JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1981, p. 266-272
0095-1137/81/090266-07$02.00/0

Vol. 14, No. 3

Evaluation of Blood Culture Media for Isolation of Pyridoxal-Dependent *Streptococcus mitior* (*mitis*)

KATHARINE C. GROSS,[1] MARION P. HOUGHTON,[1] AND RICHARD B. ROBERTS[2]*

Laboratory of Microbiology[1] and Division of Infectious Diseases, Department of Medicine,[2] The New York Hospital-Cornell Medical Center, New York, New York 10021

Received 2 March 1981/Accepted 22 April 1981

Nutritionally variant streptococci identified as pyridoxal-dependent *Streptococcus mitior* (*mitis*) account for 5 to 6% of streptococcal endocarditis and may be a cause of "culture-negative" endocarditis. Hence, growth of three variant strains in 11 commercial blood culture broths was compared to that in fresh heart infusion broth. For simulation of clinical specimens, culture bottles were injected with 5 ml of human blood, inoculated with approximately 500 colony-forming units (CFU) per bottle, and monitored for 7 days with Gram stains and viable counts. Only Thiol broth (Difco Laboratories, Detroit, Mich.) supported growth without blood at this low inoculum. In media containing blood, maximal growth of 10^9 CFU/ml was reached within 2 days of incubation, and heavy turbidity was consistently observed in only heart infusion broth, Thiol broth, and media supplemented with pyridoxal hydrochloride. Columbia broth (BBL Microbiology Systems, Cockeysville, Md.) with increased cysteine, thioglycollate broth, and one brain heart infusion broth produced moderate growth (1×10^8 to 5×10^8 CFU/ml), whereas Columbia broth, another brain heart infusion broth, and two brands of tryptic soy broth showed fair growth (1×10^7 to 4×10^7 CFU/ml). The poor growth (1×10^6 to 3×10^6 CFU/ml) observed in three other brands of tryptic soy broth was often not apparent macroscopically or by Gram stain. Furthermore, no growth occurred in 40% of tryptic soy broth cultures inoculated with 50 CFU. Therefore, to ensure isolation of these variant streptococci from clinical blood cultures, a medium containing thiol compounds or supplemented with pyridoxal should be used. Subcultures should be made within 2 days of incubation to blood agar enriched with pyridoxal or containing a *Staphylococcus* sp. streak for satellitism.

Nutritionally deficient viridans streptococci are a significant cause of systemic disease in man and account for 5 to 6% of streptococcal endocarditis at The New York Hospital (14, 15). These deficient organisms are commonly called "satelliting streptococci" because they are unable to grow on various blood or chocolate agars except as colonies around another bacterial species (3, 4, 8, 9, 11). They have also been described as L forms of streptococci (8) or as thiol-requiring (4, 8, 11), symbiotic (9), or vitamin B_6-dependent (3, 14, 15) streptococci. Previous studies at this institution have shown that the active forms of vitamin B_6, either pyridoxal or pyridoxamine but not pyridoxine, replace the requirement for certain sulfhydryl (thiol) compounds (3, 15). Supplementation of media with 10 to 20 μg of pyridoxal hydrochloride per ml facilitates identification of these streptococci by biochemical tests and determination of their susceptibility to antimicrobial agents (2). To date, all nutritionally deficient viridans streptococcal isolates examined in our laboratory have been identified as pyridoxal-dependent strains of *Streptococcus mitior* (*mitis*) by the Colman and Williams classification (5).

Although differences as to the identification of satelliting streptococci exist (6), their possible role as one cause of "culture-negative" endocarditis is well recognized (7, 12-15). Due to their nutritional variation, these organisms do not grow well, if at all, in certain commercial media. For example, pyridoxal-dependent strains have varied in their growth pattern in the blood culture media routinely used at our hospital (15). These media are fresh heart infusion broth (HI) and two commercially prepared broths, thioglycollate and tryptic soy broth (TSB). From six patients seen over the past 10 years with pyridoxal-dependent streptococcal endocarditis, 36 sets of blood cultures were obtained. The six isolates grew 100% in HI and 78% in thioglycol-

late, but only 8% in TSB cultures. The purpose of this study, therefore, was to investigate the growth of these organisms under various cultural conditions and to evaluate the ability of certain commercial blood culture media to support their growth.

