Effect of Hypertonic Sucrose on the Growth of *Salmonella typhi* in Experimental Blood Cultures

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**ABSTRACT**

Slower growth of *S. typhi* in hypertonic media, reported previously by the authors, was contradictory to other workers' results which showed better growth of some species of bacteria. To evaluate further the effect of hypertonic sucrose on the growth of *S. typhi*, organisms were suspended in saline or in blood with or without sodium polyanethol sulfonate (SPS) and stored up to 24 hours. And then viable counts were determined on tryptic soy agar (TSA) and experimental blood cultures were done in tryptic soy broth (TSB) and in TSB with 10% sucrose (TSB-H). *S. typhi*, suspended in blood and kept for 24 hours, were inoculated into TSB and TSB-H and after 4 hour incubation viable counts were made on TSA and on TSA with 10% sucrose (TSA-H). In this study it was found that, during the 24 hour storage, the viable counts of *S. typhi* suspended in saline with or without SPS were similar and those suspended in blood with SPS were increasing.

Comparison of the growth in TSB and in TSB-H did not show hyperonic media was better for the cultivation of *S. typhi* which was kept up to 24 hours before inoculation. On the contrary the growth was slower. Viable counts made on TSA and on TSA-H from the TSB and TSB-H, which were inoculated with *S. typhi* suspended in blood and incubated for 4 hours, showed similar results indicating TSB-H did not support faster growth.

From the results of this experiment and of the previous clinical blood cultures, it is concluded that 0.1% SPS does not give adverse effect on *S. typhi* during the 24 hour storage and that hypertonic sucrose does not give better result in the cultivation of *S. typhi*.

**INTRODUCTION**

Cell wall-defective forms, known in many species of bacteria including *S. typhi* (Dienes *et al.*, 1950) and formed spontaneously or when exposed to antibody and complement or to some antibiotics (Feingold, 1969), can be cultured with the use of hypertonic media (Dienes and Sharp, 1956; Seeberg, 1973). Hypertonic media were reported to support growth of the osmotically fragile cell wall-defective bacteria from some clinical materials (Mattman and Mattman, 1965; Neu and Goldreyer, 1968; Louria *et al.*, 1969; Louria *et al.*, 1976) and to increase frequencies of isola-
tion of bacteria from blood and various body fluids (Rosner, 1972; Sullivan et al., 1972; Henrichsen and Bruun, 1973; Phair et al., 1974; Rosner, 1975). However, hypertonic media were not always reported as beneficial (Washington et al., 1975) and the authors (Chong et al., 1975) found them inferior in the cultivation of *S. typhi* from clinical blood cultures.

In this study, artificial blood culture was done to evaluate further the effect of hypertonic sucrose on the growth of *S. typhi*. Viable counts of the suspension with sodium polyanethol sulfonate (SPS) was tested to determine the effect of SPS on *S. typhi* during the storage of blood.

**MATERIALS AND METHODS**

SPS (ICN Pharmaceutical Inc., Plainview, N.Y.), 1% solution in distilled water, was sterilized at 121°C for 15 minutes. Tryptic soy broth (TSB, Difco) was prepared according to the directions. Hypertonic TSB (TSB-H) was prepared by adding sucrose (1st grade, Ishizu Pharmaceutical Co., Japan) to TSB to make a 10% concentration. The broth was distributed in 4 ml amounts in 13×100 mm test tubes with Bacti-Capalls. TSA and TSA-H were prepared by adding 1.5% agar to TSB and to TSB-H.

Four rows of screw cap test tubes were set up. Serial ten-fold dilution suspensions were prepared in saline from 24 hour culture of *S. typhi* (clinical isolate, 75-12-708). Each dilution, in 0.05 ml amount, was placed into one of the tubes in each row. SPS solution in 0.1 ml amounts was placed in the tubes of the 2nd and the 4th row. To the tubes of the 1st and the 2nd row, 1 ml of saline was added and to the tubes of the 3rd and the 4th row 1 ml of fresh blood was added immediately after drawing. Thus the final concentration of SPS in the tubes of the 2nd and the 4th row was 0.1%. The blood, taken from a healthy male, agglutinated Widal antigen only up to 1:4 dilution and contained 5,800 WBC/mm³.

