DETECTION OF Q FEVER ANTIBODIES IN WHEY BY THE ANTI-GLOBULIN SENSITIZATION TEST AND OTHER TECHNIQUES

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Q fever in man is derived chiefly from a reservoir of infection in cattle, sheep and goats. The disease is inapparent in these animals; consequently diagnosis, and ultimately control, rest on laboratory procedures, such as the isolation of *Rickettsia burneti* from the milk, or the detection of specific complement-fixing antibodies in the blood serum. A survey of the geographical distribution of Q fever in cattle over a wide area is greatly assisted if bulked specimens of milk from whole herds can be examined initially. The usual method of testing for infection in herds is by the attempted demonstration of rickettsiae in bulked milk by injecting it into guinea-pigs and testing for subsequent development of specific antibodies. Because this method of testing is both expensive and time-consuming, it was decided to investigate the *R. burneti* antibody content of whey from infected cattle with the object of devising a test similar to that used in brucella infections.

Early experiments suggested that the standard haemolytic complement-fixation test (H.C.F.T.) using guinea-pig complement was rather insensitive for this purpose so other serological techniques were used and compared. In addition to the direct agglutination test, these included the conglutinating complement-absorption test (C.C.A.T.) using horse complement, and the anti-globulin sensitization test (A.G.S.T.).

The conglutinating complement-absorption test was developed and applied to the diagnosis of glanders by Hole & Coombs (1947). Subsequently, the C.C.A.T. has been used in studies of *Salmonella pullorum* (Blomfield, Coombs & Hole, 1950) and in serological tests for vaccinia, lympho-granuloma venereum and influenza (Stoker, Coombs & Bedson, 1950; Stoker & Marmion, 1950). In all these systems the C.C.A.T. was never less and usually much more sensitive than the classical H.C.F.T. The test was applied to the diagnosis of Q fever in man and cattle by Wolfe & Kornfeld (1948), who found that the C.C.A.T. gave higher titres than the H.C.F.T., particularly with bovine antisera. It, therefore, seemed possible that this technique might be suitable for the detection of *Rickettsia burneti* antibodies in bovine whey.

The anti-globulin sensitization test was developed by Coombs, Mourant & Race (1945) for the detection of incomplete rhesus antibodies. It employs an anti-human globulin serum to agglutinate red cells which have been sensitized, but not agglutinated, by rhesus antibody. This principle has now been used for the detection of human anti-shiga and anti-typhoid antibodies (Morgan & Schütze, 1946; Stewart & McKeever, 1950) and anti-brucella antibodies (Jones & Wilson,

1951). In all these systems the A.G.S.T. showed considerably greater sensitivity than the direct agglutination test.

Recently Coombs & Stoker (1951) applied the A.G.S.T. to the detection of Q fever antibodies in human serum and found the test more sensitive than the H.C.F.T. and much more sensitive than the direct agglutination test. The A.G.S.T. appeared to be another possible technique for detection of Q fever antibodies in whey, and for this purpose suspensions of *R. burneti* were incubated with increasing dilutions of whey suspected to contain antibodies. These suspensions were then washed by centrifugation and re-suspension in saline and finally tested for adsorbed antibody by addition of an anti-bovine globulin serum which agglutinated the sensitized rickettsiae.

This paper shows a comparison of the haemolytic and conglutinating complement-absorption tests, the direct agglutination test and the anti-globulin sensitization test when used for the examination of specimens of whey for Q fever antibodies. Both infected and uninfected individual cow and herd samples were used.

MATERIALS AND METHODS

Rickettsia burneti *antigen*

Suspensions of *R. burneti* (Henzerling strain) were made from infected yolk sacs by Wolfe & Kornfeld's (1949) modification of Plotz's (1943) technique. The final washed suspension was made up in 0·25 % formol saline buffered at pH 7·0 and appeared microscopically to contain very little extraneous material. A 1/5 dilution was found to be optimal for all the tests except the C.C.A.T. for which the optimum was 1/10 (see Results, §1). At the dilutions used the antigens showed little or no anti-complementary action for guinea-pig or horse complement.

