

'False positive' complement fixation with psittacosis-trachoma antigens due to antibodies in complement preparations*†

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In the course of serological studies with psittacosis and trachoma during the last few years, first in Sarajevo and later in Bethesda, difficulties have been experienced attributable to the presence of antibodies to the psittacosis-group agents in guinea-pig sera used as source of complement. In view of the increasing interest in the serology of trachoma and psittacosis group agents, it is worth while to describe briefly the observations made and to point out an important source of potential error.

METHODS

Complement fixation (c.f.) and c.f.-inhibition tests have been performed as described by Hoyle (1948), and by an adaptation of the same technique (Terzin, 1958), respectively. The 'units' of antigen and antibody were determined by checkerboard titration, as described elsewhere (Terzin, 1960).

Samples of psittacosis-positive and psittacosis-negative sera of rabbits, as well as guinea-pig sera primarily intended for use as complement, were inactivated for 30 min. at 56° C. before testing for presence of antibodies.

Samples of guinea-pig sera tested for their complement titres were stored lyophilized at 4° C., or frozen at -60° C. and rehydrated or melted, respectively, immediately before titration. Samples of at least ten guinea-pig sera intended for use as complement were absorbed with red blood cells of sheep as recommended by Mayer (Kabat & Mayer, 1961). Samples of absorbed and unabsorbed guinea-pig sera, when inactivated and titrated for the presence of Bedsonia antibodies, showed no detectable difference in c.f. titres or anticomplementary activity. Consequently, the following findings observed in samples of unabsorbed guinea-pig sera apply also to the absorbed specimens.

RESULTS

Comparison of different batches of complement

A total of fifteen batches of complement, originating from five different sources, were inactivated and tested by c.f. test against influenza, mumps and typhus or

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Q-fever antigens. Twelve of these showed completely negative results in dilutions 1/2 or higher, while three samples revealed anticomplementary activity as shown in Table 1. The same fifteen batches of guinea-pig sera were tested also against Bedsonia antigen with known antibody-free guinea-pig complement. The c.F., c.F.-inhibiting and anti-complementary titres, found in tests repeated several times, are shown in Table 1. Ten batches showed measurable c.F. or c.F.-inhibiting titres, three of them as high as 1/16 or 1/32. Two c.F. negative samples showed c.F.-inhibition titres of 1/4 and 1/16, respectively.

Table 1. *Anti-Bedsonia titres found in fifteen batches of guinea-pig sera intended for use as complement**

Batches	Commercial sources						Bethesda laboratory				Sarajevo laboratory				
	a		b			c									
	1	2	1	2	3	1	2	1	2	3	4	1	2	3	4
c.F.	8	16	< 2	4	4	2	16	< 2	< 2	4	4	< 2	4	4	32 [‡]
c.F. inhibition	< 2	< 2	< 2	< 2	< 2	< 2	< 2	4	4	< 2	< 2	16	< 2	< 2	4
A.C. [†]	4	2	< 2	< 2	< 2	< 2	4	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2

* Each was titrated with 2 and 9 units of homologous antigen.

† Anticomplementary activity (saline control).

‡ Specimen showing prozone in the c.F. test.

When c.F.-positive guinea-pig sera, with anti-psittacosis titres as high as 1/16 and 1/32, were used as sources of complement in Bedsonia anti-Bedsonia c.F.-systems, they caused apparently nonspecific positive reactions. When the guinea-pig serum with a c.F.-inhibiting titre of 1/16 was used as a source of complement, it caused a two- to fourfold decrease in the c.F. titres of Bedsonia positive sera.

As seen from Table 1, both the highest c.F. and c.F.-inhibiting titres were obtained with sera of guinea-pigs from Sarajevo. These titres could be attributed to an otherwise inapparent, accidental cross-infection of the complement-donating animals with ornithosis strain P-4.

Effect of the anti-Bedsonia c.F. titre of a complement preparation on antigen and antibody titres

Table 2 shows the increment of antigen titres, and of rabbit antibody titres in c.F. tests when different samples of Bedsonia-positive guinea-pig sera (C' 2, C' 3, C' 4, C' 5 and C' 6) were used as sources of complement. These are compared with the respective titre increments obtained in similar tests performed with a Bedsonia-negative guinea-pig serum as source of complement (C' 1).

