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Effect of Dilution on Recovery of Bacteria from Blood

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The multiplication rate of bacteria in undiluted blood containing sodium polyanethol sulfonate was compared with growth rates obtained in dilutions of blood ranging from 1:2 to 1:8. Although all organisms tested grew in the undiluted blood, increased growth rates were seen in the 1:2 dilution. Further dilution resulted in growth rates equivalent to that obtained with the 1:2 dilution. In view of these results, we question the present recommendations that blood be diluted 1:10 or 1:20.

It is generally believed that blood specimens for culture must be diluted with the broth medium. It is currently recommended that the ratio of blood to medium should be 1:10 to 1:20 (1; R. C. Bartlett, P. D. Ellner, and J. A. Washington II, in *Cumitech 1*, Am. Soc. Microbiol., Washington, D.C., 1974). This opinion is based in part upon the fact that fresh human serum is bactericidal (7, 9-11), and immediate dilution or neutralization of this effect is necessary for the optimal recovery of bacteria from blood (13).

It has been shown by Lawrance and Traub (8) that this bactericidal effect can be abolished by the use of sodium polyanethol sulfonate (SPS) as the anticoagulant. Since SPS is widely employed when culturing blood (1), we believe that the present recommendation for dilution should be reevaluated.

The present study was undertaken to examine the multiplication of organisms in lower-than-recommended dilutions and in undiluted blood containing SPS.

MATERIALS AND METHODS

The bacterial strains utilized were recent clinical isolates obtained from specimens submitted to the Clinical Microbiology Service. Organisms employed included *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus* Viridans group, *Streptococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacteroides fragilis*. All organisms were grown overnight in modified Columbia broth. Aerobic organisms were diluted 10^{-4} with sterile saline. *B. fragilis* was diluted 10^{-3} in modified Columbia broth. Each organism was tested with the unpooled blood of four individuals. After skin preparation with 2% tincture of iodine, 40 ml of blood was drawn from each healthy volunteer into two Vacutainer tubes (165 by 16 mm), containing approximately 3.2 ml of 0.35% SPS, and permitted to stand at room temperature for 20 min. Duplicate samples of blood containing 10, 5, 2.5, and 1.25 ml were removed from the Vacutainer tubes and added to tubes (16 by 125 mm, which contained 0, 5.0, 7.5, or 8.75 ml of modified Columbia broth, thus providing undiluted blood, as well as 1:2, 1:4, and 1:8 dilutions. One-tenth milliliter of the diluted organism was added to each tube, and after mixing, the tubes were incubated at 35°C. One-tenth milliliter of the inoculum was also added to 10 ml of saline as a control. Plate counts were performed on portions removed from each tube immediately after inoculation and at 4, 12, and 24 h, with use of Columbia agar, containing 5% sheep blood or chocolate agar. Experiments with *B. fragilis* were performed in a 20-ml Vacutainer tube with a rubber stopper. Plate counts were done on reduced blood agar plates (2) incubated in GasPak jars. Sampling was done at 24, 48, 72, and 96 h.

RESULTS

All organisms grew in undiluted blood, as well as in the various dilutions. The mean results of these experiments are shown in Fig. 1-6.

At 4 h, lower counts were seen with *S. pneumoniae* and *S. faecium* in undiluted blood as compared with the various dilutions. Lower counts were seen in undiluted blood and the 1:2 dilutions with *Streptococcus* Viridans as compared with the higher dilutions.

By 12 h, the colony counts of the aerobic organisms in the undiluted blood were an average of 2.5 logs lower than counts in the diluted blood.

At 24 h, no significant differences in colony counts were detected between undiluted and diluted blood with *S. pneumoniae*, *S. aureus*, and *P. aeruginosa*. With the remaining aerobic species, the counts in the undiluted blood were still an average of 2.1 logs lower than counts in the diluted blood. No significant donor differences could be detected with the aerobic organisms.