Preparation of whey

10·0 ml. of milk were shaken with 5·0 ml. of chloroform in a screw-capped bottle and 0·5 ml. of rennet* was added. The mixture was then incubated in a water-bath for 1 hr. at 37° C. After this the mixture was centrifuged at 3000 r.p.m. for 15 min., whereupon it separated into three layers: whey at the top, casein clot in the middle, and chloroform at the bottom. The whey was pipetted off and before testing was heated at 56° C. for 30 min. This heating sometimes caused the formation of a precipitate which was removed by centrifugation at 4000 r.p.m. for 10 min. in an angle centrifuge. Appropriate experiments showed that heating at 56° C. for 30 min. had no effect on the whey antibodies, as measured by the H.C.F.T.

Tests of milk and bovine blood serum

Samples of milk were tested for infective *R. burneti* by the inoculation of guinea-pigs, and blood sera from the cows giving the milk were tested by the H.C.F.T. using the techniques described by Marmion & Stoker (1950).

* Real Devonshire Essence of Rennet, Unichem Ltd.

Haemolytic complement-fixation test (H.C.F.T.)

0·1 ml. vol. containing two minimal haemolytic doses of guinea-pig complement (previously determined by overnight titration in the presence of antigen) were added to serial 0·1 ml. doubling dilutions of whey in 0·9 % saline (9 % NaCl, w/v, diluted 1/10 with distilled water). 0·1 ml. of the optimal dilution of antigen (see Results, § 1) was then added to each tube, and to control tubes containing 2, 1 and $\frac{1}{2}$ units of complement. A titration of a standard positive human serum was also included as a control, as were the usual anti-complementary controls of the whey. The tests were allowed to remain at 4° C. overnight before the addition of 0·1 ml. of 1 % sheep cells and 0·1 ml. of 5 M.H.D. horse haemolysin, and were subsequently incubated at 37° C. for 30 min. Degrees of haemolysis were recorded after centrifugation.

Conglutinating complement-absorption test (C.C.A.T.)

The technique was the same as for the H.C.F.T. with the substitution of horse complement for guinea-pig complement, and after standing overnight the addition of a conglutinating instead of a haemolytic indicator system. The conglutinating system consists of 0·3 % sheep cells and 1/20 bovine serum inactivated at 56° C. (which contains both conglutinin and a natural anti-sheep cell antibody). After incubation at 37° C. for 30 min. the tubes were centrifuged and the cells re-suspended by tapping. Unconglutinated cells form a homogeneous suspension and conglutinated cells remain in large discrete clumps.

Direct agglutination test (D.A.T.)

0·1 ml. quantities of 1/5 rickettsial suspensions were added to 0·1 ml. serial dilutions of whey in Dreyer's tubes. After incubation in a 37° C. water-bath overnight, readings were made with a hand-lens in indirect light against a dark background.

Anti-globulin sensitization test (A.G.S.T.)

The technique used for whey was the same as that described by Coombs & Stoker (1951) for detecting *R. burneti* antibodies in human serum. 0·2 ml. quantities of 1/5 rickettsial suspension were added to equal volumes of doubling dilutions of whey in small round-bottomed tubes (internal dimensions 7 × 50 mm.). After the whey-antigen mixtures had been incubated at 37° C. for 30 min., the tubes were centrifuged in an angle centrifuge at 4000 r.p.m. for 1 hr. in order to deposit the rickettsiae. After withdrawal of the supernatant fluid the deposit was resuspended in saline. This washing procedure was repeated twice and the rickettsiae were finally resuspended in 0·2 ml. of saline. Each suspension was distributed in two Dreyer's tubes in 0·1 ml. volumes. To one series was added an equal volume of rabbit anti-bovine globulin serum diluted 1/160 and to the other normal rabbit serum diluted 1/160 (both sera inactivated at 56° C. for 30 min.). The tubes were then incubated in a water-bath at 37° C. Agglutination of the sensitized rickettsiae was first observed after 1–2 hr. and reached a

maximum after 5 hr. In practice it was most convenient to read the test after incubation at 37° C. overnight. Readings were made in the same way as in the direct agglutination reaction. The titre of *R. burneti* antibodies in a whey was expressed as the highest dilution which so sensitized the rickettsiae that definite agglutination occurred with anti-globulin serum.

