FACTORS INFLUENCING THE RESULTS OF BLOOD CULTURE IN ENTERIC FEVER

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Vilchur, in 1887, was the first to isolate the typhoid bacillus from the blood stream during life, and early studies of the diagnostic value of this procedure were published by Castellani (1900, 1902) and Schotmüller (1902). In 1906, Conradi and Kayser reported on the improved results which accompanied the inclusion of bile in the culture medium, and in the following year Coleman & Buxton (1907) analysed the results of blood culture in 1602 cases of typhoid fever, on the basis of their own experiences and those hitherto recorded in the literature. During the 1914–18 war further studies on the results of blood culture were published by Mann, Rainsford & Warren (1915), Le Boeuf & Braun (1917), Gay (1918) and others; in recent years Chatterjee (1942) and Stuart & Pullen (1946) have been among those to report the largest series.

The majority of physicians would concur with Manson-Bahr (1950) that blood culture 'is unquestionably the most satisfactory method of diagnosis (in enteric fever)', but few recent studies have been made of those factors which influence the results so obtained. Such an investigation was undertaken during an outbreak of seventy-six cases of enteric fever at Acre, Palestine, in 1948. The epidemic, which involved British troops and members of the Palestine Police, was unusual in that it was caused by a double enteric infection with *Salmonella typhi* and *Salm. paratyphi B* (Batty Shaw & Mackay, 1951). This paper is based on an analysis of the results of blood culture which were obtained, and their relation to body temperature, stage and severity of disease, previous inoculation, and the length of incubation required in order to obtain a positive result. In the majority of cases the results of blood culture are only available for the first 3 weeks of the disease; for during the fourth week of the epidemic the evacuation of British troops, and our patients, took place from Palestine to Egypt.

METHOD

Blood culture was performed within the first 2 days of admission to hospital in all cases. If the result was negative, the culture was repeated after a few days or at a week's interval. In addition to such diagnostic blood cultures, a blood culture was performed in each of the first 3 weeks of disease in all cases. Five ml. of blood were withdrawn into 15 ml. of taurocholate broth, which, after 24 hr. incubation, was subcultured on to MacConkey's medium. This subculture was repeated on the third, fifth, ninth and eleventh days of incubation, before regarding the result as negative.

It was intended to make a simultaneous study of the results of stool and urine culture in each week of the disease. But on account of the labour and difficulty

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of collecting specimens from so large a number of patients, this part of the investigation was considered unjustified in the circumstances which were associated with the evacuation from Palestine. A few positive results were obtained before this part of the investigation was abandoned. Thereafter stool and urine culture were employed only in those cases where blood culture had been negative. Variations in the supply of media available prevented a uniform technique for urine culture. Selenite F, when available, was the medium of choice, and was used in approximately one-third of the cultures. In its absence, tetrathionate broth, or enriched tetrathionate broth, was employed. The latter medium, devised by the Central Pathology Laboratory, Fayid, contained, in addition to the normal formula for tetrathionate broth, 1 % sodium desoxycholate, 1 % sodium citrate and 1:100,000 brilliant green. Ten ml. of one of these media were added to 10 ml. of urine and, after 24–48 hr. of incubation, were subcultured on to MacConkey's agar and desoxycholate citrate agar.

For stool culture the same media were used, approximately 20 ml. of each being added to 2 ml. of faeces and incubated after thorough mixing.

During the epidemic, slide agglutination with typhoid Vi antiserum or paratyphoid B 'H' antiserum, together with the appropriate sugar reactions, were used in diagnosis. All isolated organisms were sent to Fayid, Egypt, for confirmation and phage typing. All the specimens of *Salm. typhi* were shown, in Egypt, to be of Vi-phage type T. With the paratyphoid-B Vi-typing phages then available in the Middle East, it was not found possible to type the specimens of *Salm. paratyphi B* that were isolated, but Dr A. Felix informs us that the strain was subsequently identified at the Central Enteric Reference Laboratory, London, as belonging to Vi-phage Type 'Dundee', according to the extended typing scheme of Felix & Callow (1943).

