen prepared from uninoculated tissue cultures.

The anti-psittacosis sera were obtained from the Medical Faculty, Sarajevo. There they were prepared by intraperitoneal inoculation of rabbits with organs (20 % suspension of mixed brain and spleen homogenates in saline solution) of mice infected intracerebrally with the 25th mouse passage of the psittacosis strain P-4.

Absorption of sera

Three volumes of the boiled MoPn antigen were centrifuged for 40 min. at about 30,000*g*. The supernatant was separated and used successfully as a group-specific C.F. antigen in other studies. The sediment was resuspended and well homogenized

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in 1 volume of the serum intended for absorption. The suspension was kept for about 3 days at 4° C., and occasionally shaken. The absorbed serum was separated at about 70,000*g* for 50 min., decanted and passed through a Millipore filter (porosity 0·45μ). Although only the sediment of the boiled E.B. suspension was used for absorption, and the serum after absorption was centrifuged for 50 min. at 70,000*g*, the absorbed serum showed an anticomplementary activity as a rule as high as 1/16. After filtering it through Millipore pads, we could reduce its anti-complementary activity to less than 1/4 (apparently due to removal of the flooding and 'soluble' serum-antigen complexes which tend to fix complement). Serum specimens from which the anti-group titres were not removed by a single absorption were absorbed for a second time. In terms of C.F.-units, we were unable to define a generally applicable agent–antibody ratio which would be satisfactory for complete absorption. Experiments repeated with several serum samples of rabbits and guinea-pigs and with different preparations of boiled MoPn antigen showed, that for the removal of all detectable group antibodies in some instances it was enough to use 1 C.F.-unit, while in other cases it was necessary to use 20 C.F.-units of the antigen per 1 C.F.-unit of group antibody. We decided to use E.B. of mouse pneumonitis agent in our absorption experiments because, as far as we know, the similarity of the group antigens contained in mouse pneumonitis and TRIC organisms has not previously been investigated.

RESULTS AND CONCLUSIONS

(1) *Correlation of* C.F. *titres revealed by sera titrated by psittacosis and mouse-pneumonitis antigens*

Of the 85 serum samples tested, 63 (74 %) were taken from cases of trachoma (mostly convalescents), diagnosed on the basis of both clinical and epidemiological evidences and a few of them confirmed also by positive smears or isolation of the agent from the conjunctival lesions. Many of the adult patients have spent periods of their life in areas where psittacosis is known to be prevalent. Regardless of the proportion in which trachoma infection can be made responsible for the development of anti-Bedsonia antibodies in the serum specimens of these individuals, the fact remains that these specimens showed the same proportion of C.F. positive reactors and revealed comparable titres with group antigens prepared both from psittacosis and mouse pneumonitis organisms. This appears to be suggestive evidence for a very similar, if not identical specificity of the group antigens of psittacosis, mouse pneumonitis and the group antibodies found in the sera of trachoma patients and trachoma infected monkeys.

These 85 serum specimens all showed negative results ($< 1/4$) when tested both for anticomplementary activity and against Q fever antigen.

Each of the 85 serum specimens was tested against the ether soluble psittacosis antigen diluted 1/60 (which represents about six homologous group antigenic units) and against the boiled E.B. suspension of MoPn antigen diluted 1/140 (representing about 7·3 homologous group antigenic units). Figure 1 shows the correlation table of the titres revealed against the two antigens. Statistical analysis of

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these data revealed a positive correlation coefficient 0·896. The probability that this positive correlation of a high degree might have occurred by mere chance was found to be much less than 0·001. Also by inspection of Fig. 1, one can note that except for one specimen (which showed a titre of < 1/4 with the psittacosis and a titre of 1/8 with the MoPn antigen), all sera showed identical titres with both antigens in the limits of ± one tube difference. This variation is considered tolerable in the serial twofold dilution C.F. test, if the tests are repeated on different days. As seen from Fig. 1, out of the 85 specimens tested, 46 (or 54%) were positive with psittacosis, and 42 (or 49%) with the MoPn antigen. A comparison of the significance of the proportion of positives and negatives revealed by the two antigens, gave a chi-square value as low as 0·196 indicating with great probability ($0{\cdot}7 > P > 0{\cdot}5$) that the results obtained with the two antigens were indistinguishable.