MATERIALS AND METHODS

Strains. The three isolates of pyridoxal-dependent streptococci selected for this study were from a collection of viridans streptococci previously described (15). All were recovered from blood cultures of patients with endocarditis. The physiological characteristics which identify these strains as *S. mitior* (*mitis*) have also been reported previously (15). Strain MB was used as the prototype strain throughout these studies, whereas the other two strains (MC and HD) were used for confirmation.

Isolates were grown on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) enriched with 5% sheep blood and supplemented with 20 μg of pyridoxal hydrochloride (Sigma Chemical Co., St. Louis, Mo.) per ml or in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) also supplemented with pyridoxal hydrochloride. Pyridoxal was sterilized by filtration before addition or by autoclave after addition to broth or agar medium. Stocks were maintained on heart infusion agar (Difco) slants with 5% sheep blood and 20 μg of pyridoxal hydrochloride per ml or frozen at −70°C in HI (Difco) with 15% glycerine. For each experiment, satellite tests were used to monitor the purity of each strain. The surface of a plate containing Trypticase soy agar supplemented with 5% sheep blood was streaked with the pyridoxal-dependent strain and then overlaid with perpendicular streaks of *Staphylococcus aureus*. Colonies of the test strain only grew adjacent to the staphylococcus streak. Cultures were incubated for 18 to 48 h in a CO_2 incubator at 35°C.

Blood culture media. The broths tested for their ability to support the growth of these pyridoxal-dependent streptococci are listed in Table 1. HI, prepared weekly in our laboratory from fresh beef hearts (3, 15), Scott TSB (Scott Laboratories, Inc., Fiskeville, R.I.), and thioglycollate medium (Scott) constitute the blood culture set routinely used at The New York Hospital.

Experimental procedure. To simulate blood cultures from patients, broth culture bottles were injected with 5 ml of fresh human blood from normal volunteers and then inoculated with approximately 500 colony-forming units (CFU) of the test strain per bottle. Defibrinated sheep, horse, bovine, and rabbit blood (Mayer and Myles, Coopersburg, Pa.) were also used for comparison. Inocula were prepared by transferring a small amount of growth from an 18-h culture on Trypticase soy agar supplemented with 5% sheep blood and pyridoxal hydrochloride to a tube of Gey balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.). This cell suspension was adjusted to an optical density equivalent to a concentration of 5×10^8 CFU/ml (Coleman 44 Spectrophotometer) and diluted to 5×10^2 CFU/ml, and 1.0 ml of this latter dilution was added to each bottle. Bottles were vented with sterile, plugged hypodermic needles and incubated at 35°C in a CO_2 incubator. Inocula were standardized and diluted in Gey solution because preliminary studies showed a 6- to 32-fold decrease in viability within 30 min when pyridoxal-dependent streptococci were diluted in 0.9% NaCl. Quantitative monitoring for each experiment ensured that the initial bacterial concentration was 70 to 200 CFU/ml of blood. In addition, the inoculum was decreased 10-fold to approximately 50 CFU per bottle, representing 10 CFU/ml of blood in some experiments.

Cultures were examined macroscopically after 1, 2, and 6 or 7 days of incubation for turbid or flocculent growth. Bottles were then shaken to disperse organisms, and samples were removed for Gram stain and viable counts. All additions to and sampling from the culture bottles were made with a sterile needle and syringe after wiping the rubber stopper with povidone-iodine surgical scrub and 70% isopropyl alcohol. Viable counts were determined by streaking with calibrated loops appropriate dilutions of the sample to Trypticase soy agar supplemented with 5% sheep blood and pyridoxal hydrochloride. Colonies were counted after 48 h of incubation in CO_2. Data comprise the mean of at least four determinations.