RESULTS

(1) *Optimum antigen dilutions for the haemolytic and conglutinating complement-absorption tests*

In order to determine the optimum antigen dilutions for the H.C.F.T. and C.C.A.T., a sample of whey was used from a cow ('Nigger') which was excreting *R. burneti* in the milk, and whose blood serum showed an antibody titre against *R. burneti* of 1/320 by the H.C.F.T. Doubling dilutions of whey from this cow were titrated in 'chessboard' fashion against doubling dilutions of *R. burneti* antigen both by the H.C.F.T. and C.C.A.T. The results are shown in Table 1, and it will be seen that in the H.C.F.T. the whey titre remained at 1/128 with antigen dilutions from

Table 1. *Titration of Q fever antibodies in whey from a cow 'Nigger' by haemolytic and conglutinating complement-absorption tests*

Test	Antigen dilution	Whey dilution 16	32	64	128	256	512	1024	2048	5096
Haemolytic C.F.T.	5	4	4	4	4	2	0	0	0	0
	10	4	4	4	4	2	0	0	0	0
	20	4*	4*	4*	3	2	0	0	0	0
	40	0	0	0	0	0	0	0	0	0
Conglutinating C.A.T.	5	4	4	4	4	4	4	0	0	0
	10	4	4	4	4	4	4	0	0	0
	20	4	4	4	4	4	4	0	0	0
	40	4	4	4	4	4	4	0	0	0
	80	0	0	0	0	0	0	0	0	0

* A zone of inhibition appears at an antigen dilution of 1/20 in some tests.
4 = complete fixation of complement; 0 = no fixation of complement; 2, 3 = intermediate degrees of fixation of complement.

1/5 to 1/20. The whey titre was higher in the C.C.A.T. (1/512) and was unaltered between antigen dilutions of 1/5 to 1/40. Subsequent tests were carried out with four minimal reacting units of antigen; that is a dilution of 1/5 for the H.C.F.T. and 1/10 for the C.C.A.T. These dilutions of antigen showed little or no anti-complementary effect in the two tests.

Several other wheys were titrated against the antigen by the H.C.F.T. in 'chessboard' fashion and all gave similar patterns except that zones of inhibition were sometimes more marked at lower antigen concentrations. There was, however, one exception. The whey from cow 'Muriel III' showed better fixation with lower antigen concentrations of 1/16 and 1/32 than with higher concentrations of 1/4 and 1/8 (Table 2). This curious zone of fixation was reproducible in repeated tests and

was apparently due to specific antibody, as there was no fixation with a control typhus vaccine antigen. The presence of antibody in this sample of whey was further shown by the results of the direct agglutination and sensitization tests (Table 5).

Table 2. *'Chessboard' titration of whey from a cow, 'Muriel III', in* H.C.F.T.

		Dilutions of whey					
		2	4	8	16	32	64
Dilution of *R. burneti* antigen	4	tr	0	0	0	0	tr
	8	tr	0	0	0	0	0
	16	4	4	0	0	0	0
	32	4	4	tr	0	0	0
Control antigen (typhus vaccine diluted 1/10)		0	0	0	0	0	0

4 = complete fixation; 0 = complete haemolysis; tr = partial haemolysis.