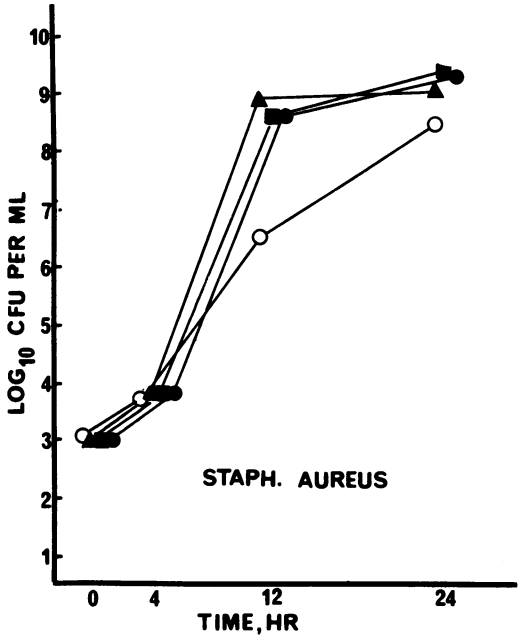

FIG. 1. *Growth of S. aureus in diluted and undiluted blood. Symbols: Undiluted blood (○); 1:2 dilution (▲); 1:4 dilution (■); 1:8 dilution (●).*

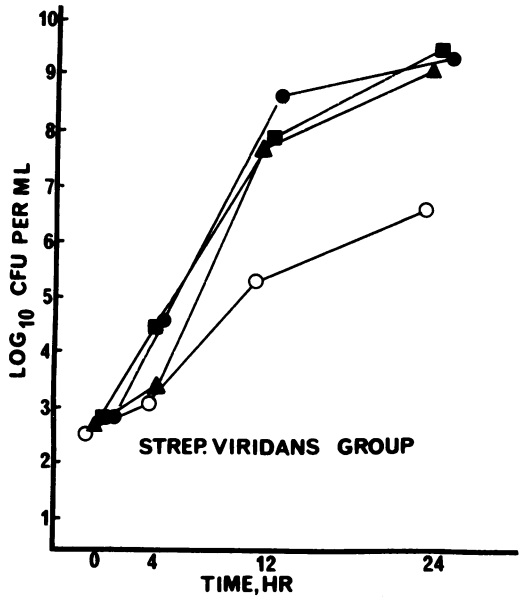

FIG. 3. *Growth of Streptococcus Viridans group in diluted and undiluted blood. Symbols as in Fig. 1.*

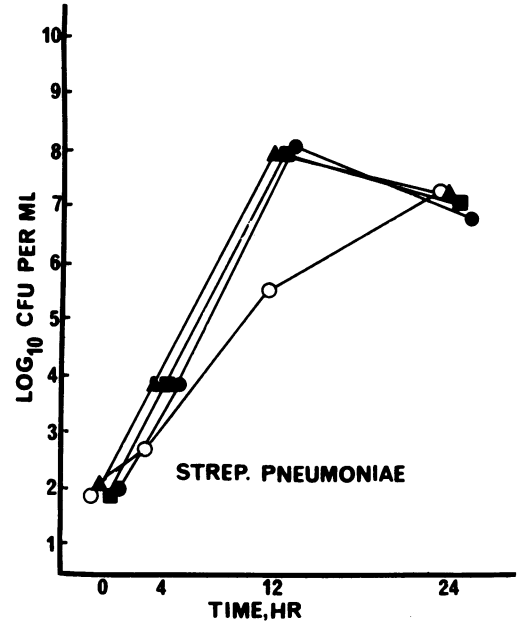

FIG. 2. *Growth of S. pneumoniae in diluted and undiluted blood. Symbols as in Fig. 1.*