RESULTS

Comparative growth of strain MB in HI and Scott TSB. Initially, strain MB was isolated clinically from all five of our HI and thioglycollate cultures, but not from Scott TSB. The ability of this isolate to grow in HI and Scott TSB media in our test system was therefore examined, and the results are shown in Fig. 1. Differences in maximum viable count and rate of growth are readily seen. Maximal growth of 1×10^9 to 4×10^9 CFU/ml in HI occurred after 1 to 2 days with obvious heavy turbidity and Gram staining that showed many (greater than 50 per high-power field) gram-positive cocci in pairs and chains. On the other hand, growth in TSB was occasionally visible after 2 to 3 days as flocculent growth, and only rare to few bacteria were seen on Gram stain. Maximal viable counts in TSB were 10^6 to 10^7 CFU/ml after 2 to 5 days of incubation. With prolonged incubation, loss of viability was observed in HI so that by day 5 to 7 of incubation, viable counts in both media were similar. Similar growth patterns were observed when the other pyridoxal-dependent isolates (strains CM and HD) were grown in HI and TSB. Since maximal growth in HI was observed within 2 days of incubation, all subsequent quantitative determinations were performed after this incubation period.

Supplementation of media with fresh human blood and pyridoxal hydrochloride. The effect of supplementation of HI and Scott TSB with pyridoxal hydrochloride in the presence and absence of 5% fresh human blood is

TABLE 1. *Source of blood culture bottles used in growth studies*

Manufacturer	Media[a]
Microbiology Laboratory, The New York Hospital, New York, N.Y.	Heart infusion broth (HI), 70 ml
Scott Laboratories, Inc., Fiskeville, R.I.	Tryp-soy broth (Scott TSB), 90 ml Thioglycollate medium without indicator, 90 ml
Difco Laboratories, Detroit, Mich.	Thiol broth, 100 ml Brain heart infusion broth with *p*-aminobenzoic acid (Difco BHI), 100 ml Tryptic soy broth (Difco TSB), 100 ml
BBL Microbiology Systems, Cockeysville, Md.	Brain heart infusion broth with *p*-aminobenzoic acid (BBL BHI), 100 ml Columbia broth (BBL Columbia), 100 ml Columbia broth with increased cysteine, 100 ml Trypticase soy broth (BBL TSB), 100 ml
GIBCO Diagnostics, Chagrin Falls, Ohio	Tryptic soy broth (GIBCO TSB), 100 ml
Pfizer Diagnostics Division, New York, N.Y.	Evac tryptic soy broth (Pfizer TSB), 50 ml
Lederle Diagnostics, Pearl River, N.Y.	Lederle blood culture bottle, 50 ml
Johnston Laboratories, Inc., Cockeysville, Md.	BACTEC tryptic soy broth aerobic hypertonic, 30 ml BACTEC tryptic soy broth anaerobic, 30 ml

[a] All commercially prepared broths contained sodium polyanetholesulfonate and CO_2 under vacuum.

shown in Table 2. In the absence of both the vitamin and blood, strain MB was unable to grow in either HI or TSB. When pyridoxal was added to either medium without blood, maximal growth of 10^9 CFU/ml was seen in both media. In media containing 5% blood, maximal growth of 10^9 CFU/ml was achieved in HI, but not in TSB. The addition of pyridoxal to HI containing blood neither enhanced nor inhibited growth. However, growth in TSB supplemented with blood and pyridoxal was visibly turbid, and the viable count reached 1×10^9 to 6×10^9 CFU/ml.

No variation in maximal growth was observed in HI containing blood from different human donors or containing different amounts of supplemented blood (0.5 to 5.0 ml). When blood components were substituted for 5 ml of whole blood, maximal growth was observed in media containing erythrocytes, but not in media containing plasma or serum. Indeed, marked variability in growth was observed between experiments using media with plasma or serum. Growth ranged from 10^7 to 10^9 and from $<10^2$ to 10^8 CFU/ml in media supplemented with 5% plasma and human serum, respectively.