(2) *Optimal dilution of anti-globulin serum in the sensitization test*

Previous experience with brucella and other antigen-antibody systems has shown that there may be an optimal dilution of anti-globulin serum for detecting sensitization, and that if the serum is used in too strong a concentration a zone of inhibition can occur. Nevertheless, in testing for *R. burneti* antibodies in human serum, Coombs & Stoker (1951) found that concentrated solutions of the anti-globulin serum used gave the best results.

To determine the optimal concentration of anti-bovine globulin serum for detecting sensitization by whey antibodies, experiments were carried out with whey from a herd Devon '6'. Milk from this herd contained *R. burneti*, but no whey antibodies could be detected by the H.C.F.T., C.C.A.T. or direct agglutination test.

The anti-globulin technique was carried out as described with antigen and doubling dilutions of whey, but each reagent was used in four times the normal amount (total 0·8 ml. instead of 0·2 ml.). After incubation, centrifugation and washing of the antigen, the treated rickettsial suspensions were finally resuspended in 0·8 ml. of saline and distributed in 0·1 ml. vol. in eight parallel series. Each series thus consisted of suspensions of rickettsiae which had originally been treated with increasing dilutions of whey. To each of the first four series was added rabbit anti-bovine globulin serum diluted 1/20, 1/40, 1/60 and 1/640 respectively. To each of the four remaining series was added normal rabbit serum in the same four dilutions. After 5 hr. incubation at 37° the tests were read with results which are shown in Table 3. A dilution of anti-globulin serum of 1/160 was taken to be optimal for future tests.

Washed rickettsiae treated with this particular whey were also agglutinated by dilute, but not concentrated, normal rabbit serum. A possible explanation of this agglutination by normal rabbit serum was that the repeated centrifugation used in washing the rickettsiae enhanced the direct spontaneous agglutination

Table 3. *Titrations of rabbit anti-bovine globulin serum and normal rabbit serum used in testing* Rickettsia burneti *for sensitization with antibodies from whey*

		Dilutions of whey used for treating rickettsial suspension			
Dilutions of rabbit serum ...		Undiluted	2	4	8
Rabbit anti-bovine globulin serum	20	+	−	−	−
	40	++	+	−	−
	160	++	++	++	++
	640	++	++	++	−
Normal rabbit serum	20	−	−	−	−
	40	+	−	−	−
	160	++	++	−	−
	640	++	++	−	−

++ = complete agglutination; − = no agglutination.

of the organisms sensitized with whey antibodies. To test this possibility, rickettsial suspensions were treated with dilutions of whey (from herd 'Wm'), and after subsequent incubation and washing were mixed with normal saline and normal undiluted whey, as well as normal rabbit serum and rabbit anti-globulin serum both diluted 1/160.

It will be seen from Table 4 that simple centrifugation and resuspension in saline or normal whey enhanced the direct agglutination titre. Presumably the enhancement of rickettsial agglutination in normal rabbit serum diluted 1/160 was due to the same effect. Stronger concentrations of rabbit serum inhibited the agglutination (see Table 2).

Table 4. *Effect of saline, normal whey, normal rabbit serum, and rabbit anti-bovine globulin serum, on rickettsiae which had been sensitized with* Rickettsia burneti *antibodies from whey*

	Dilutions of whey used for sensitizing rickettsial suspension						
	2	4	8	16	32	64	128
Direct agglutination in saline	++	++	++	−	−	−	−
Sensitized rickettsiae washed by centrifugation and resuspended in:							
Saline	++	++	++	++	+	−	−
Normal whey undiluted	++	++	++	++	++	−	−
Normal rabbit serum 1/60	++	++	++	++	++	−	−
Rabbit anti-globulin serum 1/160	++	++	++	++	++	++	−

Similar enhancement of direct agglutinating titres was seen with most samples of whey, as will be apparent from the results given in the next section. For convenience it is referred to as the enhanced agglutination reaction.