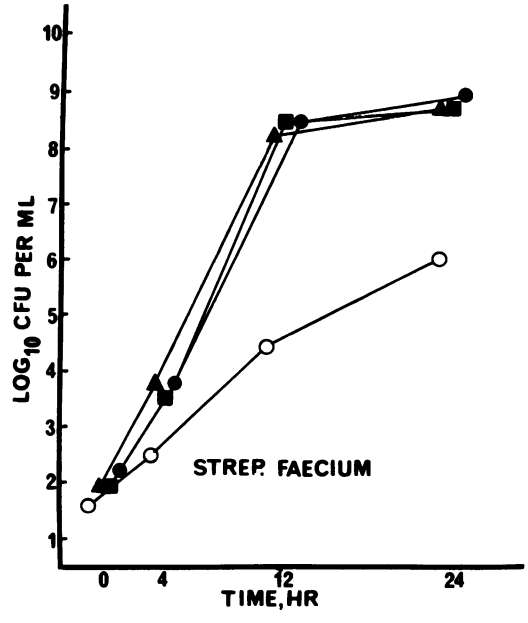

FIG. 4. *Growth of S. faecium in diluted and undiluted blood. Symbols as in Fig. 1.*

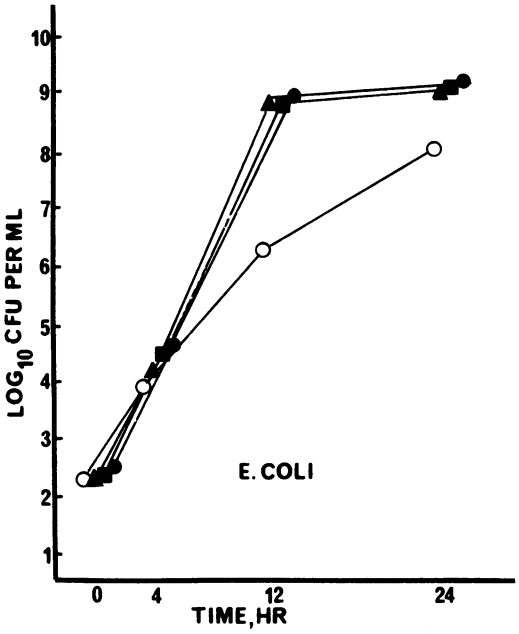

FIG. 5. *Growth of E. coli in diluted and undiluted blood. Symbols as in Fig. 1.*

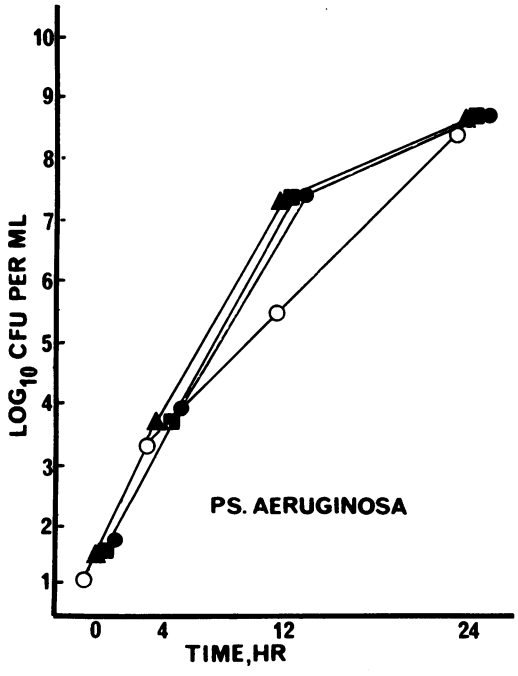

FIG. 6. *Growth of P. aeruginosa in diluted and undiluted blood. Symbols as in Fig. 1.*

With *B. fragilis*, some donor variation was observed. The growth of *B. fragilis* was poor or lagged in the undiluted blood of all four donors and in the 1:4 and 1:8 dilutions of three donors. Good growth occurred in the 1:2 dilution of all donor blood (Fig. 7).

DISCUSSION

Prior to the introduction of SPS, a blood-broth ratio of at least 1:10 was considered necessary to counteract the bactericidal properties of blood, which could inhibit bacterial growth. After the demonstration that SPS neutralizes these bactericidal properties (8), it was shown by Ellner and Stoessel (4) that bacteria survive and multiply in undiluted blood containing SPS. The present study of confirmed these findings. Further, a 1:2 dilution of the blood resulted in an increased growth rate of all the organisms tested as compared with the growth in undiluted blood. Dilutions greater than 1:2 did not result in further increases in the growth rate of these organisms. In the case of *B. fragilis*, decreased growth sometimes occurred with increasing dilutions of the blood, suggesting that the redox potential may have been raised to unfavorable levels.

