CLOT CULTURE IN TYPHOID FEVER

BY

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In early diagnosis of typhoid fever, the emphasis has for long been on the isolation of *S. typhi* from blood culture. Clot culture techniques have not enjoyed the same vogue, although they are largely used in areas where the disease is endemic. Felix (1924) found that clot culture was hardly ever unsuccessful if blood culture was successful, but that the reverse was frequently true. He attributed this to the removal of any bactericidal serum factors before culturing the clot.

In an earlier publication (Thomas, Watson, and Hewstone, 1954) we recommended the use of streptokinase bile broth (containing 100 units/ml. of streptokinase) as a culture medium for blood clot. Our experience with this method since then has amply borne out the initial favourable results, and as a result of its application it soon became apparent that the results of clot culture were considerably better than those of blood culture. Thus, in a series of 48 cases where both clot culture and blood culture were performed before the introduction of streptokinase and one or both yielded a positive isolation, there were 38 positive blood cultures and 36 positive clot cultures. In a further series of 99 cases since the introduction of streptokinase there were 48 positive blood cultures and 93 positive clot cultures, an increase of 45 in favour of the latter.

Two possibilities arose that might be responsible for these results: (1) streptokinase might exert a specific stimulating effect on the growth of *S. typhi*, or (2) removal of the serum before culturing the clot increases the chances of obtaining a positive isolation. We have investigated (1) at some length, but have been unable to demonstrate any specific effect on growth, and the action of streptokinase appears to be, as we previously reported, purely an enzyme one concerned with fibrinolysis.

A study was therefore undertaken of the effect of serum factors on the isolation of *S. typhi* from blood clot and whole blood.

Serum Factors in Clot Culture

Preparation of Artificially Infected Clots.—Samples of blood, each of 5 ml., from a healthy donor were added to 1.0 ml. quantities of a saline suspension of a freshly isolated strain of *S. typhi* containing approximately 30 viable organisms as estimated by plate count. The blood-bacterium suspension was thoroughly mixed and the blood allowed to clot. After two hours at room temperature the separated serum was removed. Each clot was then bisected, using a sterile Petri plate and two microscope slides for each, the clot being tipped into the plate, held by the edge of one slide and divided with the other. One half of each plus the total serum volume was incubated in 15 ml. of streptokinase bile broth in a universal container, and the other half clot alone in another 15 ml. Subcultures were plated daily on to Wilson and Blair's medium. Results are detailed in Table I.

TABLE I

Days' Incubation	Clot with Serum		Clot Without Serum	
	Positive	Negative	Positive	Negative
1	17	11	28	Nil
7	17	11	—	—
14	17	11	—	—

After 14 days' incubation 11 of the 28 half-clots incubated with serum gave a negative result, whereas all 28 were positive where half-clots alone were cultured. Since these were corresponding halves of the same clots it is improbable that the former were initially sterile.

When the above method was applied to clots from clinical cases of typhoid fever the results in Table II were obtained in a series of 35 consecutive cases.

Table II shows that nine of the 35, with added serum, gave a negative result after 14 days' incubation where the corresponding half-clot was positive, but only one gave a negative result without added serum where the other half was positive. The chance occurrence of such a result has been estimated at 1 in 93. Analysis of the number of days required to produce a positive result in those cases where both

TABLE II

No. of Cases	Half-clot with Serum		Half-clot Alone	
	No. of Cases	Days' Incubation	No. of Cases	Days' Incubation
35	15	+1	26	+1
	3	+2	5	+2
	4	+3	3	+3
	2	+4	1	-14
	1	+6		
	1	+7		
	9	-14		

+ = Positive isolation. - = Negative result.

halves were positive gives a mean difference of 0.80 ± 0.283 days. The ratio of the mean difference to the standard error is thus 2.83, which is significant. Agglutinin titres were estimated in all the above 35 cases, but were not significantly higher, where a negative result was obtained with added serum, than in those which were positive.

Serum Factors in Blood Culture

A study of the effects of serum factors in blood cultures was performed as follows.