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(3) *Comparison of the haemolytic and conglutinating complement-absorption tests, the direct agglutination test, and the anti-globulin sensitization test in the detection of rickettsial antibodies in whey*

A. *Whey from individual cows*

Samples of whey were obtained from ten cows which had *R. burneti* antibodies in their blood serum and from eleven cows with no detectable antibody. Four of the ten positive cows were also excreting *R. burneti* in the milk.

The results of the various tests on the wheys are seen in Table 5. Wheys from

Table 5. *Tests for Q fever antibodies on wheys from individual cows*

Name or number of cow	Blood serum titre (H.C.F.T.)	*R. burneti* in milk	Whey				
			H.C.F.T.	C.C.A.T.	Direct agglutination	Sensitization test: Normal rabbit serum	Sensitization test: Anti-globulin serum
Nigger	320	+	128	512	128	256	2048
Trixie	80	+	32	256	32	64	1024
Polly	160	+	0(8)*	64	64	64	512
Muriel III	160	+	0(4)†	0	1	16	32
Nora	40	−	1	4	16	64	128
Old Nancy	320	−	2	8	4	16	64
Ida	80	−	1	16	2	4	32
Frances	20	−	1	<2 AC	1	16	32
Ginger Poll	10	−	0	1	4	4	8
Rose 'A'	10	−	0	<2 AC	0	2	4
5753	<5	NT	0	0	0	0	0
5756	<5	NT	0	0	0	0	0
5757	<5	NT	0	0	0	0	0
930	<8	NT	0	0	0	0	0
968	<8	NT	0	0	0	(1)	(1)
1185	<8	NT	0	<2 AC	0	0	0
1187	<8	NT	0	0	0	0	0
1492	<8	NT	0	0	0	0	0
1528	<8	NT	0	0	0	0	0
1529	<8	NT	0	0	0	0	0
1531	<8	NT	0	0	0	0	0

H.C.F.T. = haemolytic complement-fixation test.
C.C.A.T. = conglutinating complement absorption test.
AC = anticomplementary.
(8)* = 1/8 in earlier test with lower antigen dilution.
(4)† = 1/4 at higher dilutions of antigen (see Table 2).
NT = not tested.
0 = no reaction by undiluted whey.
1, 2, etc. = reaction by undiluted or 1/2, etc. dilution of whey.
(1) = doubtful reaction by undiluted whey.

cows with no *R. burneti* antibodies in the blood serum were negative by all tests except for a very doubtful reaction in the A.G.S.T. with undiluted whey from cow 968.

The different tests varied considerably in their sensitivity for *R. burneti* antibody

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in wheys from the group of infected cows. Using the H.C.F.T., antibody was detected in specimens from eight of the ten infected cows, but, in four of these, the titre was very low (less than 1/4) and in a further two specimens, fixation was only obtained by varying the antigen dilution. Only two wheys (from 'Nigger' and 'Trixie') were unequivocally positive by the H.C.F.T.

Antibody titres of 1/4 or over were found in six specimens of whey by the C.C.A.T. and in seven by the direct agglutination test. It should be noted that in this series of wheys the ordinary agglutination reaction was more sensitive than the H.C.F.T. This is contrary to our experience with human Q fever antisera in which the H.C.F.T. reveals much higher antibody titres than the direct agglutination test.

Only the A.G.S.T. was sensitive enough to detect antibodies at dilutions of 1/4 or higher in specimens of whey from all ten infected cows. The enhanced agglutination reaction (as shown in the normal rabbit serum controls of the A.G.S.T.) was also positive for all ten specimens, although titres were generally lower than in the A.G.S.T. and one was less than 1/4.

B. *Whey from herd bulked milk samples*

Whey was obtained from six herd milk samples from which *R. burneti* had been isolated, and from two samples which had yielded no rickettsiae. The number of cows and proportion of serologically positive animals was only known for three of the herds, and from one of these ('We' 1545) there was insufficient whey to complete all the tests.