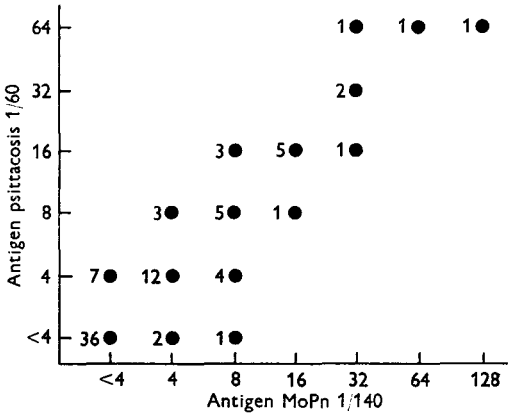

Fig. 1. Two-way frequency of reciprocals of the C.F. titres shown by 85 serum specimens with two antigens.

The data in Fig. 1 also show that the mean titre of the positive serum samples against the psittacosis antigen was 1/12·26 as compared with 1/14 of the MoPn antigen.

When analysed by *t*-test, the difference between these means is shown to be not significant. The *t* value obtained was 0·4394, at a probability level of significance of $0{\cdot}7 > P > 0{\cdot}6$, indicating with great probability that the difference observed between the two means might have been obtained just as well by re-titrating the same serum specimens twice with the same antigen.

All three statistical examinations suggest a great similarity, if not identity of the specificity of the two antigens employed, although each was prepared in a different way (ether soluble fraction of psittacosis and boiled E.B. suspension of MoPn), with the specificity of group antibodies found in sera of trachoma patients and trachoma infected monkeys.

(2) *Experiments with absorbed anti-trachoma sera*

The E.B. suspensions (with stabilized serological activity of the species-specific antigen) as well as the absorbed antisera (which were free both of the Bedsonia-

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group antibodies and of anticomplementary activity) enabled us to study the specificity of the antigens (contained in TRIC organisms) not only at the group-genus level, but also at the species-specific level, and to make comparisons of the serological cross-reactivity of different TRIC strains.

Different samples of boiled elementary body suspensions prepared from mouse pneumonitis agent removed or reduced very significantly the capacity of immune sera against trachoma to react with group antigens prepared from psittacosis or other Bedsonia agents. However, the absorbed sera retained their capacity to react with elementary body suspensions prepared from trachoma or inclusion blennorrhoea strains.

Table 1. *Reciprocals of antigen titres with unabsorbed and absorbed sera*

	Antiserum (rabbit)								
	MRC-1/G			TE-55		Psittacosis			
		Absorbed			Absorbed				A.C. of
Antigens	1:40	1:6	1:20	1:256	1:20	1:20	1:40	1:80	antigen
E.B. susp. (yolk grown)									
MRC-1/G	1024	64	32	512	128	256	128	64	< 8
Cal-1	256	16	8	256	128	n.t.	n.t.	n.t.	< 8
TE-55	512	256	128	512	256	256	256	128	< 8
Psittacosis	128	< 8	< 8	64	< 4	64	32	32	< 4
E.B. susp. (TC grown)									
MRC-1/G	32	n.t.	8	n.t.	n.t.	32	n.t.	n.t.	< 4
TE-55	64	n.t.	32	n.t.	n.t.	32	n.t.	n.t.	< 8
Boiled E.B. susp. MoPn	2048	< 8	< 8	1024	< 16	1024	1024	512	< 8
Ether soluble psittacosis	256	< 8	< 4	256	< 16	256	256	32	< 4
A.C. of respective serum dilution	−	−	−	−	−	−	−	−	.

A.C., Anticomplementary activity; n.t., not tested; −, negative.

The sera used in absorption experiments were prepared by immunization of rabbits with elementary body suspensions grown in tissue culture, and the elementary bodies of mouse pneumonitis used for serum absorption were grown in yolk sacs of chick embryos. It is obvious that the specificity shared by these two systems may be only that of the Bedsonia antigen shared by both organisms (trachoma and mouse pneumonitis). Consequently, the data presented in Tables 1 and 2 seem to offer strong additional evidence for the notion that the specificity of group antibodies formed against trachoma organisms is serologically indistinguishable from the specificity of the group antigen produced by mouse pneumonitis organisms.

