THE VALUE OF NON-SPECIFIC AGGLUTINATION IN THE DIFFERENTIATION OF THE GENUS *BRUCELLA*.

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The recognised difficulty of differentiating between *Brucella melitensis* and *Br. abortus* except by complicated serological methods has led to attention being given to non-specific agglutination as a possible means of establishing the identity of the various members of the *Brucella* group. It has been recognised for some time that, on heating to 90° C., agglutination is produced in a saline suspension of *Br. paramelitensis*, whereas a similar suspension of *Br. melitensis* remains stable. Since, however, the serological differentiation between these two organisms is clear cut, there is little need to emphasise this interesting thermo-agglutination of *Br. paramelitensis* as more than a confirmatory test.

Most recent work, however, has been directed towards the separation of *Br. abortus* and *Br. melitensis* by such non-specific tests. In the case of the colon-typhoid and dysentery groups the acid agglutination test devised by Michaelis (1915) has received a measure of praise from bacteriologists as a method of discrimination between *B. dysenteriae* Flexner and *B. dysenteriae* Shiga. With this example there was justification for testing the *melitensis-abortus* group on similar lines. Vercellana and Zanzucchi (1926) tested suspensions of these organisms with various chemical substances. They employed formalin, corrosive sublimate (1 in 1000), alcohol (95 per cent.), caustic soda, lactic acid, acetic acid, butyric acid, and hydrochloric acid each in 1 per cent. dilution. They found that, in the case of lactic acid, after incubation at 37° C. for 30 minutes, agglutination of *Br. melitensis* with 1 per cent. lactic acid was marked, and with 0·2 per cent. after 24 hours, whereas *Br. abortus* gave a negative result.

With eight strains of each organism, *Br. melitensis* reacted strongly in six instances, and weakly in the remaining two, whereas *Br. abortus* was negative except in one instance where there was weak agglutination in 1 in 20 in 24 hours. Trentini (1926), who repeated these experiments with six strains of *Br. melitensis* and eight of *Br. abortus*, was unable to corroborate these results completely. Two strains of *Br. melitensis* gave complete and four partial agglutination. Four strains of *Br. abortus* failed to agglutinate but the remaining four gave definite though only partial agglutination. Favilli (1927) investigated twenty-two strains of *Br. abortus*, seven of *Br. melitensis*, and five of *Br. paramelitensis* by thermo-agglutination, agglutination in peptone, and agglutination in lactic acid. He came to the conclusion that

similar results were obtained by the first two methods, but that lactic acid agglutination had a wider range. With one or two exceptions *Br. melitensis* and *Br. abortus* did not react to thermo-agglutination or agglutination by peptone but positive results were obtained with *Br. paramelitensis*. He considers that the lactic acid test is valueless in distinguishing between *Br. melitensis* and *Br. paramelitensis*.

The present communication records the results obtained in the investigation of non-specific agglutination in strains of *Br. melitensis*, *Br. abortus*, *Br. paramelitensis* and *Br. paraabortus*. The series was partly composed of strains which had undergone artificial cultivation for some time, and partly of strains recently isolated from cases of undulant fever in Southern Rhodesia. Their identification was first established by means of agglutination, and absorption of agglutinin tests. It was found by absorption that two main groups could be differentiated; one comprising the majority of the stock *Br. melitensis* and *Br. abortus* strains as well as the majority of the Rhodesian strains; the other consisting of a stock *Br. paramelitensis*, a so-called *Br. melitensis*, a so-called *Br. abortus* strain and two of the Rhodesian strains. On absorption of agglutinin tests being applied the groups were subdivided as follows:

Group A	*Br. melitensis*	Br. M. 1	(S.)
		Br. M. 2	(S.)
		Br. M. 3	(S.)
		Br. M. 4	(S.)
	Br. abortus	Br. A. 1	(S.)
		Br. A. 3	(S.)
		Du Rand	(R.)
		Narcee	(R.)
		Collinson	(R.)
		Williams	(R.)
		Gray	(R.)
		Kenny	(R.)
Group B	*Br. paramelitensis* (A)	Br. PM. 1	(S.)
	Br. paramelitensis (B)	Br. M. 5	(S.)
	Br. paraabortus	Br. A. 2	(S.)
		Lockie	(R.)
		Weldon	(R.)

S. = Stock strain. R. = Rhodesian strain.

Groups A and B were clearly defined by the fact that, with immune rabbit serum, the serum prepared against group A did not agglutinate the organisms of group B, or did so only in low dilution. The reverse was also the case.

The non-specific agglutination of these strains, by heat, by peptone, by lactic acid, and by Michaelis' original technique was investigated.

