THE EFFECT OF OPSONIZATION WITH ANTISERUM ON THE SURVIVAL OF *BRUCELLA ABORTUS* WITHIN BOVINE PHAGOCYTES

H. SMITH AND R. B. FITZGEORGE

From the Microbiological Research Establishment, Porton, Salisbury, Wilts.

Received for publication July 1, 1964

IT is agreed that opsonization with antiserum increases the rate at which bacteria are phagocytozed but its effect on their intracellular survival seems to vary according to which organism and which phagocytic cell is used. Some workers have reported that opsonization increases the intracellular destruction of bacteria (*e.g.* Jenkin and Rowley, 1963), others that it has a protective effect (*e.g.* Cohn, 1963) and others that there is no significant alteration of intracellular behaviour after opsonization (Mackaness, 1960; Thorpe and Marcus, 1964). For work on the chemical basis of the intracellular growth of *Brucella abortus*, a test was established for estimating the degree of survival and intracellular growth of this organism in the phagocytes of its natural host—the bovine (Pearce, Williams, Harris-Smith, FitzGeorge and Smith, 1962; Smith and FitzGeorge, 1964). This paper describes the use of the test to examine the effect of opsonization on the phagocytosis and intracellular survival of a virulent and an avirulent strain of *Br. abortus*. Two antisera against live virulent *Br. abortus* were used; one was prepared in rabbits and the other in a heifer.

MATERIALS AND METHODS

Rabbit and bovine antiserum against live Br. abortus (544).—This was prepared as described by Smith, Keppie, Pearce and Witt (1962) and Macrae and Smith (1964).

Test for estimating the degree of survival and intracellular growth of Br. abortus *in bovine phagocytes.*—This was described by Pearce *et al.* (1962) and Smith and FitzGeorge (1964).

Treatment of Br. abortus (*strains* 544 *and* 45/0) *with antisera.*—The strains were grown on tryptic meat agar slopes for 18 hr. at 37° in 10 per cent CO_2/air and washed off with saline containing 10 per cent v/v tryptic meat broth. Equal parts (0·2 ml.) of the suspension (standardized to 8×10^9 organisms/ml.) and of Locke solution or a dilution ($\frac{1}{3}$) of the appropriate serum, were mixed and left at 37° for 5 min. The mixtures were diluted with saline (10 ml.), centrifuged at $750 \times g$. for 15 min. and the deposits resuspended in saline (2·5 ml.). A few clumps of organisms were dispersed by shaking (15 cm. strokes, 500 strokes per min.) with ballotini beads for 5 min. The suspensions of organisms were counted, standardized (8×10^5/ml. for "544" and $9{\cdot}6 \times 10^6$/ml. for "45/0") and used in the test described by Smith and FitzGeorge (1964).

RESULTS

Table I summarizes the results of 6 similar tests using rabbit antiserum and the strains 544 and 45/0. Treatment with antiserum increased the proportion of organisms which were phagocytozed and therefore increased the number that were protected from the extracellular destruction that occurs during this period of phagocytosis (Smith and FitzGeorge, 1964). However the opsonization had no significant effect on the progressive intracellular destruction of the avirulent

TABLE I.—*The Survival and Growth of* Br. abortus (*strains* 544 *and* 45/0) *during Phagocytosis and Residence within Bovine Phagocytes, after Pre-opsonization with Rabbit Brucella Antiserum*

	Bacterial count; survival (per cent) of original inoculum					
	Strain 544 pretreated with:			Strain 45/0 pretreated with:		
Stage in test*	Locke solution	Rabbit normal serum	Rabbit brucella antiserum	Locke solution	Rabbit normal serum	Rabbit brucella antiserum
After 3 hr. phagocytosis and before killing of extracellular organisms	27	31	81†	17	18	40†
After incubation with an extracellular bactericidal mixture (hr.)						
1	7	8	59	3·0	3·2	23
18	5	6	57	1·2	1·3	10
40	13	14	135	0·7	0·8	7

This test is representative of 6 similar tests.

* For details of test see Smith and FitzGeorge (1964).

† Viable counts on deposits and supernatants produced by centrifugation of samples at this stage (Smith and FitzGeorge, 1964) indicated that pretreatment with antiserum increased the proportion of organisms phagocytozed.

TABLE II.—*The Survival and Growth of* Br. abortus (*strains* 544 *and* 45/0) *during Phagocytosis and Residence within Bovine Phagocytes, after Pre-opsonization with Bovine Brucella Antiserum*

	Bacterial count; survival (per cent) of original inoculum					
	Strain 544 pretreated with:			Strain 45/0 pretreated with:		
Stage in test*	Locke solution	Normal bovine serum	Bovine brucella antiserum	Locke solution	Normal bovine serum	Bovine brucella antiserum
After 3 hr. phagocytosis and before killing of extracellular organisms	35	27	57†	13	14	21†
After incubation with an extracellular bactericidal mixture (hr.)						
1	9	11	46	2·5	2·3	8·8
18	15	21	82	2·3	2·5	8·3
40	41	44	155	2·0	1·5	4·7

Similar results were obtained in a second test.

* For details of test see Smith and FitzGeorge (1964).

† Viable counts on deposits and supernatants produced by centrifugation of samples at this stage (Smith and FitzGeorge, 1964) indicated that pretreatment with antiserum increased the proportion of organisms phagocytozed.

smaller number of experiments. Here, the bovine phagocytes were somewhat less destructive for both strains of *Br. abortus* than in the experiments with rabbit antiserum. The overall result in both series of experiments was that the final level of intracellular infection (after 40 hr. incubation) had been increased as a result of opsonization with specific antiserum.

DISCUSSION AND SUMMARY

In a phagocytosis and intracellular growth test *in vitro*, opsonization with rabbit and bovine brucella antiserum increased the phagocytosis by normal bovine phagocytes of both a virulent and an avirulent strain of *Br. abortus*. However, there was no significant effect on their subsequent intracellular behaviour and hence in this test *in vitro*, the final level of intracellular infection was greater for the opsonized organisms. It is difficult to judge what relevance the results of these experiments *in vitro* have to the situation *in vivo*. The difficulties in demonstrating differences in the behaviour of *Br. abortus* within the white blood cells of normal and immune cattle have been described in the previous paper (Macrae and Smith, 1964). Furthermore, Henderson (1964) showed that although *in vitro*, *Brucella suis* grew better in monocytes (and serum) from immune guinea-pigs than in a similar system from normal guinea-pigs, the immune system had a bactericidal effect *in vivo* when injected into normal guinea-pigs.

REFERENCES

COHN, Z. A.—(1963) *J. exp. Med.*, **117**, 43.
HENDERSON, D. W.—(1964) *Symp. Soc. gen. Microbiol.*, **14**, 241.
JENKIN, C. R. AND ROWLEY, D.—(1963) *Bact. Rev.*, **27**, 391.
MACKANESS, G. B.—(1960) *J. exp. Med.*, **112**, 35.
MACRAE, R. M. AND SMITH, H.—(1964) *Brit. J. exp. Path.*, **45**, 595.
PEARCE, J. H., WILLIAMS, A. E., HARRIS-SMITH, P. W., FITZGEORGE, R. B. AND SMITH, H.—(1962) *Ibid.*, **43**, 31.
SMITH, H. AND FITZGEORGE, R. B.—(1964) *Ibid.*, **45**, 174.
Idem, KEPPIE, J., PEARCE, J. H. AND WITT, K.—(1962) *Ibid.*, **43**, 538.
THORPE, B. D. AND MARCUS, S.—(1964) *J. Immunol.*, **92**, 657.