Since each of the six C' samples showed a complement titre 1/60, all six complements were used in 1/30 dilution. Consequently, each of the test tubes set up with C' 2-C' 6 contained additional Bedsonia antibody units which amounted to about 1/30 of the antibody units contained per volume of the respective undiluted guinea-pig serum used as complement. Such additional amounts of antibody originating from the sera of Bedsonia-positive complement donors, when added to

the antibody amounts contained in the sera of humans or rabbits actually titrated against the *Bedsonia* antigen, account for the change of titres observed in C.F. tests, set up with the same antigen-antiserum system versus different complements (e.g. with C' 1 or with C' 2-C' 6, respectively).

Table 2. *Tests with six complement samples showing different anti-Bedsonia titres*

Complement samples ...	C' 1	C' 2	C' 3	C' 4	C' 5	C' 6
Anti-Bedsonia titres (c.f.) ...	< 1/2	1/2	1/4	1/8	1/16	1/32
c.f. units per 1 vol. of a 1/30 dilution of C'	≤ 0.033	0.066	0.133	0.266	0.533	1.066
One volume of the respective C' 1/30 causes in c.f. test						
Titre increase of <i>Bedsonia</i> antigen*	× 1	× 1	× 1	× 1	× 1-2	× 2-4
Titre increase of <i>Bedsonia</i> positive serum†	× 1	× 1	× 1-2	× 2	× 2-4	× ∞
Apparent <i>Bedsonia</i> titre in a negative serum	< 1/1	< 1/1	< 1/1	< 1/1-1/2	1/1-1/4	N.E.‡

* Titrated with *ca.* 3 units of homologous test serum.

† Titrated with *ca.* 3 units of homologous antigen.

‡ No end-point (all tubes show positive c.f.).

Although, in general, the results presented in Table 2 hold true for all the observations made in our experiments, in some instances the results observed showed a departure from the details given in that table.

In some instances the increased antigen titres did not appear in the series containing the one or more additional antibody units per tube, but rather in those containing 0.53 or 0.26 antibody units per volume of complement. It is known that sera of some species of animals, including guinea-pigs (Terzin, 1960) as well as certain preparations of the *Bedsonia* group antigen (Terzin, Matuka, Fornazarić & Hlača, 1961) show prozones in the area of antibody excess when checkerboard titrated by c.f.-test. In such cases, a small increment of antibody near a critical point may result in an increase of antigen titre greater than that seen with a larger increment of antibody (see Fig. 1).

As one would expect, if in serum-titration tests one uses complement dilutions containing one or more units of homologous antibody per volume (C' 6 in Table 2), the c.f. test will show positive results in all tubes, whether the serum serially diluted was *Bedsonia* positive or negative, and regardless of how far the dilutions were carried (no end point). However, with *Bedsonia*-negative serum samples even if enriched by 0.26 or 0.53 antibody unit per test tube (as in tests set up with the C' 4 or C' 5 samples of complement) one would not expect a positive c.f. reaction in dilutions higher than 1/1. In contrast to that, as shown in Table 2, the *Bedsonia* negative sera enriched with samples of C' 4 and C' 5 (each containing less

than 1 full unit of homologous antibody) did show c.f.-titres of 1/2 or 1/4, respectively.

Due to possible errors of the twofold serial dilution technique, small amounts of originally undetected antibody contained in the 'Bedsonia-negative' serum, associated with added small amounts of antibody contained in the complement, may account for the antibody titre higher than expected in specimens of 'Bedsonia-negative' sera.