From the data presented in Tables 1 and 2 it is easy to calculate the factors of the titre decrease which were induced by serum absorption. When titrated against constant amounts of the unabsorbed and absorbed samples of the respective sera, the E.B. suspensions prepared from MRC-1/G, Cal-1 and TE-55 organisms grown

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Table 2. *Reciprocals of titres revealed by unabsorbed and absorbed sera*

	Antigens							
	MRC-1/G E.B. susp. grown		TE-55 E.B. susp. grown		Psittacosis E.B. susp. grown in yolk,	MoPn grown in yolk boiled,	Psittacosis grown in yolk ether extract,	A.C. of serum
Anti-sera (rabbit)	In TC, 1:20	In yolk, 1:20	In TC, 1:30	In yolk, 1:50	1:30	1:140	1:100	
MRC-1/G	512	64	256	256	128	128	128	< 4
MRC-1/G absorbed	128	8	32	16	< 8	< 4	< 4	< 4
TE-55	n.t.	512	n.t.	1024	n.t.	1024	512	< 16
TE-55 absorbed	n.t.	16	n.t.	32	n.t.	< 16	< 16	< 16
A.C. of respective antigen dilution	–	–	–	–	–	–	–	–

A.C., Anticomplementary activity; n.t., not tested; –, negative.

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both in yolk and tissue culture showed an average of 10·25-fold drop of the C.F. titre (varying from 2- to 32-fold in individual examples shown in Table 1).

Similarly, titrations of the unabsorbed and absorbed samples of anti-MRC-1/G and anti-TE-55 sera against constant amounts of E.B. suspensions prepared from MRC-1/G and TE-55 organisms, showed on the average a 16·66-fold decrease of the C.F.-titres (varying from 4- to 32-fold decrease in individual cases shown in Table 2).

In contrast to that, the results of C.F.-tests run with ether soluble antigen and E.B. suspension prepared from psittacosis, as well as of the tests run with the MoPn antigen, all titrated against both unabsorbed and absorbed specimens of anti-MRC-1/G and anti-TE-55 sera, revealed an average 144-fold decrease of the antigen titres (varying from 32- to 512-fold in different cases) and an average 70-fold decrease of the respective serum titres (varying from 32- to 128-fold in different examples). In most titrations the amounts of the reagents which were kept constant represented 4–16 homologous C.F.-units of antiserum (Table 1) and 1–10 units of the homologous antigen (Table 2). An exception is seen in the 1/20 dilution of the yolk grown MRC-1/G E.B. suspension, which contained about 50 C.F. units of the group reactive antigen component (Table 2).

The data presented in Tables 1 and 2 seem to show clearly that the serological specificity of the group antigens of inclusion blennorrhoea and trachoma is indistinguishable from that of the mouse pneumonitis and psittacosis organism.

In contrast to that, if tested against absorbed (group-negative) sera, the species-specific antigen activity of the E.B. suspensions prepared from strains of trachoma or inclusion blennorrhoea discriminate clearly between TRIC-species and other Bedsonia agents (psittacosis, mouse pneumonitis, etc.).

The serological activity of E.B. suspensions prepared from TRIC and psittacosis agents both against unabsorbed and absorbed anti-TRIC sera, as well as against the anti-psittacosis serum, shows that the species-specific antigens of TRIC agents are different from the species antigen of the psittacosis agent (Table 1). However, a differentiation of various strains within the TRIC-species could not be demonstrated by the use of C.F.-test and purified E.B. suspensions prepared from strains of trachoma and inclusion conjunctivitis. Data shown in Tables 1 and 2 actually indicate that the species-specific antigens of the TRIC strains we compared were indistinguishable. If confirmed by more extensive studies, this finding would put strains isolated from trachoma and inclusion conjunctivitis cases in the same, serologically homogeneous, species.