Supplementation of media with blood from different species. The comparative growth after 2 days of incubation of strain MB in HI and Scott TSB supplemented with 5% blood from different animal species is shown in Fig. 2. Maximal growth of 10^9 CFU/ml was obtained in HI supplemented with blood from any of the species. Marked differences in maximal viable counts were observed, however, in blood-supplemented TSB. Growth of 5×10^7 CFU/ml in TSB with rabbit blood, 1×10^7 CFU/ml in TSB with human blood, and only 2×10^4 CFU/ml in TSB with horse blood was seen. No growth occurred in TSB supplemented with either sheep or bovine blood.

Comparative growth of strain MB in HI and in various commercial media containing 5% human blood. Based on the above findings, growth of strain MB in various media supplemented with 5% human blood was then examined (Fig. 3). Three commercial media (BBL brain heart infusion broth [BHI], Difco Thiol broth, and BBL Columbia broth plus cysteine) supported growth with viable counts greater than 4×10^8 CFU/ml after 2 days of incubation. All three inoculated broths were visibly turbid, and many gram-positive cocci in pairs and short chains were seen on Gram stain. Although Scott thioglycollate was only slightly turbid, with no flocculent growth, and Gram staining showed few to moderate numbers of cocci, the mean viable count was 2×10^8 CFU/ml in this medium. No turbidity was apparent in the other commercial media, although some flocculent cell growth could be visible on careful inspection of Difco BHI, BBL Columbia, BBL TSB, and Scott TSB. Few organisms were seen on Gram stain, and mean viable counts ranged from 1×10^7 to 4×10^7 CFU/ml in these media.

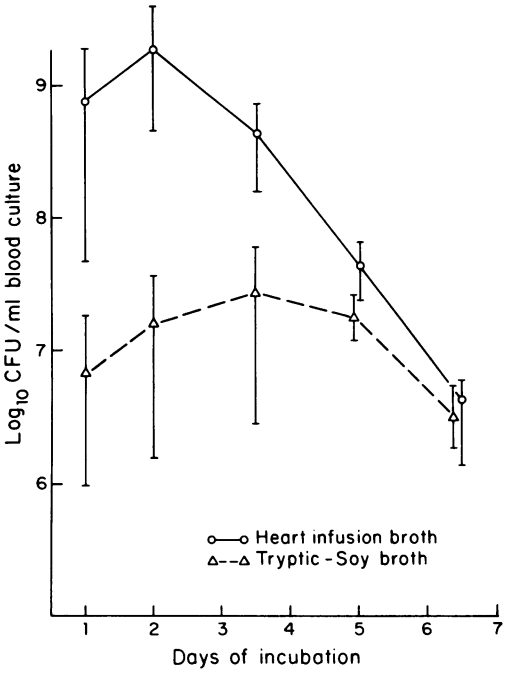

FIG. 1. *Growth curves showing the range and mean viable counts of strain MB in HI and TSB containing 5% human blood during 7 days of incubation at 35°C.*

TABLE 2. *Comparative growth of strain MB in HI and TSB supplemented with pyridoxal hydrochloride and containing 5% human blood*

Prepn	Supplement	CFU/ml of culture after 2 days of incubation in:	
		HI	Scott TSB
No blood	None	$<10^2$	$<10^2$
No blood	Pyridoxal hydrochloride	1.5×10^9	1.2×10^9
Blood added	None	1.2×10^9	9.6×10^6
Blood added	Pyridoxal hydrochloride	2.1×10^9	5.8×10^9

Growth was minimal in the TSB's from Difco, GIBCO, and Pfizer, Inc., New York, N.Y. These broths usually were clear, no to rare cocci were seen on Gram stain, and the mean viable counts ranged from 1×10^6 to 3×10^6 CFU/ml.

Comparable growth patterns with the additional pyridoxal-dependent strains (MC and HD) were observed in these media. The mean viable counts for all three strains were essentially the same in the five media which supported growth greater than 10^8 CFU/ml, i.e., HI, BBL BHI, Difco Thiol, Scott thioglycollate, and BBL Columbia broth with cysteine. Minor differences, however, were observed between strains when grown in Difco BHI, BBL Columbia broth, and the tryptic soy broths.