The tubes were kept at room temperature and after 0, 4, and 24 hour standing, 0.05 ml amounts of the suspension in each tube were inoculated into TSB and TSB-H tubes and TSA and TSA-H plates. Blood clots, formed in the tubes of the 2nd row, were broken before the contents were used as inocula. Growth in TSB and TSB-H were compared macroscopically. To determine viable counts, colony counts were done from plates having 30 to 300 colonies, and multiplied by the reciprocal of the original dilution.

**RESULTS**

After the 24 hour storage, the viable count of *S. typhi* suspended in saline decreased from 4,200 to 1,300 and that suspended in saline

![Graph](image)

**Fig. 1.** Effect of the addition of SPS into saline and blood on the viable count of *S. typhi*. 
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Table 1. Comparison of the growths, after 24 hr incubation, in TSB and TSB-H of *S. typhi* which is suspended in saline, saline with SPS, blood, and blood with SPS.

<table>
<thead>
<tr>
<th>Suspended in</th>
<th>Storage time (hr)</th>
<th>Media</th>
<th>Suspension</th>
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<tr>
<td></td>
<td>0</td>
<td>TSB</td>
<td>3+</td>
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<td></td>
<td>TSB-H</td>
<td>3+</td>
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<tr>
<td>Saline</td>
<td>4</td>
<td>TSB</td>
<td>3+</td>
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<td></td>
<td></td>
<td>TSB-H</td>
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<td></td>
<td>24</td>
<td>TSB</td>
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<tr>
<td></td>
<td></td>
<td>TSB-H</td>
<td>3+</td>
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<tr>
<td>Saline with SPS</td>
<td>4</td>
<td>TSB</td>
<td>3+</td>
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<td></td>
<td></td>
<td>TSB-H</td>
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<td></td>
<td>24</td>
<td>TSB</td>
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<td></td>
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<td>TSB-H</td>
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<tr>
<td>Blood</td>
<td>4</td>
<td>TSB</td>
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<td>Blood with SPS</td>
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<td>3.2×10^6</td>
<td>3.2×10^7</td>
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* Viable count/0.05 ml of saline suspension at time 0-hr.
* b Symbols: 3+, heavy growth; 2+, moderate growth; 1+, light growth; --, no growth.
* c Viable count was 2/inoculum.
* d Viable count was 0.6/inoculum.

With SPS from 4,700 to 1,100. The viable counts of the organism suspended in blood was only 390 at 0 hour (actually it took about 15 minutes from the time of adding blood to the organism until the completion of inoculation), 6 after 4 hours, and none after 24 hours. The viable counts of the organism suspended in blood with SPS showed increase from 5,000 to 46,000 during the 24 hour period (Fig. 1).

Comparison of the growth after 24 hour incubation in the TSB and in the TSB-H, which were inoculated with 0, 4, and 24 hour-old suspension in saline with SPS and in blood with SPS, showed the end point of the growth were equal in both media (Table 1). The TSB tube which was inoculated with 24 hour-old saline suspension, which contained viable count 2 (Table 1, foot note c) showed no growth while the counterpart TSB-H tube showed growth. The TSB-H tube which was inoculated with 4 hour-old blood suspension, which con-
tained viable count 0.6 (Table 1, foot note d), showed no growth while the counterpart TSB showed growth.

Rapidity of the growth in TSB and in TSB-H were compared by observing the turbidity of the tubes which were inoculated with the smallest number of viable organism. Although, on table 1, differences were shown only in the tubes which were inoculated with the 0 hour suspensions, in reality most of the TSB-H tubes showed lesser turbidity than TSB tubes and, when the observations were made after 18 hour incubation, the differences were even greater, indicating slower growth in TSB-H.

TSB and TSB-H, which were inoculated with the suspension in blood and incubated for 4 hours, were found to have similar viable counts whether they were inoculated on TSA or on TSA-H.