The results are shown in Table 6. It will be observed that neither of the two

Table 6. *Tests for Q fever antibodies in wheys from bulked milks*

			Whey				
						Sensitization test	
Name of herd	No. of cows with positive sera in in herd	*R. burneti* in milk	H.C.F.T.	C.C.A.T.	Direct agglutination test	Normal rabbit serum	Anti-globulin serum
Ri	1/11	+	<4 AC	<4 AC	2	16	32
We 1501	6/24	+	1	2	4	16	64
We 1545	6/24	+	1	8*	NT	8	>16<64
To 11–15	UK	+	0	<2 AC	2	8	8
Wm	UK	+	0	<2 AC	8	32	64
De 6	UK	+	0	0	0	2	8
To 16–20	UK	−	0	0	0	0	(1)
Wg	UK	−	0	0	0	0	(1)

H.C.F.T. = haemolytic complement-fixation test.
C.C.A.T. = *conglutinating complement-absorption test.*
(1) = doubtful.
AC = anticomplementary.
* = *insufficient whey to test at lower dilution.*
UK = unknown.
NT = not tested.

complement-fixation tests was sensitive enough to detect antibodies in the pooled wheys except where very low titres were found in specimens 'We' 1501 and

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'We' 1545. The C.C.A.T. was particularly unsatisfactory because of the anti-complementary action of several of the specimens of whey for horse complement.

The A.G.S.T., on the other hand, detected antibodies in the pooled wheys from all six infected herds, with titres ranging from 1/8 to 1/64. The direct agglutination test only failed in one instance ('De' 6) and the enhanced agglutination reaction was positive in wheys from all infected herds. Titres revealed by these last two tests, however, were generally lower than those by the A.G.S.T.

The doubtful reactions which were obtained by the A.G.S.T. in the undiluted wheys from apparently uninfected herds do not seriously threaten the specificity of this test, but this small number of controls is obviously insufficient.

DISCUSSION

From these preliminary tests it would appear that the detection of *R. burneti* antibodies in whey is a reliable method for tracing infected cows and herds. Even with individual cows it is much simpler to obtain a sample of milk than of blood, while for surveying a large number of herds, the method would have great advantages. However, the validity and practical usefulness of the whey examination in detecting infected cows, or, in particular, infected herds of cattle, must depend on the results of examination of a larger number of wheys. Of the serological techniques the direct agglutination test was remarkably sensitive compared with the complement-fixation tests, and its sensitivity was considerably enhanced by centrifugation and resuspension of the rickettsiae in normal serum or saline.

The use of anti-globulin serum for detecting sensitization with antibody gave the highest titres, with but little loss of specificity. Since a high sensitivity is important in examination of bulked milk, where the whey antibodies from positive cows may be considerably diluted by the milk from other negative cows, the A.G.S.T. is probably the best method, although technically the least simple.

At present the source of the antibodies in the whey is unknown. It is possible that they may either come from the blood serum or be formed locally in the mammary gland. If, as suggested by Smith & Holm (1948), the immune globulin concerned is not the same as the blood serum immune globulin, the use of an antiserum against whey globulin in the A.G.S.T. might yield better results than the use of an anti-blood serum globulin as used in these experiments.

In addition to the practical applications, several points of theoretical interest emerge from this investigation. Repeated centrifugation enhanced the tendency of organisms sensitized with antibody to agglutinate spontaneously in saline or diluted normal rabbit serum. Resuspension in concentrated rabbit serum, however, reduced this enhancement. In different specimens of whey there was considerable variation between the ratio of the direct or enhanced agglutination titre on the one hand, and the titre determined by the A.G.S.T. on the other. For example, the A.G.S.T. titre of 'Ginger Poll' was only twice the enhanced agglutination titre and the ordinary direct agglutination titre (see Table 5). On the other hand, with whey from 'Nigger' the A.G.S.T. gave titres sixteen times higher than the direct agglutination and eight times higher than the enhanced agglutination reactions. It is possible that the amount of incomplete or non-agglutinating

antibody over and above the complete or agglutinating antibody is variable. It would be interesting to know if the relative amounts of the two forms of antibody are related to the stage of infection in the cow.