RESULTS

The diagnosis in seventy-four of the seventy-six cases (97·3 %) was confirmed bacteriologically, with the following results: *Salm. typhi* alone, 43 cases; *Salm. paratyphi B* alone, 3 cases; *Salm. typhi* and *Salm. paratyphi B*, 28 cases.

In seventy-one cases (93·4 %) positive blood cultures were obtained. In fifteen, both organisms were isolated from the same culture, and in one case blood culture in the third week revealed a different organism from that isolated at the start of the disease. Of the remaining twelve cases of double enteric infection ten were diagnosed by the isolation of one organism from the blood and the second from the excreta, and two by isolation of both organisms from the same specimen of excreta.

THE FACTORS INFLUENCING THE RESULTS OF BLOOD CULTURE

(i) *Body temperature*

There is a common misconception that it is of little value to perform a blood culture, in a suspected case of enteric fever, when moderate or high pyrexia is absent. This view dates from the work of Hébert & Bloch (1922), who reported only one positive result when the body temperature was below 101·2° F. in a series of 7500 cultures performed at different temperatures. We have already confirmed the results of

others that blood culture may be positive in enteric fever when the temperature is 99° F., or, in one case, subnormal (Batty Shaw & Handfield-Jones, 1948).

Table 1 shows the results obtained from 138 blood cultures at different body temperatures. These figures show that, although the percentage of positive results was greater at the higher body temperatures (100 % of sixty-five cultures between 102 and 104° F. were positive), a significant number of positive results were obtained at the lower temperatures. Of fifty-three cultures, taken at a body temperature between 97 and 101° F., forty-five (84·9 %) were positive, and three of the four cultures taken when the temperature was subnormal also grew enteric bacilli.

Table 1. *Relation between results of blood culture and body temperature*

Temp. (° F.) ...	97–98	98–99	99–100	100–101	101–102	102–103	103–104	104
Total no. of blood cultures	4	19	13	17	20	23	28	14
Positive	3	14	12	16	18	23	28	14
Negative	1	5	1	1	2	0	0	0

(ii) *Stage and severity of disease*

The largest published series of blood culture results is that collected by Coleman & Buxton (1907), in which they showed that the highest percentage of positive results was obtained in the first week of disease. Their results have since been confirmed by Mann *et al.* (1915), Jochmann (1924) and Stuart & Pullen (1946). But the experiences of Gay (1918) and Chatterjee (1942) have shown that the percentage of positive results may rise in the second and third weeks of disease. The results of these earlier studies are shown in Table 2, together with the results obtained at Acre.

Table 2. *Percentage of positive blood cultures in relation to stage of disease*

(a) Blood-culture results with highest percentage in first week of disease

	1st week	2nd week	3rd week	4th week
Coleman & Buxton (1907), 1602 cultures	89	73	60	38
Mann *et al.* (1915), 391 cases	80	62	50	36
Stuart & Pullen (1946), 361 cases	81	62	32	12

(b) Blood-culture results with highest percentage in second or third week of disease

	1st week	2nd week	3rd week	4th week
Gay (1918), 98 cases	73	80	53	40
Chatterjee (1942), 348 cases	55	70	75	55
Batty Shaw & Mackay (1951), 76 cases, 192 cultures	80	90	84	—

In the diagnosis of typhoid fever, it is a common practice to discontinue further blood cultures if their result has been negative in the first week of disease. In *Manson's Tropical Diseases*, it is stated that 'the usefulness of blood culture (in enteric fever) is limited in view of the short duration of bacillaemia' (Manson-Bahr, 1950). The validity of such a remark is not confirmed by the early series of Coleman & Buxton, where over 50 % of the cultures were positive in the second and third weeks. The findings of those authors shown in Table 2*a*, show an increase in the

percentage of positive isolations from the first to second weeks, and Chatterjee (1942) showed that a further increase might occur in the third week of disease.