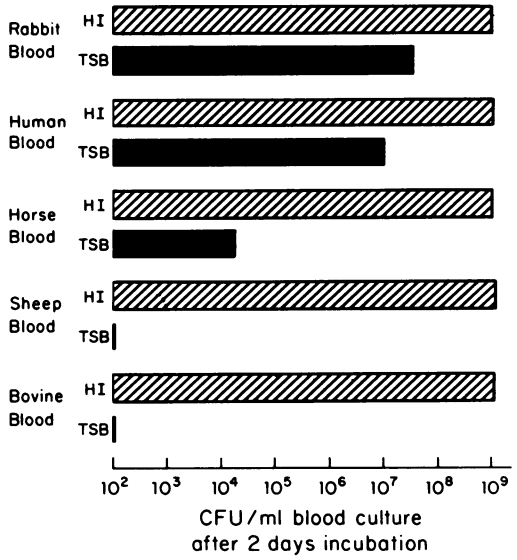

FIG. 2. *Maximal growth of strain MB in HI and TSB containing 5% blood from different animal species.*

In additional studies not shown in Fig. 3, viable counts of strain MB were followed in three Lederle blood culture bottles and in two bottles each of BACTEC tryptic soy broth aerobic hypertonic (8B, Johnston Laboratories, Inc., Cockeysville, Md.) and BACTEC tryptic soy broth anaerobic (7C, Johnston). Growth was minimal in the Lederle medium (6×10^3 CFU/ml), and these cultures were negative macroscopically and by Gram stain. In contrast, growth was readily apparent by day 1 of incubation in both BACTEC TSB media, and the viable counts ranged from 2×10^8 to 5×10^8 CFU/ml after 2 days of incubation.

Survival of strain MB during prolonged incubation. Initial studies demonstrated that maximal growth of strain MB in HI was observed after 1 to 2 days of incubation, but that a 2 log loss in viability occurred over a subsequent 4- to 5-day incubation period (Fig. 1). Similar studies were performed in Difco Thiol broth and in BBL Columbia broth with cysteine, and the comparable results are shown in Fig. 4. A 2.5 log loss in viability occurred in BBL Columbia broth with cysteine, but only a minimal loss in viability was observed in Difco Thiol broth. Indeed, Difco Thiol broth was the only medium tested that supported the growth of pyridoxal-dependent streptococci at this low inoculum in the absence of blood. Growth curves

