

INCOMPLETE ANTIBODIES IN EXPERIMENTAL BRUCELLA INFECTION

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(With 3 Figures in the Text)

It was shown by Wiener (1944) that some human anti-*Rh* sera contained incomplete antibodies which combined with but did not agglutinate the corresponding red cells. They appeared to be more avid than agglutinating antibodies, so that, if the two kinds of antibody co-existed in the same serum, the incomplete or blocking antibody might account for failure of agglutination or for a zone of inhibition in the tubes containing the greatest amount of serum. Inhibition of this nature has long been known to occur with the sera of some patients and animals suffering from brucella infection. Griffiths (1947), Cox & Kutner (1950), Renoux (1950), Schuhardt, Woodfin & Knolle (1951) and Jones & Wilson (1951) all found blocking antibodies to titres ranging from 1/10 to 1/128 in the sera of persons or of cattle suspected of brucella infection, these antibodies being absent from normal sera or present in low titres only.

Incomplete antibodies have also been demonstrated by the antiglobulin technique of Coombs, Mourant & Race (1945*a, b*). Jones & Wilson (1951) and Wilson & Merrifield (1951) applied this method to the diagnosis of brucella infections and found that the Coombs test revealed significant antibody titres in a considerable number of brucella cases in which the sera were negative to the ordinary agglutination test. Inhibition zones were also eliminated by the Coombs test. Ferris, Stevenson & Lewis (1953) found the test of greater value than the agglutination test for the detection of subclinical or past infection in abattoir workers. Corticelli (1952), Wagner & Kuhns (1953), Clapp (1953), Fey & Bürki (1954) and Brahic, Tamalet, Tamalet & Madet (1955) all report on the examination of sera from persons suspected of brucella infection in which Coombs antibodies were present in the absence of ordinary agglutinins or were present to higher titres.

Of special interest is the work of Hall & Manion (1953) who compared the titres of agglutinins, blocking antibodies and Coombs antibodies in eighty persons. They found no correlation between agglutinins and blocking antibodies, but found that the Coombs method increased sensitivity without destroying specificity, the increase in titre being often fourfold.

The object of the present work was to investigate the appearance of incomplete antibodies in experimentally infected guinea-pigs.

METHOD

Guinea-pigs were inoculated subcutaneously with suspensions of *Brucella abortus* in different dosages ranging from 500,000 to 500,000,000 organisms. Two strains were used, B 437 and B 412, both isolated from milk. The guinea-pigs were bled at

intervals by heart puncture and the sera tested for the presence of antibodies. In one series the animals were bled twelve times, at intervals of a few days in the early stages, then at longer intervals up to the 95th day. In another series, thirteen bleedings were made, up to the 209th day.

The titre of agglutinins was determined against a formalized suspension of the homologous organism by the usual method, with the use of doubling dilutions of the serum from 1/10 upwards. Readings were made after 24 hr. in the water-bath at 50° C. Similar results were obtained in tests incubated at 37° C. Tubes showing no agglutination, or substandard agglutination, were submitted to the Coombs technique. The tubes were centrifuged, the deposit was washed three times and resuspended in saline. A drop of anti-guinea-pig serum was then added and the tubes reincubated overnight and read. The anti-guinea-pig serum was prepared by giving a series of antivenous injections of pooled guinea-pig serum to rabbits, the antiserum being titrated by the method recommended by Proom (1943). These sera were appropriately diluted so that their final concentrations in the test were well above their titres.

RESULTS

A typical result is shown in Table 1, and charts obtained with six guinea-pigs inoculated with various dosages of the two strains are set out in Fig. 1. Agglutinins began to appear towards the end of the first week and had usually attained a titre of 1/640 to 1/1280 by the 30th day. Between the 40th and 80th day there was frequently a further two- to fourfold rise in agglutinin titre, and, as this was sometimes preceded by a fall in titre, a curve with two peaks could be obtained. Observations over a 7-month period, of which Fig. 2 is an example, showed the gradual disappearance of agglutinins in recovered animals, but titres of 1/640 were still often found at the end of that time. In the individual sera a zone of inhibition of agglutination up to a dilution of 1/40 or 1/80 was usual.

By the Coombs technique incomplete antibodies were demonstrated in the zones of inhibition. Their appearance in the tubes containing serum in higher dilution than the agglutinin titres is shown clearly in Fig. 1. They were usually detectable between the 20th and 30th day after infection and increased rapidly to a peak between the 50th and 70th day, declining thereafter, though a second rise was observed in about half the guinea-pigs. Prolonged observations (Fig. 2) showed the gradual decline to a low level in recovered animals. Although this was the characteristic picture, it will be seen from Fig. 1 that in some guinea-pigs there was little or no evidence of Coombs antibodies. In these animals there was a rise of agglutinins up to about the 35th day, then a steady fall with no later response.