(3) *Observations bearing on the species-specific antigenic activity of TRIC agents*

In contrast to the E.B. suspensions of trachoma agent suspended in SPG (Terzin *et al.* 1963), the suspensions of the E.B. preserved by SPA, after storage for 3 months at $-70°$ C. or for 60 days at 4° C., showed neither detectable drop in their species-specific titres, nor detectable increase in their group titres when tested by absorbed and unabsorbed homologous sera. The finding that sucrose-albumin preserves the species-specific C.F. antigen of trachoma-inclusion blennorrhoea

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agents seems to be in agreement with the findings of Weiss & Dressler (1962) who reported that sucrose and albumin have pronounced stabilizing effect on the infectivity of purified preparations of trachoma agent.

However, these suspensions, although both stabilized and revealing pronounced species-specific activity as test-antigens for detection of trachoma or inclusion conjunctivitis antibodies, can be used only if the serum tested is free of group-specific antibodies (against absorbed or *a priori* Bedsonia-negative sera). In the presence of group antibodies the group–antigroup C.F. system would mask the distinct manifestation of the species–antispecies C.F. system.

With well-preserved specimens of normal sera of men, rabbits and guinea-pigs, the E.B. suspensions showed no signs of fixation of complement. However, with turbid, many times frozen and thawed specimens or with contaminated sera, the same suspensions tended to give nonspecific fixation of complement.

Samples of E.B. suspensions which became contaminated developed anti-complementary activity. Stock batches of E.B. suspensions should be carefully preserved, divided into small volumes and kept frozen until use. As shown in Table 1, the antigen titres obtained with anti-psittacosis serum are comparable with the respective antigen titres obtained with homologous anti-TRIC sera. Therefore, it does not seem feasible to use some 'critical' working dilutions of these E.B. suspensions which would allow a distinction between species- and group-specific serum titres. For these reasons we attempted to apply to trachoma serology the method of Jenkin, Ross & Moulder (1961) for preparing species-specific cell-wall antigens, free of the group antigen component. As starting material, we used purified suspensions of elementary bodies grown in yolk sacs. Out of seven preparations tested the first two showed promising results (species-specific reactions with absorbed sera; low or no detectable group–antigen reactivity; tests set up with boiled samples revealed both a resistance to heat of the species-specific activity and a disappearance of the initially present anticomplementary activity). However, in five additional attempts, for unknown reasons, we failed to reproduce the results which looked so promising at the beginning.

DISCUSSION

In spite of the huge amounts of money spent for treatment programmes, more than 400 million of the world's population still suffer from trachoma and its complications (Thygeson, 1962).

It is known that the clinical diagnosis of this disease requires careful examination of each patient by an eye specialist, a condition which would be very hard to procure in remote areas of Africa, Asia and South America, where trachoma is flourishing. Besides that, clinical examination may fail to diagnose cases of atypical trachoma, which nevertheless might represent sources of infection. According to Thygeson (1960) 'the obstacles in the way of detecting and treating the disease in entire populations have seemed insuperable...'.

Both the isolation of the agent from and the demonstration of inclusion bodies in scrapings and smears of the suspect conjunctiva, are the best evidences for an

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actual infection of the eye with TRIC-agents. However, the low frequency of positive results obtained by these methods, as well as the expense of adequate laboratory facilities, make these methods both unreliable and unfeasible for use as diagnostic tools. In field work on a large scale, these methods cannot be used as substitutes for complete clinical examinations by the eye-specialist.

All these circumstances seem to point out quite clearly the need for a reliable, easily performed, specific serological test, which would make it possible to diagnose trachoma from blood specimens collected in the field, and transported to central laboratories.

As summarized by Thygeson (1960), 'No serological tests of diagnostic value are yet available. The sera of many, but not all, trachoma patients show group complement-fixing antibodies for the psittacosis-lymphogranuloma venereum group of viruses, but no differentiation among members of the group is yet possible.'

The purpose of this discussion is to sketch a pattern of facts and notions which seem to indicate a definite direction of effective search for serological tests of diagnostic value in trachoma.

The serological evidence available so far seems to indicate the existence of at least three taxonomic levels of the TRIC organisms:

(1) At the level of genus or group, it is known (Bedson, 1959) that a large number of organisms share a common serological specificity of their Bedsonia-group antigen or psittacosis–LGV–trachoma-group antigen (as well as similar morphology, cultural characteristics in chick embryos, propagation cycle, resistance to streptomycin, sensitivity to many antibiotics and several other features of similarity).