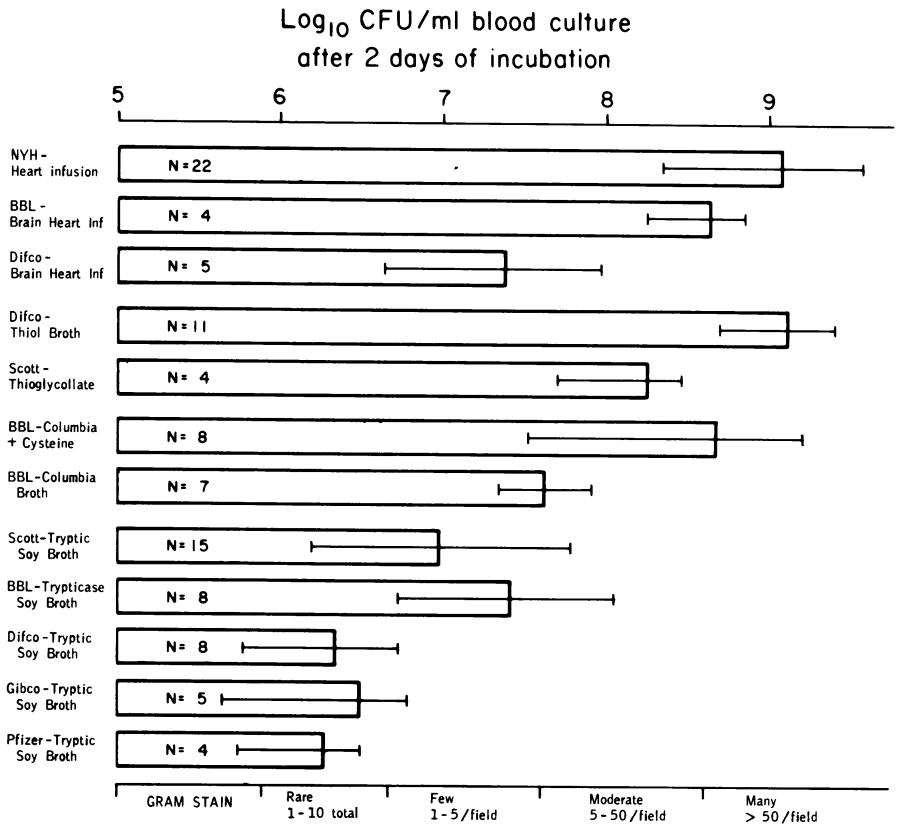

FIG. 3. *Maximal growth of strain MB in heart infusion broth and various commercial blood culture media containing 5% human blood and an inoculum of 500 CFU per bottle after 2 days of incubation at 35°C. The range and mean numbers of CFU were calculated from the number of values shown (N = number of independent experiments).*

of strain MC and HD in these media resembled those of strain MB, except that strain HD showed a 3 log loss of viability in Difco Thiol broth and a complete loss of viability ($<10^2$ CFU/ml) in BBL Columbia broth with cysteine after 6 days of incubation.

Effect of inoculum size on maximal growth of strain MB in various media. The results described above were obtained after an inoculum of approximately 500 CFU per bottle (or 100 CFU/ml of blood). Previous studies employing quantitative blood cultures have shown, however, that in patients with streptococcal endocarditis the magnitude of bacteremia may be as low as 2 to 10 CFU/ml of blood (17). Studies were therefore performed by employing 50 CFU per culture bottle or 10 CFU/ml of blood. Maximal growth of strain MB in Difco Thiol broth (n = 11 independent culture bottles) was the same with either inoculum, but slightly less (8×10^8 CFU/ml) in HI (n = 8) with the lower inoculum. Growth in TSB, however, was variable. No growth was observed, and viable counts remained $<10^2$ CFU/ml in 8 (38%) of 21 cultures in Scott TSB, in 5 (45%) of 11 cultures in Difco TSB, and in 2 (40%) of 5 cultures in GIBCO TSB. When growth did occur, viable counts ranged from 3×10^2 to 3×10^7 CFU per ml after 2 days of incubation. Viable counts in both Scott TSB and Difco TSB supplemented with 20 μg of pyridoxal hydrochloride were similar ($>10^9$ CFU/ml) to those obtained with the higher inoculum (500 CFU per bottle).

DISCUSSION

Previous studies have demonstrated that the bacteremia associated with streptococcal endocarditis is not only prolonged, but also is of small magnitude, i.e., 2 to 173 bacteria per ml of blood (17). At our institution, blood cultures are routinely prepared by placing 5 ml of whole blood in 70 to 90 ml of the three media previously described with incubation for 7 to 10 days. The design of this experimental study was therefore

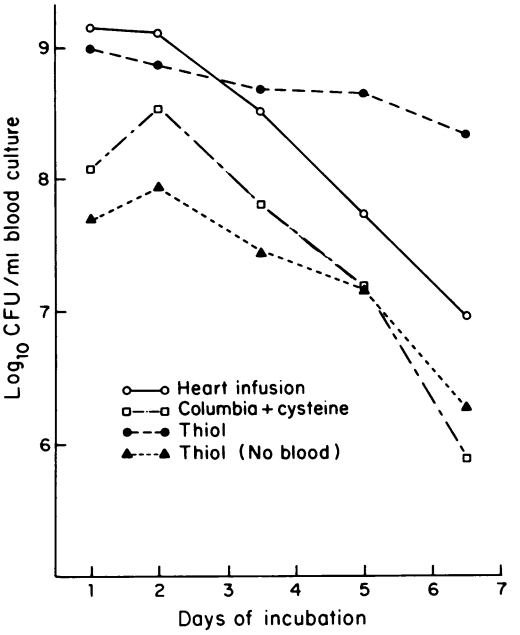

FIG. 4. *Growth curves of strain MB in various blood culture media containing 5% human blood during 7 days of incubation at 35°C.*