Selected sera from these experiments were used to make the following additional observations, which are not reported in detail, either because the findings were negative or because they were confirmatory of previous observations by other workers. Coombs antibodies were not destroyed by heating the sera to 56° C. for $\frac{1}{2}$ hr., but were no longer detectable in some sera that were retested after storage for 4–18 months at 3° C.

Table 1. *Agglutinins (A) and Coombs antibodies (C) in the serum of a guinea-pig at different times after infection with Brucella abortus*

Day	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480	Control
0	A	-	-	-	-	-
	C	-	-	-	-	-
14	A	+	+	+	+	+	+	-	-	-	-	-	-
	C	+	+	+	+	+	+	-	-	-	-	-	-
27	A	+	+	+	+	+	+	+	+	+	-	-	-
	C	+	+	+	+	+	+	+	+	+	-	-	-
37	A	-	+	+	+	+	+	+	+	+	-	-	-
	C	+	+	+	+	+	+	+	+	+	-	-	-
48	A	-	-	+	+	+	+	+	+	+	-	-	-
	C	+	+	+	+	+	+	+	+	+	-	-	-
58	A	+	+	+	+	+	+	+	+	+	+	+	-
	C	+	+	+	+	+	+	+	+	+	+	+	-
69	A	+	+	+	+	+	+	+	+	+	+	+	-
	C	+	+	+	+	+	+	+	+	+	+	+	-
79	A	+	-	-	+	+	+	+	+	+	+	+	-
	C	+	+	+	+	+	+	+	+	+	+	+	-
90	A	-	-	-	+	+	+	+	+	+	+	+	-
	C	+	+	+	+	+	+	+	+	+	+	+	-
103	A	-	±	+	+	+	-	-	-	-	-	-	-
	C	+	+	+	+	+	+	+	+	+	+	+	-
125	A	-	±	+	+	+	-	-	-	-	-	-	-
	C	+	+	+	+	+	+	+	+	+	+	+	-
174	A	-	+	+	+	±	-	-	-	-	-	-	-
	C	+	+	+	+	+	+	+	+	+	+	+	-
209	A	±	+	+	+	±	-	-	-	-	-	-	-
	C	+	+	+	+	+	+	+	+	+	+	+	-

+++ +, ++ +, + +, + = degree of agglutination. - = Negative. . = not tested.

A number of sera were set up in agglutination tests in the usual way, and the tubes after incubation were centrifuged and then read after gentle shaking. The results obtained were almost the same as with the full Coombs test, an observation previously reported by Hall & Manion (1953).

