J. Hyg., Camb. (1963), **61**, 31
Printed in Great Britain

Demonstration of complement-fixation titres against the species-specific trachoma antigen in sera of trachoma patients

By A. L. TERZIN, M. R. FORNAZARIĆ and B. V. BIRTAŠEVIĆ
Medical Faculty and HE Military Laboratory, Sarajevo, Yugoslavia

(*Received* 23 *July* 1962)

INTRODUCTION

It seems to be well established that the isolation of viable trachoma virus from conjunctival scrapings showing no inclusion bodies usually requires two or more blind passages and laboratory facilities not available in everyday routine (Hanna, 1962). At present, virus isolation is not, as a diagnostic method, much superior to the demonstration of inclusion bodies in conjunctival scrapings (Snyder *et al.* 1959; Sowa & Collier, 1960; Grayston, Wang, Woolridge, Yang & Johnston, 1960; Murray, Guerra, Abbot & McComb, 1962; and others). Consequently, much endeavour has been directed toward elaboration of a serological method that could be used in routine, both for the diagnosis of trachoma infections and for differentiating infections with trachoma virus from those caused by other agents of the same group (Bedsonia group) of viruses.

As indicated in a review by Bedson (1959), a good deal of valuable information has been accumulated on the antigenic composition of the psittacosis-lymphogranuloma group of infective agents.

However as yet only some of the observations bearing on the antigenic composition of psittacosis virus have been used fully in the efforts to perfect the serology of trachoma (or even the routine serology of psittacosis as practised by workers in the respective medical and veterinary branches). The species-specific antigenic activity of elementary body suspensions, first observed by Bedson in 1936 in his studies on psittacosis virus, seems to be confirmed also by recent studies on trachoma.

Numerous authors, using semipurified suspensions of trachoma virus as antigens, in agglutination or complement fixation (CF) reactions have succeeded in detecting species specific antibodies in sera of trachoma patients.

Some of these claims seem to be invalidated by apparent neglect of two facts:

(*a*) the possible presence of a group-reactive antigenic component in semipurified suspensions of elementary bodies, and

(*b*) the possible presence of group-reactive antibodies in sera of those trachoma patients who might have had previous contacts with other members of the psittacosis-lymphogranuloma group of viruses.

Nevertheless, several authors have found elementary-body antigens useful for demonstrating species-specific agglutination (Bernkopf, 1962; and others) or CF-reaction (Woolridge & Grayston, 1962; and others).

Recently we submitted additional evidence that in sera of trachoma patients group-reactive CF-titres are of no diagnostic value in respect to trachoma (Terzin & Birtašević, 1962). We reported then also, that trachoma infection did not produce group-specific CF-inhibiting antibodies in sera of men as detected by CF-inhibition reaction with group-reactive Bedsonia–anti-Bedsonia indicator system.

The present report shows that semipurified elementary body suspensions of trachoma virus, as prepared in our laboratory, may be used for detection of species-specific antibodies only in Bedsonia-negative samples of sera. The elementary body suspension was prepared according to a procedure used by Collier (1961) for the production of trachoma vaccine, and modified as described below.

MATERIALS AND METHODS

(1) *The trachoma strain and its propagation in ovo*

A strain of T'ang's trachoma virus, obtained from Dr F. B. Gordon (Naval Medical Research Institute, Bethesda, U.S.A.) has been used for the preparation of the various antigens, referred to in this report.

Several other strains were sent to us, from other laboratories, as non-lyophilized preparations, but from none of these, received after 10–14 days of journey, could viable trachoma virus be recovered.

From Dr F. B. Gordon, we received a yolk membrane suspension from the harvest of the 34th egg passage, lyophilized from SPG suspending medium, and sealed in nitrogen.

In our laboratory the strain was passed seven more times through eggs.

Prior to inoculation the eggs were incubated at about 38° C. Embryos found alive at the 7th day of age were inoculated into the yolk sack, and further incubated at 34–35° C. Yolk sacs were inoculated with 0·20 to 0·25 ml. of a well-homogenized suspension of infected yolk membranes (harvest of the 38th to 40th egg-passages), diluted 10^{-2} in plain broth, containing 500 units of streptomycin per ml. and clarified by centrifugation for 10 min. at 2000 r.p.m.