to simulate as closely as possible the routine preparation and processing of clinical specimens. Thus, a low inoculum of organisms was used, 5 ml of fresh blood was placed in each blood culture bottle, and specimens were examined over 7 days of incubation. The experimental growth of these organisms (Fig. 1) closely resembled their clinical isolation in that maximal growth occurred in HI, whereas minimal growth was observed in TSB with an inoculum of 100 CFU per ml of blood. Moreover, no growth was observed in 40% of TSB cultures employing an inoculum of 10 CFU/ml of blood. As shown in Table 2, optimal growth was achieved equally well in HI supplemented with blood or pyridoxal hydrochloride. However, growth in TSB also became maximal, with either size inoculum, when pyridoxal was added to the medium, in contrast to blood supplementation alone.

The three clinical isolates that were selected for this study were alike in that they do not hydrolyze either esculin or arginine and do not contain cell wall rhamnose (15), but they vary slightly in sugar fermentation patterns, suggesting different biotypes. Although fermentation of inulin is rare in typical strains of *S. mitior*, both strains MB and HD ferment this sugar. These isolates were therefore representative of the diverse species described by Cooksey and co-workers (6).

Although these pyridoxal-dependent streptococci were unable to initiate growth in HI from a small inoculum without blood, human blood at concentrations from 0.5 to 5% supported maximal growth. Furthermore, no difference in growth was observed in media supplemented with blood from five different donors. Examination of the various components of human blood revealed that erythrocytes, but not plasma or serum, could replace blood in supporting maximal growth. The growth substances in erythrocytes which are necessary for the in vitro cultivation of these variants are unknown, although it is known that erythrocytes, in contrast to plasma or serum, contain significant amounts of pyridoxal, the active form of vitamin B_6 (1, 10). Thus, the variation in maximal growth observed in plasma and serum could have been due to the release of intraerythrocyte pyridoxal by lysed erythrocytes.

The results of this study clearly demonstrate that blood culture media available from various commercial sources (Fig. 3) differ in their ability to support the growth of nutritionally variant streptococci. When compared to our freshly prepared HI supplemented with either blood or pyridoxal, maximal growth was observed only in Difco Thiol broth. BBL Columbia with cysteine, BBL BHI, and Scott thioglycollate were slightly less effective. Thiol compounds such as cysteine have been shown to support the growth of these organisms, although significantly higher concentrations are required than for the active forms of vitamin B_6 (2). The nature of the thiol compound in the Difco broth of that name is unknown. BACTEC media which contain either cysteine (0.05%) or pyridoxal hydrochloride (0.001%) also supported maximal growth (7). In all media, maximal growth was achieved within 2 days of incubation, and in general, significant loss of viability occurred with subsequent incubation.

Limited growth occurred in TSBs from different manufacturers, and this medium cannot be recommended to support the growth of these organisms unless it is supplemented with pyridoxal. These findings are in contrast to those previously reported by Sherman and Washington (16). Although results obtained by us employing three different clinical isolates with various fermentation patterns have been similar, the growth requirements for all variant streptococci may well differ. Indeed, variations in growth may also occur in similar media made by different manufacturers. For example, in our studies, maximal growth was 4×10^8 CFU/ml in BBL BHI, but only 3×10^7 CFU/ml in Difco BHI.

In general, turbidity of cultures and semiquantitative Gram stains correlated well with quantitative viable counts. One notable exception was in Scott thioglycollate cultures, in which the broth was only slightly turbid, with no flocculent cells, and few to moderate cocci were seen on Gram stain, although the viable count was 2×10^8 CFU/ml. Gram staining of these pyridoxal-dependent streptococci often revealed pleomorphic bacillary forms in addition to gram-positive cocci, especially when the bacteria were grown in TSB. Similar findings have been reported by others (4, 8, 9, 11). The possible correlation of these morphological findings indicating abnormal cell wall formation with the requirement for excess vitamin B_6 for growth is currently being investigated.