Variations in bacteriological technique or the clinical behaviour of enteric fever may account for certain of the differences observed in the stage of disease at which blood culture is most frequently positive (cf. Table 2a,b). Gay (1918) considered that the discrepancy between his results and those of earlier workers may have been due to 'the inevitable uncertainties in estimating the day of the disease correctly'. While we would concur with this difficulty, it is considered that estimations of the stage of disease are more likely to have been incorrect in the series of Coleman & Buxton (1907), the majority of whose cases were collected from the early literature on the subject, than in Gay's series, in which all the cases were personally observed. A further reason for the discrepancy in the findings between Table 2a and b may be the stage of disease at which the patients were admitted to hospital. Even in the recent series of Stuart & Pullen (1946) the average duration of disease before the patient's admission to hospital was 9–10 days, whereas in the 'typhoid-conscious' unit, with which we were concerned at Acre, the average day of disease on which a patient was admitted to hospital was the second to third. Furthermore, once the initial four cases at Acre were diagnosed as suffering with

Table 3. *Percentage of positive cultures in relation to time and severity of disease*

	1st week			2nd week			3rd week		
	Total	No. positive	% positive	Total	No. positive	% positive	Total	No. positive	% positive
All cases	73	59	80·8	74	67	90·5	45	38	84·4
Severe cases	26	22	84·6	29	27	93·1	21	20	95·2
Moderate cases	17	15	88·2	21	20	95·2	14	12	85·5
Mild cases	30	22	73·3	24	20	83·3	10	6	60

enteric fever, all further cases of fever admitted from this unit had a blood culture performed on the day of, or following, admission; whereas in many of the cases reported in other series the patients were first submitted to the normal initial investigations of a fever of unknown origin, viz. blood smears, white cell count, urine, chest X-ray, etc.

The relationship of the severity of the disease to the result of blood culture at Acre is shown in Table 3. We would emphasize that the use of terms 'severe, moderate, and mild', which we have discussed elsewhere (Batty Shaw & Mackay, 1951), is largely dependent on our clinical impressions, and that the outbreak was a mild one with an overall mortality of 3·94 %. In the moderate and mild cases the percentage of positive isolations was highest in the second week, and the severe group showed a progressive rise in positive isolations from the first to third weeks of disease. An interesting finding was that the percentage of positive isolations in the first 2 weeks was greater in the moderate than the severe group; an explanation for this somewhat unexpected finding may be that some of the moderate cases were more ill at this stage of disease than others who later developed a severe, and in three cases, fatal attack. Because of the impending evacuation

from Palestine the total number of isolations was less in the third than in the first 2 weeks of disease, and prevented us from continuing our observations into the fourth and fifth weeks in the severe cases. Jochmann (1924) has observed that the severity of the bacteraemia increases in those cases which subsequently die, and, although we did not perform quantitative blood cultures, it was certainly our experience at Acre, and in previous outbreaks, that blood culture remained positive in severe, and fatal, cases.

(iii) *T.A.B. inoculation*

Conflicting views are expressed on the effect of previous T.A.B. inoculation on the course of enteric fever and the results of blood culture. Perry (1918) stated that anti-typhoid inoculation so modified the course of the disease that it shortened the duration of bacteraemia, and thereby made it more difficult to obtain a positive blood culture. Hohlweg (1915), Labbé (1916) and Lämpe (1916) all arrived at a similar conclusion, and it appeared to be confirmed by Leishman (1923) in his analysis of the cases of enteric fever in the British Army in France during the 1914–18 war. Of 5939 cases of enteric fever so studied, 2318 (39 %) were dignosed by cultural methods; 1083 (18·2 %) were diagnosed by blood culture. The remaining cases were diagnosed by the Widal reaction. Of 2854 soldiers who had been inoculated previously, blood culture was positive in 328 cases (11·4 %); whereas in 3085 uninoculated soldiers blood culture was positive in 755 cases (24·4 %). Leishman discussed the evidence to show that T.A.B. so modified the course of enteric fever that positive blood cultures were more difficult to obtain. The total number of positive blood cultures is low for the whole series. This may have been due to limited bacteriological facilities, or to the prevalent use of the Widal test rather than cultural methods. But, before accepting these figures, it must be recalled that the H agglutinins only were customarily estimated, and reference has already been made (Batty Shaw & Mackay, 1951) to the work of Felix (1924) and others who have shown the unreliability of this method in diagnosing enteric fever in previously inoculated persons. We would agree with Felix that it is probable that a number of these cases were wrongly diagnosed by agglutination tests. With the methods available at that time, Ledingham (1920, 1921) in Mesopotamia, experienced no difficulty in obtaining positive results from blood cultures in previously inoculated persons, and expressed doubt of many of the diagnoses recorded by Leishman. Ledingham's views were supported by the experience of Vaughan (1920), and Hébert & Bloch (1922), who reported a positive result from 1000 blood cultures in inoculated persons. Harvey (1929) suggested that the growth of *Salm. typhi* in blood cultures was more likely to be delayed in the case of the vaccinated subject, but little further work on this subject has since been published. In a review of 238 cases of enteric fever in British troops in the Middle East between 1941 and 1943, Dick (1946) obtained a positive blood culture in 189 (79 %); Hayes & Freeman (1945) reported that only 30 % of the cases of enteric were proved bacteriologically in India between 1942 and 1943. Jordan & Everley Jones (1945) reported thirty-two (72·8 %) positive isolations in forty-four cases of enteric fever which they investigated in France, and Anderson & Richards (1948) isolated