Volumes of 0.5% taurocholate broth, each of 5 ml., were placed in sterile 5 in. by 5/8 in. tubes. To each was added 0.1 ml. of a saline suspension of *S. typhi* containing approximately 25 viable organisms, together with varying volumes of fresh citrated blood from a donor. Tubes were incubated at 37° C. and subcultures plated daily on to Wilson and Blair's medium. Results are shown in Table III.

TABLE III

Volume of Medium (ml.)	Volume of Blood (ml.)	Days' Incubation							
		1	2	3	4	5	6	7	14
5.0	0.5	+	+	+	+	+	+	+	+
5.0	1.0	+	+	+	+	+	+	+	+
5.0	1.5	-	+	+	+	+	+	+	+
5.0	2.0	-	-	-	-	-	-	-	-
5.0	2.5	-	-	-	-	-	-	-	-
5.0	3.0	-	-	-	-	-	-	-	-
5.0	4.0	-	-	-	-	-	-	-	-
5.0	5.0	-	-	-	-	-	-	-	-

+ = Positive isolation. - = Negative result.

Using this particular strain of *S. typhi* (A2), a bactericidal effect was evident where the ratio of blood to medium was less than 1 in 4 to 1 in 5. When the experiment was repeated with six different strains, using approximately the same initial inoculum as above, two resulted in positive isolations where the blood-medium ratio was 1:2, and four gave results similar to those in Table III.

Discussion

Felix (1924), Soman (1932), and Compton and Said (1933) all stress the necessity for removing serum before culturing the residual clot, and the results in Tables I and II support the need for this precaution. Of even more importance in our

experience is the need to bring about quick lysis of the clot. In a series of 96 positive clot cultures before the introduction of streptokinase broth, the average period of incubation required to produce a positive result was 6.8 days with a variation of one to 16 days. After the introduction of streptokinase broth the average period required in 170 positive cultures was 1.9 days with a variation of one to four days. Recently we have obtained positive isolations in less than 24 hours by culturing the clots in streptokinase broth in 4 oz. bottles containing Wilson and Blair agar slopes.

It has been our custom to perform blood cultures by adding 8-10 ml. of blood to 35 ml. of 0.5% taurocholate broth, but, in view of the results in Table III, the volume of medium would appear to be too small in relation to the volume of blood. Volumes of culture medium reported in the literature vary considerably; Batty Shaw and Mackay (1951) used 15 ml. of taurocholate broth, whereas Treffers (1948) recommended 190 ml. medium. Pulvertaft (1930) advocated the addition of 10 ml. of blood to at least five times that volume of medium. Where the volume of medium is small the theoretical advantage of adding a large volume of blood to increase the chances of obtaining an infected sample may be outweighed by the disadvantage of increasing the concentration of bactericidal serum factors. Recently we have increased the volume of medium from 35 to 50 ml., and in a series of 30 bacteriologically proved cases of typhoid fever since then there were 23 positive blood cultures, i.e. 77%, and 30 positive clot cultures. This compares with 48 positive blood cultures in a series of 99 cases where the volume of culture medium was 35 ml., i.e., 48.4%. It would seem essential to increase both the volume of medium and the volume of blood if the most satisfactory results are to be obtained.

The failure to find any correlation between the agglutinin titres and the occurrence of positive or negative results of culture in the 35 clinical cases is in keeping with the findings of others. Khairat (1946), for example, could find no correlation between agglutinin titres and the susceptibility of the infecting strain by testing *in vitro*.

Summary

Streptokinase bile broth as a culture medium for blood clot has increased the number of positive isolations and decreased the duration of incubation required compared with ordinary bile broth.

Clot culture techniques, in our experience, have proved superior to the blood culture.

Bactericidal serum factors may exist in sufficient concentration to militate against successful isolation of *S. typhi* where the volume of medium is too small.

No correlation between the bactericidal action of serum and the level of circulating agglutinins was found.

The bactericidal action of serum on *S. typhi* is dependent on the strain of organisms as well as on the serum.

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