Embryos found dead 72 hr. after inoculation were discarded. At the 5th to the 7th day after inoculation, all embryos died or showed very sluggish movements. Embryos found dead or very sluggish were chilled for 2–4 hr., and harvested. Membranes showing congestion (the embryos themselves showing congestion and haemorrhages of the skin) were collected and stored in a deepfreezer until used for the preparation of antigens.

(2) *Preparation of the elementary body antigens*

All operations were conducted with materials cooled at 1°–4° C. and handled as sterile. All centrifugations at speeds below 3000 r.p.m. were done in horizontal heads, and those at higher speeds in angle heads.

The SPG solution (Bovarnick, Miller & Snyder, 1950) was adjusted to pH 7·2. The glycerol, formalin and other reagents used in these experiments, were all of analytical grade. The yolk membranes were homogenized in a Waring blender in three consecutive cycles, each of about 40 sec. (total time of grinding 2 min.).

A step-by-step description of the preparation is shown in the flow diagram (Fig. 1). In addition to the 'SPG-glycerol' and the 'SPG-formol' antigens, we prepared a batch of 'saline-formol' antigen. This saline antigen also, was prepared according to the procedure shown in the flow diagram, except that through the whole work sterile saline was used as diluent instead of the sterile SPG solution. Giemsa-