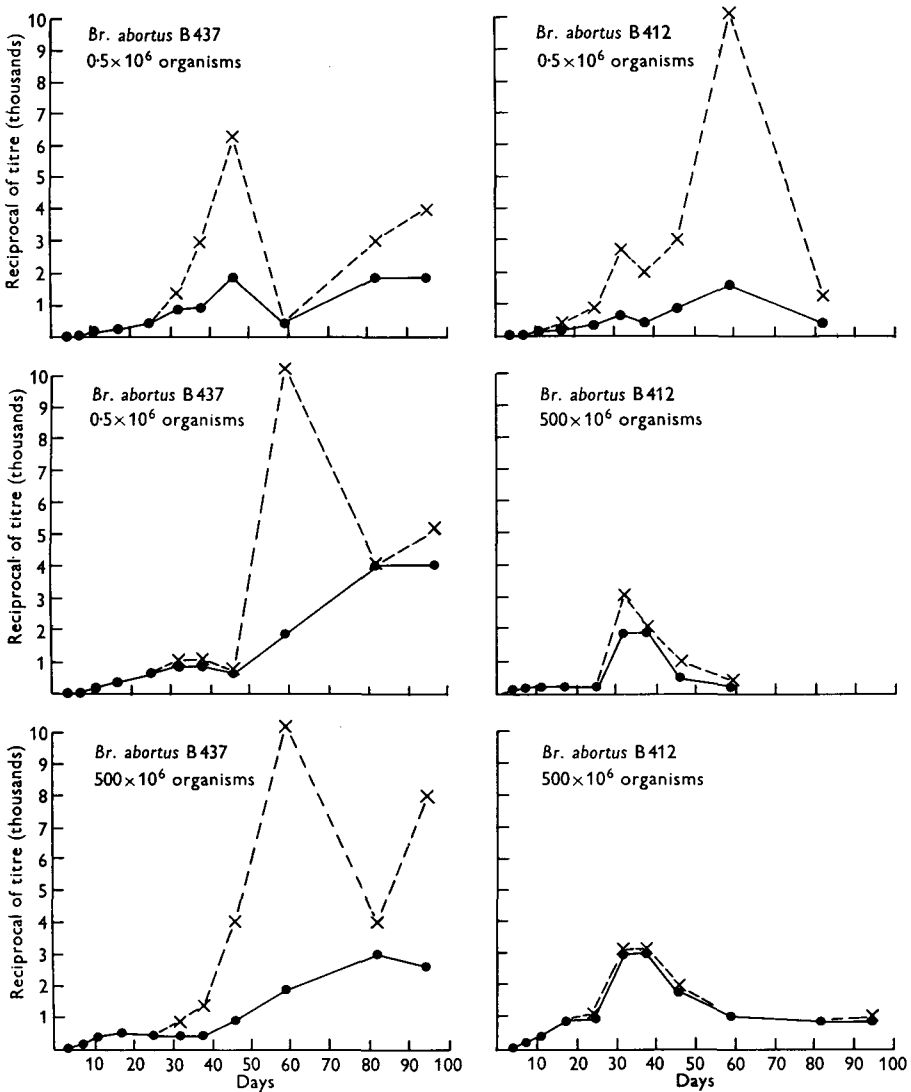


Fig. 1. Production of agglutinins and incomplete antibodies in guinea-pigs infected with *Br. abortus*. ●—●, agglutinins; x—x, incomplete antibodies.

In some experiments normal sera were used instead of saline as diluent. The results led to the finding that some substance capable of blocking agglutination by brucella antiserum was present in normal rabbit, guinea-pig, hog and some human sera to titres of 1/4 to 1/8. These blocking agents were still present in sera heated to 56°C . for $\frac{1}{2}$ hr. and were not removed by absorption with a thick suspension of *Br. abortus*. It will be noted that the titres to which these blocking effects were

observed are not such as to account for the zone effects noted in routine agglutination tests. The normal horse sera tested, on the other hand, agglutinated *Br. abortus* to titres of about $\frac{1}{8}$.

Since numerous sets of sera taken from guinea-pigs throughout the period of infection were available, the opportunity was taken to examine some of these by paper electrophoresis. Fig. 3 shows the mobility patterns obtained by the use of a scanner from a typical series of sera from an infected guinea-pig. The agglutinin

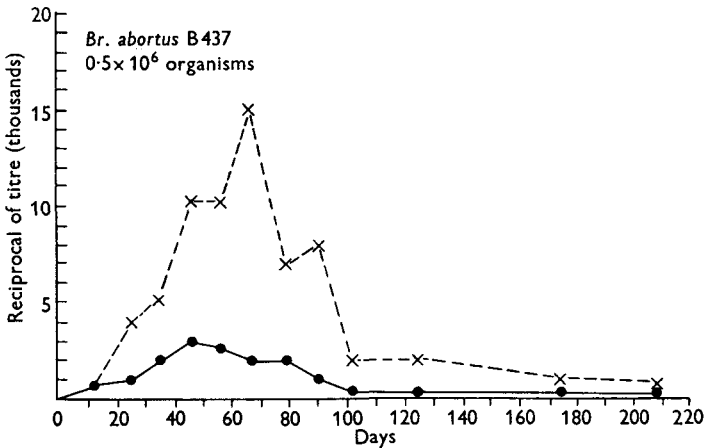


Fig. 2. Production of agglutinins and incomplete antibodies in a guinea-pig infected with *Br. abortus*. ●—●, agglutinins; ×—×, incomplete antibodies.