Salm. typhi by blood culture in sixty-two (59%) of 105 cases in British troops in Egypt. The percentage of positive isolations shown in Table 4 was obtained in the previous enteric outbreaks in inoculated soldiers with which we had been associated in Palestine between 1946 and 1948.

Table 4. *Blood cultures in enteric fever in Palestine*, 1946–8

				No. of cases with		
Place	Year	Organism	Total no. of cases	Positive blood culture	Negative blood culture	Percentage of positive cultures
Ramleh	1947	*Salm. typhi*	22	16	6	72·4
Jerusalem	1947	*Salm. typhi*	27	23	4	85·2
Nazareth*	1947	*Salm. paratyphi B*	40	22	18	55
Acre	1948	*Salm. typhi* and *Salm. paratyphi B*	76	71	5	93·4

* A very mild epidemic (Batty Shaw & Handfield-Jones, 1948).

It is our view that T.A.B. inoculation has little effect upon the course of enteric fever once the disease has been contracted, and that a positive blood culture may readily be obtained in such cases.

(iv) *Duration of incubation of cultures*

Wilson & Miles (1946) advise that, in the diagnosis of enteric fever, blood cultures should not be discarded for at least 5 days, and Butler (1937) has recommended that if typhoid bacilli have not grown after 1 week's incubation then the cultures should be kept for a month to see if further organisms can be isolated. If suitable media are employed, the appearance of enteric organisms can usually be detected after 24 or 48 hr. incubation, and this had been our previous experience in the Middle East. It was decided, however, in the Acre epidemic, to investigate whether the duration of incubation of cultures influenced the result of blood culture. The results shown in Table 5 were obtained from an analysis of 160 blood cultures.

Table 5. *Duration of incubation of cultures*

Day of incubation at which a positive result was first obtained	1	3	5	7	9	11
Number of positive blood-culture results	34	47	31	21	19	8

The average period of incubation required to produce a positive result was 4–5 days. It will be seen that slightly over 50% were positive by the third day, but that twenty-seven (17%) did not become positive until the ninth or eleventh day of incubation.

The reason for the somewhat surprising length of incubation required in a number of cases may have been due to one of several factors. First, our method of blood culture involved the withdrawal of 5 ml. of blood. Although we had obtained successful results with the removal of this quantity of blood in the past, Butler (1937) recommends that 10 ml. and whenever possible 20 ml. of blood should be used, and Wilson & Miles (1946) recommend the withdrawal of 10 ml.