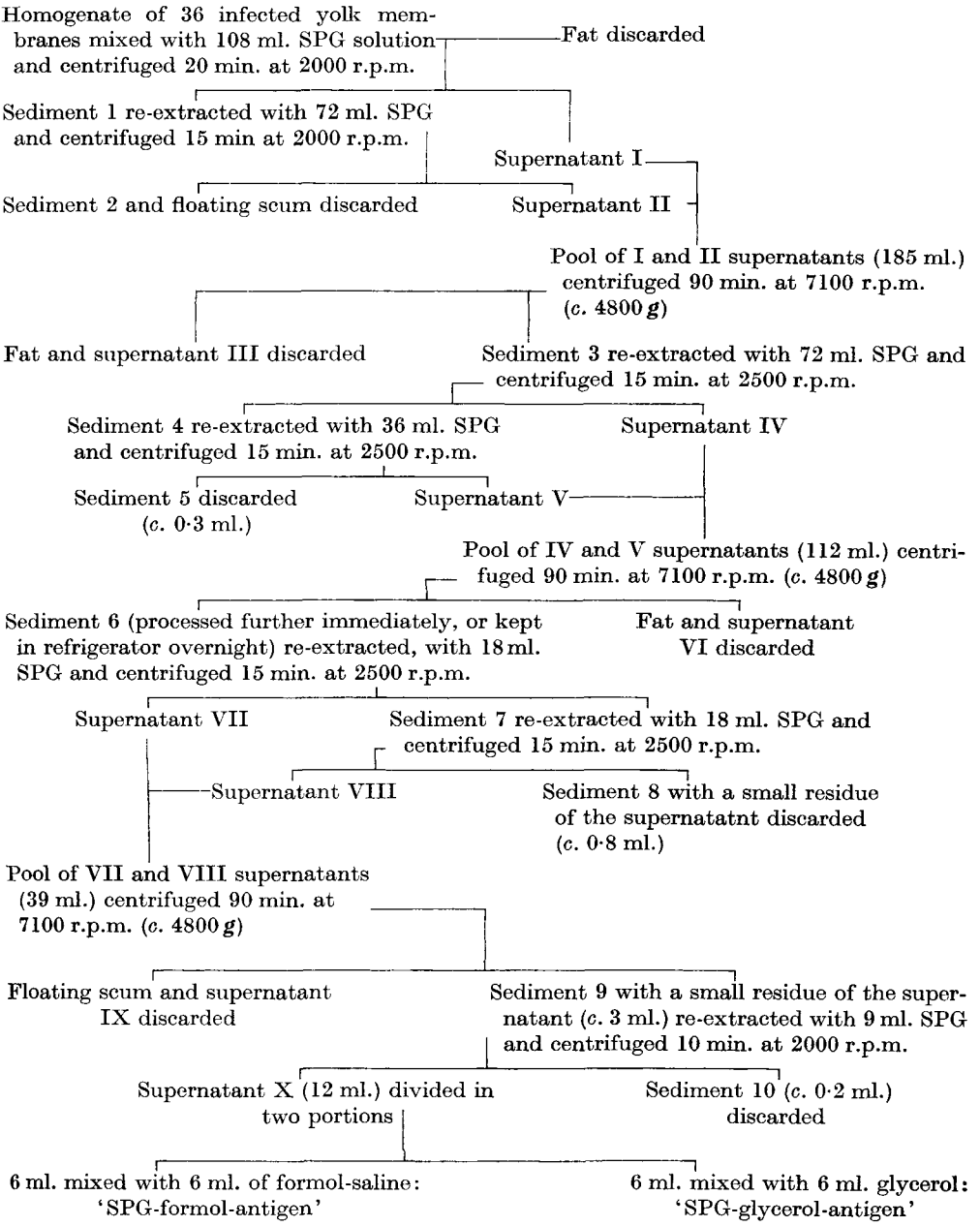

Fig. 1. Flow diagram for the preparation of elementary body antigens.

stained smears of samples from all three antigens showed under the microscope semi-purified elementary body suspensions of approximately equal density.

To both kinds of 'formol-antigen' the formalin was added in a final concentration of 0·04%.

All three antigens were tested with more than 20 Bedsonia-negative serum specimens from patients convalescent from infections with influenza A, influenza B, or mumps viruses. None of the antigens showed fixation with any of the influenza A, B or mumps-positive, nor with Bedsonia-negative serum specimens.

(3) *Preparation and comparison of group-specific soluble antigens prepared from psittacosis and trachoma viruses*

From trachoma-infected yolk membranes we prepared the group-reactive soluble antigen, according to the procedure described previously (Terzin, Matuka, Fornazarić & Hlača, 1961).

When comparing the complement fixing activity of this antigen with that of the group-reactive soluble antigen prepared from psittacosis virus, we could detect no differences in regard to specificity, sensitivity or height of the titre against sera of men and pigeons recovered from infection with ornithosis, or against sera of rabbits and guinea-pigs immunized with psittacosis virus (Table 1).

More than 60 specimens of serum obtained from men, sheep, cattle and birds have been tested with both of the group-reactive antigens prepared from trachoma and from psittacosis virus. All serum specimens showing negative CF-reactions with the first antigen were negative also with the other and those showing positive CF-tests gave the same titre with both antigens (Table 1).

(4) *Complement fixation (CF) tests and titration of sera*

Both the technique and the reagents used in performing the CF-test were described previously (Terzin & Birtašević, 1962). All sera were titrated in twofold serial dilutions from 1/2 to 1/128 in the following sequence:

(*a*) All sera were first tested for anticomplementary activity with appropriate dilutions of the control antigen;

(*b*) all sera showing no anticomplementary activity, were tested against two dilutions of the group-reactive antigen, one containing 4 and the other 16 CF-units of the antigen;

(*c*) sera showing no CF-activity against the group-reactive Bedsonia-antigen, were tested, each against two dilutions of the SPG-formol-antigen', the 'SPG-glycerol-antigen' and the 'saline-formol-antigen'. All three antigens were used in dilutions 1/6 and 1/12, containing from 2 to 6 CF-units of the respective species-specific trachoma antigen per tube. Serum titres against antigen dilutions containing more than one sixth of an anticomplementary unit, were disregarded. Chiefly for this reason the numbers of serum specimens tested with each of the three antigens were different.

All serum specimens were obtained from the same 'Working Collective T', from cases diagnosed as trachoma by the clinical and epidemiological criteria described previously (Terzin & Birtašević, 1962). The classification of trachoma