(2) At the species level, the TRIC agents may be distinguished from other Bedsonia organisms by similarity in host range and a species specific thermolabile antigen. In contrast to other species of Bedsonia, the TRIC agents are infectious for man, ape and monkey by ocular route, occasionally for mice intracerebrally, but not infectious for the guinea-pig by any route. The species specific antigen may distinguish TRIC strains from, e.g. the psittacosis species, but cannot distinguish trachoma from inclusion conjunctivitis strains.

The apparent existence of two separate varieties (trachoma and inclusion conjunctivitis) of the TRIC species might well be a manifestation of differences in the hosts and in some ecological factors.

(3) Within the TRIC species, as in the psittacosis–ornithosis species, it was possible to demonstrate (by active protection test of mice challenged intravenously) the existence of at least three serological types of toxins, associated with intact elementary bodies (Bell & Theobald, 1962; Chang, Wang & Grayston, 1962).

(4) The number of TRIC strains, isolated over all five continents, amounts to well above a hundred. The majority of these strains seem to be characterized by their origin, specific case history and nominal designation, rather than by stable and recognizable biological markers at a subspecies level. It is unfortunate that the E.B. of the TRIC organisms contain thermostable group antigens which are serologically indistinguishable from the group antigens in other Bedsonia

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organisms; that different species of Bedsonia organisms (mammalian and avian strains of psittacosis–ornithosis, LGV, etc.) are prevalent over the globe causing both clinically manifest and subclinical infections in men (followed by long-lasting persistence of group-specific antibodies in the cross-infected population). Consequently, the mere fact that an antigen was prepared from TRIC organisms cannot justify its use for diagnosing infections caused by TRIC agents, either for studies of prevalence of the TRIC infections in population groups, or for detecting reactors to a trachoma vaccine. In order to secure a specific serological test of diagnostic value, one has to provide reagents which will interact on a monospecific TRIC-species level. Taking as example the C.F.-test, there are two theoretical alternatives:

(1) One might use well-preserved E.B. suspension (or other antigen preparations in which the species-specific component is stabilized) which would react specifically with trachoma sera which are free from group antibodies.

(2) Alternatively, one could prepare a TRIC species-specific antigen, free from the Bedsonia group antigen, which would detect specific anti-TRIC activity in sera of trachoma patients, without reacting with any group-specific antibodies that might be present.

In this report we describe the preparation of stabilized E.B. suspensions which may be used for detection of the species-specific TRIC antibodies. However, these suspensions can be used as TRIC-specific reagents only with sera free from group-reactive antibodies.

A successful separation of the TRIC-specific antigen from the Bedsonia-group antigen would provide a reagent which might be used either in C.F.-test, or in some other serological test as an invaluable serological tool, so much needed for effective trachoma research. It is unfortunate that such an antigen is not yet available.

SUMMARY

Group-reactive ether soluble psittacosis and boiled mouse pneumonitis antigens were tested in parallel, with 85 serum specimens. The results indicate that the group-specific C.F. antigens of these organisms are indistinguishable when tested against sera of trachoma patients, monkeys infected with trachoma or against sera of other individuals.

Sera of rabbits immunized with viable trachoma-inclusion conjunctivitis (TRIC) organisms, grown in tissue culture, were absorbed with boiled elementary body suspension of mouse pneumonitis agent, which removed the group reactive antibodies, and resulted in a species-specific anti-TRIC serum.

The absorbed and unabsorbed TRIC sera were titrated against purified E.B. suspensions, which were prepared both from yolk and from tissue culture grown organisms, of homologous TRIC strains and from other heterologous Bedsonia organisms.

Results of absorption experiments indicate that group reactive antigens prepared from mouse pneumonitis and psittacosis are indistinguishable by C.F. test from the group-specific component of the TRIC antigens. The species-specific antigen of the

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TRIC agents was well distinguished from the species-specific antigen of the psittacosis agent. However, the C.F. test did not distinguish the strains isolated from trachoma from those isolated from cases of inclusion conjunctivitis.

The stabilizing effect of sucrose and albumin upon the species-specific C.F. antigen of purified elementary bodies of TRIC organisms was found to be pronounced.

Our attempts to produce a species-specific antigen preparation, free from group component, failed.

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