1. *Thermo-agglutination.* Forty-eight hour cultures of the organisms were washed off in 0·75 per cent. saline following Favilli's technique, standardised to the same opacity (8 T.M.) and were heated to 90° C. After five to ten minutes agglutination was obvious in both *paramelitensis* strains and was complete in thirty minutes. With *paraabortus* Br. A. 2 very slight agglutination was observed after one hour's heating, but this could not be compared to the clear cut result obtained with the *paramelitensis* strains. The

remaining strains were not affected. All the reacting strains had been in cultivation for some time. The same result was obtained when physiological saline was employed. This result is confirmatory of previous findings that thermo-agglutination is a feature of *Br. paramelitensis*.

2. *Peptone agglutination.* Forty-eight hour agar cultures of the organisms were suspended in distilled water, standardised as above, and were tested in the presence of Parke Davis and Co. bacteriological peptone. Each organism was added in 1, 2 and 3 c.c. amounts to 1 c.c. of 1 per cent. peptone. After eighteen hours at 37° C. no agglutination was observed with any strain.

This test is more liable to give different results in the hands of different observers than any other, owing to the fact that commercial peptones vary so much in their constitution. The test then is one which does not seem of sufficient value to warrant its general application.

3. *Lactic acid agglutination.* A 1 per cent. solution of pure lactic acid was made up in distilled water. Forty-eight hour cultures of the organisms were suspended in distilled water, standardised as above, and killed by heating to 60° C. for thirty minutes. The lactic acid solution was distributed in four tubes in 0·5 c.c. amounts of 1, 0·5, 0·25 and 0·125 per cent. concentrations. To these were added 0·5 c.c. amounts of organism suspension. Incubation was at 37° C. Results were read at 30 minutes, 1 hour, 2 hours, and 18 hours intervals.

The results were as recorded in Table I.

Table I.

			30 minutes				1 hour				2 hours				18 hours			
			1 %	·5 %	·25 %	·125 %	1 %	·5 %	·25 %	·125 %	1 %	·5 %	·25 %	·125 %	1 %	·5 %	·25 %	·125 %
Group A	*Br. melitensis*	Br. M. 1	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
		Br. M. 2	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
		Br. M. 3	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
		Br. M. 4	−	−	−	−	−	−	−	−	−	−	−	−	ǂ	ǂ	(+)	−
	Br. abortus	Br. A. 1	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
		Br. A. 3	−	−	−	−	+	−	−	−	+	+	−	−	ǂ	ǂ	+	−
		Du Rand	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Narcee	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Collinson	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Williams	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Gray	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		Kenny	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Group B	*Br. paramelitensis* (A)	Br. PM. 1	−	−	−	−	ǂ	ǂ	−	−	ǂ	ǂ	ǂ	−	ǂ	ǂ	ǂ	ǂ
	Br. paramelitensis (B)	Br. M. 5	−	−	−	−	ǂ	+	+	−	ǂ	ǂ	ǂ	−	ǂ	ǂ	ǂ	ǂ
	Br. paraabortus	Br. A. 2	−	−	−	−	+	+	−	−	ǂ	ǂ	−	−	ǂ	ǂ	ǂ	+
		Lockie	−	−	−	−	+	−	−	−	ǂ	ǂ	−	−	ǂ	ǂ	ǂ	+
		Weldon	−	−	−	−	ǂ	+	−	−	ǂ	ǂ	−	−	ǂ	ǂ	ǂ	+

ǂ = Complete agglutination. (+) = Doubtful agglutination.
\+ = Partial agglutination. − = No agglutination.

These results were also obtained with a suspension of live organisms in distilled water, and with a suspension which had been brought to the boiling point.

The sharp differentiation between organisms of group A and group B in their response to the presence of lactic acid is noteworthy. At the end of two hours only one organism in group A showed slight agglutination, whereas all the organisms in group B showed complete agglutination, the two *paramelitensis* strains in the 0·25 per cent. and over concentrations, and the three *paraabortus* strains in the 0·5 per cent. and over concentrations. The eighteen hours results are equally striking. All the group B organisms show what can be regarded as complete agglutination in all concentrations. The group A organisms divide themselves into two categories; those that show absence of agglutination which class is composed entirely of the recently isolated strains, and those which show agglutination complete or partial in some tubes of the series but not to the lowest concentration of lactic acid as in group B. This class is composed of strains which have been in cultivation for some time.