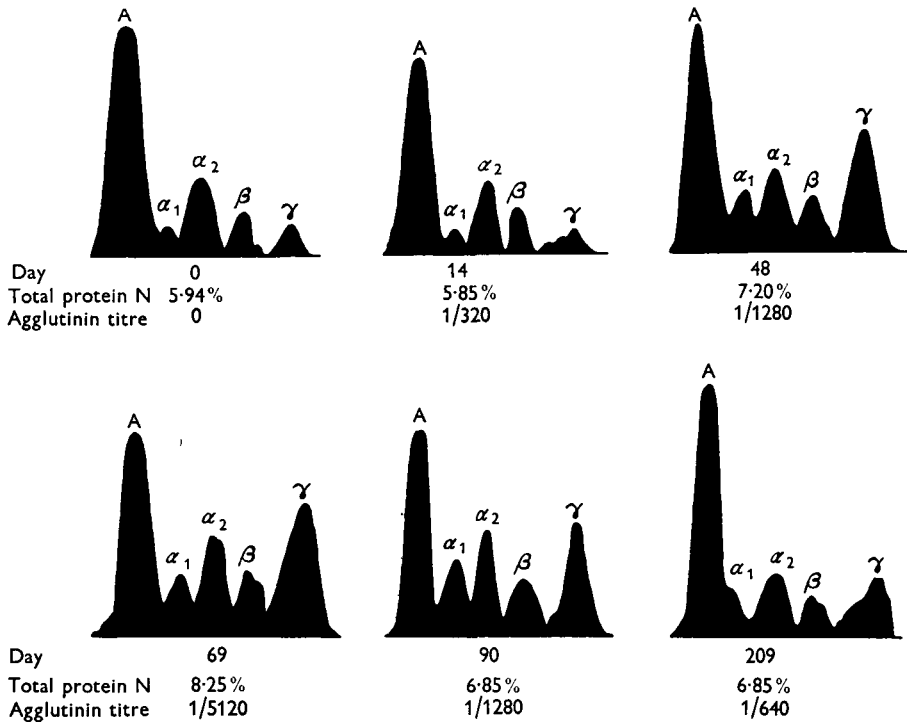


Fig. 3. Electrophoretic patterns of sera from a guinea-pig bled at various times after infection with *Br. abortus*.

titres are also shown. The most striking change as infection develops is the progressive increase in γ -globulin, from about 5% of the total protein in the uninfected guinea-pig to 20–25% between the 50th and 90th day, decreasing slowly thereafter. Much smaller rises were observed in the α_1 and β fractions. Similar results were obtained by Awad (1955). There was no difference in the pattern given by sera with and without detectable Coombs antibodies.

DISCUSSION

It is clear from the experiments now reported that in most guinea-pigs infected with *Br. abortus* incomplete antibodies of the Coombs type are detectable about 30 days after infection, and that they reach a maximum at about 50–60 days, after which they diminish rapidly as the animals recover. In some animals no Coombs antibodies were detected. These antibodies thus appear to be elaborated in the later stages of infection. Jones (1953), in the course of studies of the antigenicity of mucoid and smooth forms of *Br. abortus*, has also reported that infection with S, but not M, organisms led to the appearance of antibodies detectable by the antiglobulin technique. The maximum difference between the direct and antiglobulin titres was found 5–6 weeks after infection, the latter titre being 4–32 times higher than the former. When the present work was nearly completed, Brahic, Tamalet, Madet & Girault (1955) published the results of similar experiments made in guinea-pigs with *Br. suis*. They detected incomplete antibodies about a fortnight after infection, rising to a maximum after about 30–50 days. In their experiments the direct agglutinins had disappeared in practically all instances by the 60th day, at which time the Coombs antibodies were only beginning to fall.

A small number of serial observations in man were made by Wagner & Kuhns (1953), who bled eleven patients with chronic brucellosis, confirmed by blood culture, three times during a period of 3 weeks. All the sera were negative with the direct agglutination test (except in three patients who had titres not higher than 1/40), but all had Coombs antibodies at titres of 1/40 to 1/320, which did not vary significantly in the three bleedings from the individual patients. The authors conclude tentatively that the production of incomplete antibodies is a modified tissue response to reinfection which increases up to a point depending on its severity. The primary infection in their cases had been suppressed by chemotherapy, and it was thought that relapse was due to emergence of organisms from intracellular foci of infection.

It is of some interest that the responses in the present experiments resemble those obtained by Diamond (1948), who submitted *Rh*-negative volunteers to a series of injections of *Rh*-positive cells. Direct or 'saline' agglutinins appeared after the second injection, and incomplete or 'albumin' agglutinins only after the fourth injection (at 4–5 months). As the course of injections was continued the saline agglutinins diminished and disappeared, while the albumin antibodies remained at high titre. The albumin antibodies are the same as those detectable by the Coombs antiglobulin technique and are also referred to by Diamond as late or hyperimmune antibodies.

From the viewpoint of practical diagnosis of brucella infection, the consensus seems to be that detection of blocking antibodies is of little value. In a small number of established cases, however, Coombs antibodies have been demonstrated in the absence of direct agglutinins. Wilson & Merrifield (1951) suggested that, since the technique eliminates prozones, it could be used as a one-tube screening test for suspected sera. It seems probable, however, that the routine laboratory will continue to rely on the full direct agglutination test which, as the W.H.O. Expert Committee on Brucellosis (*Report*, 1953) has emphasized, almost always gives significantly positive results in the presence of active infection, when carried out with a suitable antigen and a satisfactory technique.

SUMMARY

Incomplete antibodies demonstrable by the Coombs antiglobulin technique were found in almost all guinea-pigs inoculated subcutaneously with *Brucella abortus*. They were detected between the 20th and 30th day after infection and reached a peak between the 50th and 70th day, at which time their titre was 4–8 times that of the ordinary agglutinins.

Paper electrophoresis of sera from infected guinea-pigs showed a great increase in γ -globulin as infection developed. Smaller increases in α_1 - and β -globulin were observed.

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