DISCUSSION

Blood culture is the most important bacteriological procedure in various bacterial infections, but in this country it is used most frequently to establish the diagnosis of typhoid fever. Therefore, the method should allow rapid growth of S. typhi.

Hypertonic media only have been reported to yield growth of bacteria in a small number of clinical cases when routine media failed to support growth (Louriа et al., 1969; Louriа et al., 1976). More important seemed to be the value of hypertonic media in its ability to support faster and more frequent growth of bacteria and yeast (Rossner, 1972; Sullivan et al., 1972; Henrichsen and Bruun, 1973; Phair et al., 1974). However, for the cultivation of S. typhi the authors (Chong et al., 1975) have experienced slower growth when hypertonic media were used in clinical blood cultures as well as in experimental cultures.

It is difficult to compare the results of different workers, since various media and methods were used. In the studies of Henrichsen and Bruun (1973) and of Ellner et al., (1976) blood were collected into SPS containing tubes. It took up to 24 hours before inoculation was made. This was different from our method of blood culture which was to draw 10 ml of blood using a syringe and needle and immediately inoculate 5 ml amounts into two 50 ml bottles of broth. Based on the fact that SPS inhibits growth of some species of bacteria (Graces et al., 1974; Eng and Iveland, 1975), and that blood possess antibacterial activity (Adler, 1953; Rowley, 1960), it can be assumed that during the storage or transport of blood in SPS containing tube, the cell wall of the bacteria is damaged. If this happens then the use of hypertonic media might result in more frequent or in faster growth of bacteria as suggested by Rosner (1972).

In these experimental cultures it was shown that viable counts were similar whether S. typhi was suspended in saline with or without SPS. S. typhi suspended in blood with SPS showed increase rather than decrease while that in blood showed rapid decrease (Fig. 1). These facts were thought to indicate that SPS in final concentration of 0.1% was not toxic to S. typhi during the 24 hour exposure period, whether it was in saline or in blood.

Henrichsen and Bruun (1973) reported more frequent isolation of E. coli and S. aureus. Ellner et al. (1976) showed more frequent isolation of anaerobes and facultative bacteria including Klebsiella. However, in this study, the comparison of the growth in TSB and in TSB-H, which were inoculated with stored suspension, the end points of growth were
similar and no advantage with the use of hypertonic media was found (Table 1). The minor differences observed in the four tubes were considered due to chance, because the inocula contained a small number of viable organisms. The ratio of the inoculum to the broth was 1:80 to eliminate possible antibacterial activity of the inoculum. This unfavorable effect of hypertonic media was in agreement with the previous result of the authors in S. typhi cultivation from blood. Unfavorable effects were also reported by Washington et al. (1975), who showed less frequent isolation of Staphylococcus and Bacteroides and by Ellner et al. (1976) who analysed the effect of hypertonic media and concluded it to be inferior in the cultivation of Pseudomonas and yeasts.

In the rapidity of growth, no advantage was noted for TSB-H. In reality the growth in TSB-H was usually slower when compared after 24 hour incubation and the difference was greater when the observation was made after 18 hour incubation. Such a result was reported in clinical blood cultures of S. typhi (Chong et al., 1975).

Rosner (1972) stated that the effect of hypertonic media was different in clinical blood cultures and in experimental blood cultures. However, to evaluate the effect of hypertonic media, it was considered there was no other way than do experimental blood cultures to minimize variables, which are inevitable in clinical blood cultures.

Rosner (1972) making subculture 4 hours after inoculation of blood showed gram-positive cocci, which were fewer in number in blood broth mixture, grew more frequently in hypertonic media. In our study viable counts were determined on TSA and TSA-H from the TSB and TSB-H which were incubated for 4 hours after inoculation of the blood suspension, to prove the possibility of the presence of osmotically fragile bacteria in the suspension. However, the two media were observed to have similar number of viable organisms.

From the result of this study and previous clinical blood cultures, it is concluded that 0.1% SPS in blood does not give an adverse effect on S. typhi during 24 hour storage and that addition of 10% sucrose to brain heart infusion, to TSB or to thioglycollate medium does not result in faster or more frequent growth of S. typhi from blood culture.

REFERENCES