Finally, there is the problem of the infectivity of the rickettsiae in the milk in the presence of this antibody. One might expect that these organisms would be neutralized but, as in brucella infections, higher antibody titres were usually found in those very milks from which the causative organism had been isolated. Infectivity of positive milks is admittedly low and this may be due to partial neutralization. If this is so, however, dilution of the milk and its antibody should enhance the infectivity, and this effect is not generally observed. Further experimental investigations of the protective effect of whey antibody are necessary to decide this point.

SUMMARY

1. Samples of whey from individual cows and herds infected with *R. burneti* and from uninfected cows and herds have been tested for *R. burneti* antibodies.

2. The haemolytic complement-fixation test, the conglutinating complement-absorption test, the direct agglutination test and the anti-globulin sensitization test were compared for sensitivity in detecting whey antibody.

3. The direct agglutination test was generally more sensitive than either of the complement-fixation tests, particularly when it was enhanced by centrifugation of the sensitized rickettsiae. The anti-globulin sensitization test, however, gave the highest titres and it appeared to be specific.

4. The practical application of these techniques and certain theoretical considerations are discussed.

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REFERENCES

Blomfield, A. M., Coombs, R. R. A. & Hole, N. H. (1950). The conglutination phenomenon. VI. An experimental investigation of the factors determining the adsorption of complement by an antigen-antiserum mixture. *J. Hyg., Camb.*, **48**, 73.

Coombs, R. R. A., Mourant, A. E. & Race, R. R. (1945). A new test for the detection of weak and 'incomplete' Rh agglutinins. *Brit. J. exp. Path.* **26**, 255.

Coombs, R. R. A. & Stoker, M. G. P. (1951). Detection of Q fever antibodies by the anti-globulin sensitisation test. *Lancet*, ii, 15.

Hole, N. H. & Coombs, R. R. A. (1947). The conglutination phenomenon. I. An introduction to the conglutination phenomenon and an account of the observations and views of previous investigators. *J. Hyg., Camb.*, **45**, 480.

Jones, L. O. & Wilson, M. M. (1951). Serum agglutinins in brucellosis. *Nature, Lond.*, **167**, 558.

Marmion, B. P. & Stoker, M. G. P. (1950). Q fever in Great Britain. *Lancet*, ii, 611.

Morgan, W. T. J. & Schütze, H. (1946). Non-agglutinating antibody in human antisera to *Sh. shiga* and *S. typhi*. *Brit. J. exp. Path.* **27**, 286.

Plotz, H. (1943). Complement fixation in rickettsial diseases. *Science*, **97**, 20.

Smith, E. L. & Holm, A. (1948). The transfer of immunity to the newborn calf from colostrum. *J. biol. Chem.* **175**, 349.

Stewart, F. S. & McKeever, J. D. (1950). The antiglobulin technique applied to the detection of non-agglutinating antibody against *Salmonella typhi* O in human sera. *J. Hyg., Camb.*, **48**, 357.

STOKER, M. G. P., COOMBS, R. R. A. & BEDSON, S. P. (1950). The application of the conglutinating complement-absorption test to virus systems. *Brit. J. exp. Path.* **31**, 217.

STOKER, M. G. P. & MARMION, B. P. (1950). A comparison of the conglutinating complement-absorption test and the haemolytic complement-fixation test in the serological diagnosis of influenza. *Brit. J. exp. Path.* **31**, 217.

WOLFE, D. M. & KORNFELD, L. (1948). Conglutinating complement-absorption test compared with haemolytic complement-fixation reactions using Q fever immune bovine serum. *Proc. Soc. exp. Biol., N.Y.*, **69**, 251.

WOLFE, D. M. & KORNFELD, L. (1949). The application of a quantitative complement-fixation method to a study of Q fever strain differentiation. *J. Immunol.* **61**, 297.

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