Table 1. *Group-specific and species-specific complement fixation*

		Antigens												
					Trachoma									
		Psittacosis soluble			Soluble			SPG-formol			SPG-glycerol			
		1/256	1/512	1/1024	1/128	1/256	1/512	1/8	1/16	1/32	1/8	1/16	1/32	1/64
Anti-psittacosis serum (guinea-pig)	1/16	+ + +	+ + +	−	+ + +	+ + +	+ +	+ + +	+ + +	+	+ + +	+ + +	+ +	−
	1/32	+ + +	+ + +	−	+ + +	+ + +	−	+ + +	+ + +	−	+ + +	+ + +	+	−
	1/64	+ + +	+ + +	−	+ + +	+ + +	−	−	−	−	−	−	−	−
	1/128	+ + +	+ +	−	+ + +	+ + +	−	−	−	−	−	−	−	−
	1/256	+ +	+	−	+ +	−	−	−	−	−	−	−	−	−
	1/512	−	−	−	−	−	−	−	−	−	−	−	−	−
Bedsonia-negative trachoma serum (human)	1/4	−	−	−	−	−	−	+ + +	+ + +	−	+ + +	+ + +	+ +	−
	1/8	−	−	−	−	−	−	+ + +	+ + +	−	+ + +	+ + +	+ +	−
	1/16	−	−	−	−	−	−	+ + +	+ + +	−	+ + +	+ + +	+	−
	1/32	−	−	−	−	−	−	+ + +	−	−	+ + +	+ + +	−	−
	1/64	−	−	−	−	−	−	−	−	−	+ + +	+	−	−
	1/128	−	−	−	−	−	−	−	−	−	−	−	−	−

Table 2. *Analysis of the duration of trachoma in various stages and the findings revealed by CF-test with three trachoma antigens*

			SPG-formol-antigen				SPG-glycerol-antigen				Saline-formol-antigen			
				Positives found				Positives found				Positives found		
Stages	Duration (years)	Mean duration (years)	No. of cases tested	No. of cases	%	Mean titres	No. of cases tested	No. of cases	%	Mean titres	No. of cases tested	No. of cases	%	Mean titres
II	1–5	3·11	18	13	72·2	1/18	5	4	80·0	1/32	13	4	30·8	1/2
III	3–11	5·33	15	12	80·0	1/20	5	5	100·0	1/42	16	7	43·8	1/3
IV	3–12	7·61	18	11	61·1	1/9	6	5	83·4	1/12	—	—	—	—
All	1–12	5·35	51	36	70·6	1/15	16	14	87·5	1/25	29	11	38·0	1/2·74

cases into stages was made according to the same criteria as described previously.

As shown in Table 2, the clinical stage is correlated with the duration of trachoma and the data on mean duration of the disease according to stages, as found in this material, are well comparable with the corresponding data found in our previous material (Terzin & Birtašević, 1962).

Fifty one serum specimens were selected which showed neither anticomplementary activity nor CF-reaction with the group-specific Bedsonia antigen. These 51 specimens were tested against experimental batches of the different species-specific trachoma antigens described in this report. For the titration of sera, if not stated otherwise, the species-specific antigens were used at the 3rd to 20th day after their preparation.

RESULTS AND CONCLUSIONS

The results seem to indicate significant differences in the incidence of CF-positive reactors, in the average CF-titres, and in the degrees of correlation between duration or stage of the diseases and the CF-titres against the various species-specific trachoma antigens. These results also seem to indicate differences between preparations in respect to stability of species-specific CF-activity, anticomplementary activity, and in other respects.

The results reported here were reproducible in the sense that from each of the SPG antigens investigated two batches at least gave identical results, and out of the 51 selected serum specimens from trachoma patients, about 30 were tested at least twice each with the same type of antigen giving comparable results at the first and subsequent titrations.

(1) *The 'saline-formol-antigen'*

As shown in Table 2, the antigen prepared by using saline as diluent, was significantly inferior to the other two antigens, both in the number of positive reactors it disclosed, and on the basis of the titres revealed with the same Bedsonia-negative sera.

(2) *The 'SPG-glycerol-antigen'*

As shown in Tables 1 and 2, when compared with the other two antigens, the 'SPG-glycerol-antigen' revealed the greatest incidence of positive reactors (about 90 % of the trachoma patients tested), showed higher antigenic titres than the other two species-specific antigens, and revealed the highest CF-titres in the Bedsonia-negative serum samples of the trachoma patients tested.

In contrast to the formol-antigens, the species-specific activity of the glycerol antigen was more stable. However, when retested several times subsequently, the antigen preserved with glycerol showed a gradually increasing anticomplementary activity. It is well known that serum samples mixed with glycerol gradually become anticomplementary. However many preparations of various viral, rickettsial, or leptospiral antigens have been found to keep well with glycerol (Cabasso, Markham & Cox, 1951; Terzin, 1953; Brand & Keil, 1955; Galton,

Powers, Hall & Cornell, 1958; and others), in our laboratory revealing no anti-complementary activity even after being stored for years.