If this be the reason, our results show that a high percentage of blood cultures (93·4 %) can be obtained with 5 ml. of blood provided the incubation of the culture is continued for a sufficient period. Secondly, there may have been a fault in our culture media; this is considered to be improbable. A third possibility is that positive results from blood culture may have been delayed by the previous inoculation with T.A.B. vaccine (Harvey, 1929); we were unable to assess this factor as there were no uninoculated controls. Finally our results may have been influenced by the fact that we were dealing with a double enteric infection. Rist (1916) noted during the 1914–18 war that the blood culture from a number of cases of enteric fever, grew *Salm. typhi* in the first 3 days, but, if incubated for a period of 8–15 days, produced gas-forming organisms giving the agglutination reactions of paratyphoid organisms. He explained this phenomenon by postulating the presence of a small number of paratyphoid organisms in the blood, detectable only after prolonged incubation. Although a rather prolonged period of incubation was, in our experience at Acre, needed to obtain a positive result, this need did not arise with the double infections. In our experience double infections were diagnosed with readiness in the first few days of incubation, and a prolonged period did not increase the frequency with which they were detected.

<div align="center">CONCLUSIONS</div>

The discovery of chloramphenicol has rendered the early diagnosis of enteric fever of great importance. Blood culture is the most certain method by which this diagnosis may be achieved, and the present study is yet a further addition to the published reports on the high number of positive results that may be obtained by this method. Our figures show that, although a greater percentage of positive results are attained at high body temperatures, a low body temperature or the stage of disease should not be a deterrent to blood culture in a suspected case. The myth that a positive blood culture may only be attained with difficulty in a previously inoculated subject has again been dispelled. In addition, we have demonstrated the value of prolonged incubation of blood cultures (at least up to 12 days), though our results may be due to the fact that only 5 ml. of blood were taken, or that we were dealing with the unusual occurrence of a double enteric infection in inoculated persons.

We do not intend to dispute the value of isolation of enteric bacilli from the excreta, especially in domiciliary practice (Glass & Wright, 1937; Wilson & Miles, 1946). But it has been our experience in hospital practice, that it is easier to collect blood than the stools or urine of patients who may be constipated or incontinent. Although the percentage of positive isolations from the excreta increases progressively with the stage of the disease, the figures in Table 2*b* show that the method of blood culture should not readily be abandoned if the initial results in a case of suspected enteric fever prove negative.

Recent accounts of the selective growth media available for the isolation of enteric bacilli have been given by Hobbs & Allison (1945), Wilson & Miles (1946) and Kauffmann (1950); but it is less probable that such media will have improved the results of blood culture to the same degree as culture of the stools (Glass &

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Wright, 1937). We have no experience of cultivation from blood clot, which is claimed to give satisfactory results (Fornet, 1906; Müller & Gräf, 1906; Le Boeuf & Braun, 1916; Felix, 1924) or the more recent practice of culture from the bone-marrow (Leitner, 1949). Apart from the disadvantages of a special needle and the need for experience of the technique, sternal puncture is clearly more distressing to a patient than simple blood culture. But Jordan & Jones (1945) have confirmed the results of others that a positive culture may be so obtained when repeated blood cultures have been sterile.

In the investigation of a suspected case of enteric fever blood culture should be performed early. If the first culture appears negative after 48 hr., a second culture should be performed, although the first culture should not be discarded as negative until it has been incubated for at least 12 days. The stage of disease or history of previous inoculation should not act as deterrents to this valuable diagnostic procedure.

SUMMARY

1. The results of 192 blood cultures in an epidemic of enteric fever are analysed. The epidemic was caused by a double enteric infection with *Salm. typhi* (Vi-phage type T) and *Salm. paratyphi B* (Vi-phage type 'Dundee').

2. All of sixty-five cultures between 102 and 104° F. were positive, and a significant number of positive cultures at lower body temperatures were obtained. Three out of four cultures, taken when the temperature was subnormal, were positive. In seventy-one out of seventy-six cases (93·4 %) a positive blood culture was obtained.

3. The highest percentage of positive isolations was obtained in the second week of disease. In the severe cases the highest percentage occurred in the third week. The reasons for this, and the findings of previous studies, are discussed.

4. No difficulty was encountered in obtaining positive blood cultures in previously inoculated individuals.

5. The average period of incubation of the cultures, required to produce a positive result, was 4–5 days, and 17 % did not become positive until the ninth to eleventh day of incubation.

6. The discovery of chloramphenicol has emphasized the need for the early diagnosis of enteric fever. This can most readily be achieved by the method of blood culture.

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