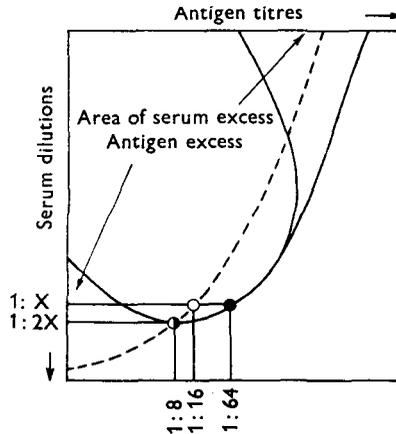


Fig. 1. Possible c.f.-titre relations of guinea-pig and rabbit serum against *Bedsonia* antigen. - - -, rabbit serum; —, guinea-pig serum.

DISCUSSION

As indicated earlier (Terzin, 1960), it is possible to find a different number of antigen units per volume of the same antigen, due to a difference in the combining capacity of the various antisera used in the respective titrations. Consequently, samples of the same antigen may show twofold or more variation in the number of antigen 'units' found per volume unit, depending on the type of serum against which the 'units' were determined (see Fig. 1). Also a twofold greater dilution of different sera (1:2× in the graph) may reveal a two- or eightfold decrease of the antigen titre, depending on the respective type of the serum and antigen used in the checkerboard titration.

Any of these facts may account for the apparent disproportion between the small amount of guinea-pig antibody present in certain complement preparations used (C'3, C'4 or C'5) and the great effect they showed upon the respective c.f. titres presented in Table 2.

Figure 1 shows possible checkerboard titration patterns of a guinea-pig and a rabbit serum titrated against samples of the same *Bedsonia* antigen (Terzin, 1960; Terzin *et al.* 1961).

From several mammalian hosts, including at least two species of rodents, agents closely related to psittacosis have been recovered (Meyer, 1959), all sharing the common soluble antigen characterizing the strains of the *Bedsonia* group. Recently, an agent belonging to the *Bedsonia* group was isolated and identified (Murray,

1964), which caused subclinical or manifest conjunctivitis in guinea-pigs. The agent seems to be widespread in guinea-pig colonies and cross-infection with the agent stimulates the production of circulating antibodies indistinguishable from *Bedsonia* antibodies. In view of these facts it seems natural to expect a high incidence of *Bedsonia*-positive guinea-pigs in colonies of animals intended as a source of complement for routine use in laboratories. Recently, we identified a common *Bedsonia*-group antigen (CBA) contained in the yolk of normal hens' eggs. When titrating the same yolk preparation with constant amounts of an anti-psittacosis serum in parallel tests, set up with samples of normal and *Bedsonia*-positive complement, we obtained CBA titres 1/4 and 1/64, respectively.

In view of the observations discussed in this report, whenever C.F. tests with systems of the psittacosis-trachoma group agents are performed, it seems to be worth while to test carefully for possible presence of *Bedsonia* antibodies in the sera of guinea-pigs to be used as source of complement.

As has been shown, under certain circumstances the presence of such small amounts as 0.53 or 0.26 C.F. units per volume of *Bedsonia* antibodies may cause apparent increase of the *Bedsonia* titres. When used as source of complement, a guinea-pig serum with a 1/32 *Bedsonia* titre may reveal a completely 'false positive' C.F. test. Of the seven commercial preparations of complement (each labelled with a different lot number), produced by firms *a*, *b* and *c*, respectively (as shown in Table 1), five were found positive when tested for *Bedsonia* antibodies. It seems to be indicated therefore, to test the sera of complement-donor guinea-pigs individually or in small pools (not exceeding four individuals per pool if testing them starting with dilution 1/1) with several dilutions of the *Bedsonia* group antigen. Larger pools to be used as source of complement may be conveniently prepared from the samples found negative.

SUMMARY

Complement-fixation tests with psittacosis-trachoma group antigens, if set up with complement prepared from guinea-pigs cross-infected with any of the *Bedsonia* agents, may give completely false positive results. The use of C.F. positive or C.F. inhibition positive samples of guinea-pig sera as a source of complement can induce also a significant increase or decrease, respectively, of the actual C.F. titres in *Bedsonia*-positive serum samples tested. Observations made both in routine serology and in experimental studies show the necessity of testing carefully, for possible presence of *Bedsonia* titres, individual sera of guinea-pigs intended for use as source of complement in C.F. tests performed with *Bedsonia* group antigens.

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