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Specificity of the Haemagglutination Test for Toxoplasmosis with reference to Infections with *Salmonella typhi* and *Salmonella paratyphi*

by

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MOST sera sent for estimation of antibodies against *Toxoplasma* bear the legend "fever of unknown origin". In addition to toxoplasmosis and a host of other conditions, continuous fever may be caused by infections with Salmonellae. In Ceylon such fever is caused by infections with *Salmonella typhi* and *S. paratyphi A*. As a result of acute disease, subclinical contact with these organisms or active immunization, antibodies at various levels against these organisms may be expected in the random population. A part of these antibodies are agglutinins notably against "H" and "O" antigens. Under these circumstances it was decided to study the effect of the presence or absence of these antibodies on the results of the haemagglutination test (Jacobs & Lunde, 1957) for toxoplasmosis.

MATERIALS AND METHODS

Sera of blood sent from various hospitals from patients suspected of typhoid fever were used in this study. The sera were collected after the Widal test (standard agglutination test) was performed and stored in the deep freeze (—17°C.) till required. This period of storage did not exceed 4 weeks but in most cases the storage was only for a week. The Widal test was carried out by the Serology section of this Institute using standard methods and their results were used.

Prior to examination with the haemagglutination test, the sera were thawed and inactivated for 30 mins. in a water bath at 56°C. They were kept in the refrigerator overnight with an equal volume of 4% sheep erythrocytes in phosphate-buffered saline of pH 7.2.

The haemagglutination test was carried out as described by Jacobs & Lunde (1957) with certain modifications, to be described later, to adapt it to a micro method. The antigen, which is a water extract of lysed *Toxoplasma* was stabilized with phosphate-buffered saline of pH 7.6 and a few drops of merthiolate solution. Sheep erythrocytes were used throughout and were collected in 3.8% sodium citrate solution. The concentration of the erythrocyte suspension was increased to 4%. Equal volumes of freshly prepared tannic acid solution and 4% erythrocyte suspension in phosphate-buffered saline of pH 7.2 (PBS) were incubated in a water bath at 37°C. for 15 mins. The tanned erythrocytes were washed twice in PBS and resuspended in one volume of PBS giving the original 4% suspension.

One volume of the antigen was mixed with one volume of tanned erythrocytes in PBS and incubated in a water bath at 37°C. for 15 mins. The sensitized erythrocytes were washed once in inactivated 1% normal rabbit serum in PBS and resuspended in two volumes of the 1% rabbit serum. The sera were diluted in twofold dilutions using a dropping pipette on a Takatsy microtitrator plate. The diluent was 1% inactivated rabbit serum in PBS. 0.05 ml. of each serum dilution was used in the test. To each of these volumes was added 0.025 ml. of the sensitized erythrocyte suspension. To each of the serum controls was added 0.025 ml. of 2% tanned erythrocytes in PBS. 0.025 ml. of sensitized erythrocytes was added to 0.05 ml. of the 1% rabbit serum in PBS to act as a diluent control. The sera and the erythrocyte suspensions were well mixed by moving the plates in a rotatory manner. The cells were allowed to sediment at room temperature and the reading taken after two hours.

The antigens used in this study were from four different batches.

RESULTS

The Table I gives in a summary form the results obtained. A total of 97 sera were tested with the haemagglutination test. Eleven agglutinated tanned erythrocytes and were not included in the table. The lowest dilution of serum at which the Widal test was carried out was 1 : 50 and sera showing agglutinins against any of the antigens below this dilution were considered as negative. The Widal test positive sera used in this study had agglutinins against " H " and/or " O " antigens of *S. typhi* and/or " H " antigen of *S. paratyphi A*. The majority of the sera had agglutinins over 1 : 100 to one or more of the antigens and 27 of the sera had over 1 : 200. None of the sera had agglutinins against " Vi " antigen. All sera that gave a positive haemagglutination test at a titre of 1 : 2 or more were considered as positive and others as negative.

TABLE I

Showing the distribution of haemagglutination results among Widal test positive and negative sera.

WIDAL TEST		Haemagglutination Test			
Description	No.	Negative	1 : 2—1 : 64	1 : 128—1 : 512	1 : 1024—1 : 4096
Positive for agglutinins at 1 : 50 or more against *S. typhi* "H" and/or "O" and/or *S. paratyphi A* "H".	46	29	12	3	2
Negative for agglutinis at 1 : 50 or more against *S. typhi* "H" and/or "O" and/or *S. paratyphi A* "H".	51	30	15	3	3

The results obtained were analysed using the modification of the " Fourfold " table by Yates (see Bradford Hill, 1961). The difference observed between the number of positives in the group where the Widal test was positive and the number of positives in the group where the Widal test was negative on statistical analysis gave a probability greater than 0.80. Therefore there was no significant difference between the two groups.

DISCUSSION

This study has shown that the presence of antibodies against " H " and/or "O " antigens of *S. typhi* and/or *S. paratyphi A* does not influence the results of the haemagglutination test of Jacobs and Lunde (1957). Some of these agglutinins detected in the Widal test may represent antibodies against other Salmonellae e.g. *S. typhi-murium* and *S. enteritidis* which have common antigens with *S. typhi*. The extent of indication of the presence of these common antibodies depends not only on the titre in the Widal test but also on the number of common antigens. What ever it may be, this study suggests that the presence of such agglutinins in the random population need not be considered in interpreting haemagglutination titres.

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