Table 3 shows the appearance of the anticomplementary activity at various time intervals in the two types of antigen compared.

Although in several respects superior to the formol antigen, the glycerol-preserved antigen had to be abandoned because of its liability to become anti-complementary on storage.

Table 3. *Titres of anticomplementary activity of antigens*

	Days after preparation				
Antigens	2	10	20	30	130
'SPG-formol'	< 1/1	< 1/1	1/1+	1/1	1/1
'SPG-glycerol'	1/1	1/2	1/4+	1/4	1/8+

(3) *The 'SPG-formol-antigen'*

As shown in tables 1, 2 and 3 the 'SPG-formol-antigen': showed fairly high species-specific-antigenic titres as detected by CF-titration against Bedsonia-negative sera of trachoma patients, revealed both a high incidence of trachoma-positive reactors (about 70 %) and reasonably high species-specific titres in the sera of trachoma patients tested. Kept at +4° C. the SPG-formol-antigen did not show anticomplementary activity in dilutions 1/2 or higher for at least four months (later not tested).

The data presented in Table 4 indicate the CF-titres revealed with the SPG-formol-antigen in Bedsonia-negative serum samples. These titres seem to increase with increasing duration of trachoma up to the 2nd or 3rd years of the disease. After the 2nd or 3rd years of trachoma, the titres revealed show a negative correlation with the duration of the disease, at a significance level of $P = 0{\cdot}01$. For the 34 pairs of data, referring to trachoma-positive serum samples obtained from 34 patients after their second year of illness, the correlation coefficient showed to be: $r = -0{\cdot}4426$ (all data needed for this calculation may be found in Table 4).

Table 4 presents also geometrical means of the positive titres for each year separately, as well as 3 years moving averages of the positive titres, in sera of patients with disease of different duration. The 3 year moving averages shown in Table 4 represent values of geometrical means of the respective CF-titres found in subsequent 3 years periods. The trend of 3 year moving means of the CF-titres shows more clearly than the trend of the 1 year mean values the correlation existing between the average CF-titres and the duration of the disease.

When analysing the data presented in Tables 2 and 5, neither the percentage incidence of positives, nor the CF-titres were found to show significant correlation with the clinical stage of trachoma.

Also the percentage incidence of positives showed no significant correlation with the duration of trachoma (see Tables 4 and 5).

As seen from the data presented, both the clinical stage of trachoma and the heights of the CF-titres, showed significant correlation with the duration of the

Table 4. *Data indicating a correlation between CF-titres against 'SPG-formol' antigen and duration of trachoma*

Reciprocals of titres	No.	Percentage	Duration (years) 1	2	3	4	5	6	7	8	9	10	11	12
< 2	15	29·4	—	1	5	1	3	—	1	1	1	—	1	1
2	1	2·0	—	—	—	—	1	—	—	—	—	—	—	—
4	7	13·7	—	—	—	1	1	—	—	2	2	—	1	—
8	6	11·8	1	1	—	1	—	—	1	1	—	—	1	—
16	10	19·6	—	—	2	3	2	1	—	1	—	1	—	—
32	4	7·9	1	—	1	1	1	—	—	—	—	—	—	—
64	8	15·7	—	1	2	2	2	1	—	—	—	—	—	—
2–64	36	70·7	2	2	5	8	7	2	1	4	2	1	2	—
Geometric mean titres	36		16·0	22·6	32·0	19·0	16·0	32·0	8·0	6·7	4·0	16·0	5·7	—
3 years moving means of titres	36		19·0	25·3	23·1	20·4	18·8	17·2	10·8	6·0	6·6	6·1	8·0	—
All	51	100	2	3	10	9	10	2	2	5	3	1	3	1

Table 5. *Results of CF-tests with formalin antigen (SPG)*

(Data on 51 specimens.)