The statement of Favilli that the lactic acid test has a wider range than thermo-agglutination is confirmed as is also his further statement that the test is of no value in aiding in the differentiation of *Br. melitensis* from *Br. abortus*. From the above results, however, it would appear that, in group A organisms, the length of time the organism has been in cultivation determines to some extent the degree of agglutination. The introduction of a variable of this nature is in itself sufficient to make the test unsuitable for general application. The contrast between the *Br. abortus* strains of recent origin and those which have been in cultivation for some time is of significance in this connection.

Where the test is of value, however, in the present instance is that it confirms the result of orthodox serological methods in separating the organisms investigated into two groups. The group B organisms stand as clearly defined by this method of non-specific agglutination as by agglutination and absorption of agglutinin tests. It would appear that the length of time of cultivation is not as important a factor in group B as in group A. "Lockie" and "Weldon," both of Rhodesian origin, reacted exactly as strains which have been in culture for years.

4. *Acid agglutination.* The technique which was followed in these experiments was the original technique of Michaelis (1915) described in the paper cited and *References*. The following six solutions were employed:

Sol.	*N*/1 NaOH c.c.	*N*/1 CH_3COOH c.c.	
1	5	7·5	Each made up into 100 c.c. with distilled water.
2	5	10	
3	5	15	
4	5	25	
5	5	45	
6	5	85	

A forty-eight hours culture of the organisms to be tested was suspended in 20 c.c. of distilled water and 3 c.c. of the emulsion placed in each of six test-tubes. One c.c. of each of the six solutions was then added to each of the tubes and well shaken. The tubes were incubated at 37° C. and results

read at 2 hours and 18 hours. The results recorded in Table II are those obtained after 18 hours. It was found that this length of incubation extended the range of agglutination in the case of those organisms which gave a positive result after 2 hours without producing agglutination in those which did not react at the end of that period.

Table II.

Organism			Tubes 1	2	3	4	5	6
Group A	*Br. melitensis*	Br. M. 1	–	–	–	–	–	–
		Br. M. 2	–	–	–	–	–	–
		Br. M. 3	–	–	–	–	–	–
		Br. M. 4	–	–	–	–	–	–
	Br. abortus	Br. A. 1	–	–	–	–	–	–
		Br. A. 3	(+)	(+)	(+)	(+)	(+)	(+)
		Du Rand	–	–	–	–	–	–
		Narcee	–	–	–	–	–	–
		Collinson	–	–	–	–	–	–
		Williams	–	–	–	–	–	–
		Gray	–	–	–	–	–	–
		Kenny	–	–	–	–	–	–
Group B	*Br. paramelitensis* (A)	Br. PM. 1	–	–	ǂ	ǂ	ǂ	ǂ
	Br. paramelitensis (B)	Br. M. 5	–	–	ǂ	ǂ	ǂ	ǂ
	Br. paraabortus	Br. A. 2	–	–	+	ǂ	ǂ	ǂ
		Lockie	(+)	+	ǂ	ǂ	ǂ	ǂ
		Weldon	–	–	+	ǂ	ǂ	ǂ

The results obtained by acid agglutination are remarkably parallel to those obtained by lactic acid agglutination. Here again there is the clear-cut differentiation into the two groups, group A with one exception showing no agglutination in any of the tubes, and group B organisms all showing agglutination in the tubes 3, 4, 5 and 6. This, however, is the only differentiation that can be made. The result given by Br. A. 3 must be regarded as a peculiarity. The flocculation observed in all the tubes was uniform but of very slight degree and not at all to be compared to the very definite agglutination observed in the group B organisms. It is interesting to note that this strain reacted peculiarly in the lactic acid tests. Excluding this result, however, no difference was observed between the strains of *Br. melitensis* and *Br. abortus* in group A, nor between the *Br. paramelitensis* and *Br. paraabortus* strains in group B. Acid agglutination then ranks like the lactic acid agglutination solely as a means of distinguishing group A organisms from group B organisms.

Summary.

(1) Non-specific agglutination as tested by thermo-agglutination, peptone agglutination, lactic acid agglutination and Michaelis' acid agglutination failed in the primary object of differentiating between *Br. melitensis* and *Br. abortus*.

(2) Thermo-agglutination was only observed in strains of *Br. paramelitensis*.

(3) With peptone agglutination no strain gave a positive result.

(4) The results of lactic acid agglutination and Michaelis' acid agglutination ran practically parallel. No distinction could be observed between *Br. melitensis* and *Br. abortus*. Strains of both of remote origin gave slight agglutination with lactic acid while recently isolated strains of *Br. abortus* were negative. Very definite agglutination, however, was observed by both methods with *paramelitensis* and *paraabortus* strains, but no differentiation was possible between such strains.

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