Although there is increasing evidence (3, 6) that larger samples of blood result in an increased recovery rate, we question the necessity for concomitant increases in the volume of media to maintain the 1:10 to 1:20 dilution factor generally recommended. In a recently reported series (3), 3,000 blood cultures were performed using a 1:7 dilution, with no decrease in the recovery rate. Since the numbers of organisms per milliliter of blood in bacteremic states tends to be low (5, 12, 14), further dilution might well prolong the lag phase or even completely eliminate growth.

The numbers of organisms per milliliter of blood recovered in clinical situations is sometimes as much as 1 log lower than the numbers used as inocula in this study. These higher inocula were chosen to permit more accurate quantitation.

It is indeed possible that the optimal dilution of blood for culture is considerably less than 1:10 when SPS is employed as the anticoagulant, but the resolution of this question must await a carefully controlled clinical study.

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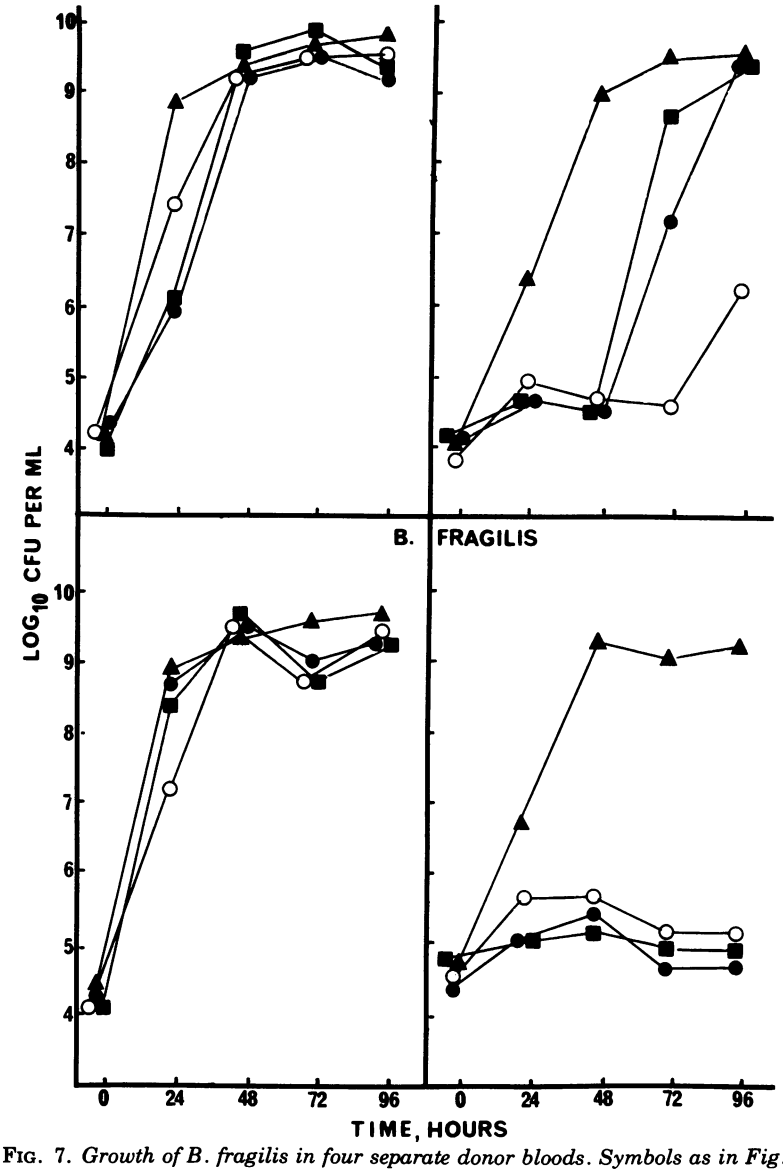

FIG. 7. *Growth of B. fragilis in four separate donor bloods. Symbols as in Fig. 1.*

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