Stage	Titre	No.	Percentage	(Duration (years)) 1	2	3	4	5	6	7	8	9	10	11	12
II	< 2	5	27·8	—	1	4	—	—	—	—	—	—	—	—	—
	2	1	5·6	—	—	—	—	1	—	—	—	—	—	—	—
	4	1	5·6	—	—	—	1	—	—	—	—	—	—	—	—
	8	2	11·1	1	1	—	—	—	—	—	—	—	—	—	—
	16	3	16·7	—	—	2	1	—	—	—	—	—	—	—	—
	32	3	16·7	1	—	—	1	1	—	—	—	—	—	—	—
	64	3	16·7	—	1	1	1	—	—	—	—	—	—	—	—
	All	18	100·2	2	3	7	4	2	—	—	—	—	—	—	—
III	< 2	3	20·0	—	—	1	—	2	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4	1	6·7	—	—	—	—	—	—	—	—	—	—	1	—
	8	2	13·3	—	—	—	1	—	—	—	1	—	—	—	—
	16	5	33·3	—	—	—	2	2	—	—	1	—	—	—	—
	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	64	4	26·6	—	—	1	1	1	1	—	—	—	—	—	—
	All	15	99·9	—	—	2	4	5	1	—	2	—	—	1	—
IV	< 2	7	38·9	—	—	—	1	1	—	1	1	1	—	1	1
	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	4	5	27·8	—	—	—	—	1	—	—	2	2	—	—	—
	8	2	11·1	—	—	—	—	—	—	1	—	—	—	1	—
	16	2	11·1	—	—	—	—	—	1	—	—	—	1	—	—
	32	1	5·6	—	—	1	—	—	—	—	—	—	—	—	—
	64	1	5·6	—	—	—	—	1	—	—	—	—	—	—	—
	All	18	100·1	—	—	1	1	3	1	2	3	3	1	2	1

disease. However no correlation was found between the height of the CF-titres and the clinical stage of the disease. The percentage incidence of CF-positives showed no correlation either with the clinical stage or with the duration of the disease. The lack of significant correlation between the last three pairs of parameters, could be attributed to some of the following reasons:

(1) The number of specimens observed might be too small to allow for a statistically significant calculation.

(2) The respective pairs of parameters might be not associated causally.

(3) A proportion of the sero-positive reactors might not have been detected by the CF-test. As shown previously (Terzin, Hlača & Fornazarić, 1958), serum specimens of some hosts (e.g. pigeons), depending on the stage of the infection caused by ornithosis virus, may react positively either in CF or in CF-inhibition reaction only. One might suppose by analogy that some positive reactors against the species-specific trachoma antigen reveal just CF-titres, while others would show CF-inhibition titres only, and that the incidence of those reacting in CF-inhibition test only was restricted chiefly to certain stages of the disease. The united incidence of both types of reactors (CF and CF-inhibition positives), under the conditions assumed, certainly would show a correlation with the respective stage of trachoma. In order to verify the validity of this assumption it would be necessary to test sera of trachoma patients both by CF and CF-inhibition reaction, against species-specific trachoma-antigen and trachoma–anti-trachoma indicator systems respectively.

Although quite useful for testing Bedsonia-negative serum samples for species-specific antibodies, the 'SPG-formol-antigen' lacks many qualities of a satisfactory CF-antigen which might be recommended for use in routine. Its main disadvantages seem to be the following:

(*a*) It can be used only to test Bedsonia-negative serum samples for the presence of species-specific anti-trachoma CF-antibodies. With all psittacosis-positive or other serum samples containing group-reactive CF-antibodies against the Bedsonia antigen, the 'SPG-formol-antigen' would react positively because of its group-reactive component as shown in Table 1.

(*b*) The species-specific component of our antigens was thermolabile, a property making the 'SPG-formol-antigen' liable to deterioration.

According to the few observations made so far, the formol antigen kept at $+4°$ C. is gradually losing its species-specific activity, at the same time apparently gaining in its group-reactive activity.

(*c*) Due to insufficient concentration and purity the 'SPG-formol-antigen' prepared by the technique described has both a turbid appearance and relatively low working titres.

SUMMARY

Semi-purified elementary body suspensions, prepared or preserved in three different ways, have been compared as to their species-specific CF-activity as revealed both against Bedsonia-negative sera of trachoma patients (of known stage), and against psittacosis-positive sera of men and animals.

Two of these antigens (the SPG-formol-antigen and the SPG-glycerol-antigen) revealed a positive species-specific CF-reaction in 61–100% of the Bedsonia-negative serum samples obtained from trachoma patients, with titres ranging from 1/2 to 1/128.

Various parameters (as percentage proportion of positive reactors, CF-titre, duration of the disease, clinical stage of trachoma, etc.) have been analysed for correlation, and the results obtained with the different antigens are compared with one another.

The most useful of the three antigens, showing a good species-specific CF-reactivity and no anticomplementary activity, was found to be the 'SPG-formol-antigen'. However because of its group-specific reactivity, for detection of species-specific trachoma antibodies in routine, it could be used only with Bedsonia-negative samples of sera. Because of the thermolability of its species-specific component, the SPG-formol-antigen proved to be liable to deterioration.

We have pleasure in thanking to Dr F. B. Gordon for help in procuring bibliography and the viable strain of trachoma virus. We wish to express our thanks also to Mrs R. Šepetavic and to Miss M. Krečo for their technical assistance.

REFERENCES

Bedson, S. P. (1936). Observations bearing on the antigenic composition of psittacosis virus. *Brit. J. exp. Path.* **17**, 109.

Bedson, S. P. (1959). The Harben Lectures. The psittacosis-lymphogranuloma group of infective Agents. *J. R. Inst. publ. Hlth*, **22**, 67.

Bernkopf, H. (1962). Discussion. *Ann. N.Y. Acad. Sci.* **98**, 345.

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.

Brand, G. & Keil, A. W. (1955). Konzentrierung von Komplementbindenden Influenza-Antigen durch Glyzerindialyse. *Zbl. Bakt.* (I., Abt. Orig.), **162**, 1–2, 13.

Cabasso, V. J., Markham, F. S. & Cox, H. R. (1951). Stabilizing action of glycerine on hemagglutination of egg-adapted mumps, Newcastle disease and influenza viruses. *Proc. Soc. exp. Biol., N.Y.*, **78**, 791.

Collier, L. H. (1961). Experiments with trachoma vaccines. *Lancet*, i, 795.

Galton, M. M., Powers, D. K., Hall, A. D. & Cornell, R. G. (1958). A rapid macroscopic-slide screening test for the serodiagnosis of Leptospirosis. *Amer. J. vet. Res.* **19**, 71, 505.

Grayston, J. T., Wang, S. P., Woolridge, R. L., Yang, Y. F. & Johnston, P. B. (1960). Trachoma: studies of etiology, laboratory diagnosis and prevention. *J. Amer. med. Ass.* **172**, 1577.

Hanna, L. (1962). Isolation of trachoma and inclusion conjunctivitis viruses in the United States. *Ann. N.Y. Acad. Sci.* **98**, 24.

Murray, E. S., Guerra, P., Abbott, A. G. & McComb, D. E. (1962). Isolation of viruses of trachoma from patients in Ethiopia. *Ann. N.Y. Acad. Sci.* **98**, 14.

Snyder, J. C., Page, R. C., Murray, E. S., Daggy, R. H., Bell, S. D., Nichols, R. L., Haddad, N. A., Hanna, A. T. & McComb, D. (1959). Observations on the etiology of trachoma. *Amer. J. Ophthal.* **48**, 325.

Sowa, J. & Collier, L. H. (1960). Isolation of trachoma virus from patients in West Africa. *J. Hyg., Camb.*, **58**, 99.

Terzin, A. L. (1953). Glycerine as a Stabilizer of some Complement-fixing antigens of viral and rickettsial origin. *Proc. Soc. exp. Biol., N.Y.*, **84**, 215.

Terzin, A. L., Hlača, D. M. & Fornazarić, M. R. (1958). Antibodies against Bedsonia antigen in sera of animals. *Arch. ges. Virusforsch.*, **8**, 511.

TERZIN, A. L., MATUKA, S., FORNAZARIĆ, M. R. & HLAČA, D. M. (1961). Preparation of group-specific Bedsonia antigens for use in complement-fixation reactions. *Acta Virol.* **5**, 78.

TERZIN, A. L. & BIRTAŠEVIĆ, B. V. (1962). Complement-fixation and complement-fixation-inhibition titres against the Bedsonia antigen in sera of trachoma patients. *J. Hyg., Camb.*, **60**, 21.

WOOLRIDGE, R. L. & GRAYSTON, J. T. (1962). Further studies with a complement fixation test for trachoma. *Ann. N.Y. Acad. Sci.*, **98**, 314.