Although our quantitative studies demonstrate that these organisms can grow in most commercial media, blood cultures will often appear negative both macroscopically and by Gram stain if growth only reaches 10^6 CFU/ml or less. In addition, if the bacterial concentration in the patient's blood is as low as 2 to 10 CFU/ml, there may be no growth at all. Therefore, to ensure isolation of these variants from clinical specimens, a medium which promotes maximal growth within 1 to 2 days should be used. The best commercially available broths contain either a thiol compound or are supplemented with pyridoxal; this confirms previous recommendations (2, 7, 11, 12, 15). The subsequent loss of viability after 2 days emphasizes the need for prompt examination and subculture to agar supplemented with pyridoxal or containing a *Staphylococcus* sp. streak to demonstrate satellitism.

ACKNOWLEDGMENT

This work was supported in part by a grant from Difco Laboratories.

LITERATURE CITED

1. **Anderson, B. B., C. E. Fulford-Jones, J. A. Child, M. E. J. Beard, and C. J. T. Bateman.** 1971. Conversion of vitamin B_6-compounds to active forms in the red blood cell. J. Clin. Invest. **50**:1901-1909.
2. **Carey, R. B., B. D. Brause, and R. B. Roberts.** 1977. Antimicrobial therapy of vitamin B_6-dependent streptococcal endocarditis. Ann. Intern. Med. **87**:150-154.
3. **Carey, R. B., K. C. Gross, and R. B. Roberts.** 1975. Vitamin B_6-dependent *Streptococcus mitior* (*mitis*) isolated from patients with systemic infections. J. Infect. Dis. **131**:722-726.
4. **Cayeux, P., J. F. Acar, and Y. A. Chabbert.** 1971. Bacterial persistence in streptococcal endocarditis due to thiol requiring mutants. J. Infect. Dis. **124**:247-254.
5. **Colman, G., and R. E. O. Williams.** 1972. Taxonomy of some human viridans streptococci, p. 281-299. *In* L. W. Wannamaker and J. M. Matsen (ed.), Streptococci and streptococcal diseases. Recognition, understanding and management. Academic Press, Inc., New York.
6. **Cooksey, R. C., F. S. Thompson, and R. R. Facklam.** 1979. Physiological characterization of nutritionally variant streptococci. J. Clin. Microbiol. **10**:326-330.
7. **Feder, H. M., N. Olsen, J. C. McLaughlin, R. C. Bartlett, and L. Chameides.** 1980. Bacterial endocarditis caused by vitamin B_6-dependent viridans group *Streptococcus*. Pediatrics **66**:309-312.
8. **Frenkel, A., and W. Hirsch.** 1961. Spontaneous development of L-forms of streptococci requiring secretions of other bacteria or sulphydryl compounds for normal growth. Nature (London) **191**:728-730.
9. **George, R. H.** 1973. The isolation of symbiotic streptococci. J. Med. Microbiol. **7**:77-83.
10. **Hines, J. D., and D. S. Love.** 1969. Determination of serum and blood pyridoxal phosphate concentrations with purified rabbit skeletal muscle apophosphorylase b. J. Lab. Clin. Med. **73**:343-349.
11. **McCarthy, L. R., and E. J. Bottone.** 1974. Bacteremia and endocarditis caused by satelliting streptococci. Am. J. Clin. Pathol. **61**:585-591.
12. **Narasimhan, S. L., and A. J. Weinstein.** 1980. Infective endocarditis due to a nutritionally deficient streptococcus. J. Pediatr. **96**:61-62.
13. **Roberts, K. B., and M. J. Sidlak.** 1979. Satellite streptococci. A major cause of "negative" blood cultures in bacterial endocarditis? J. Am. Med. Assoc. **241**:2293-2294.
14. **Roberts, R. B., A. G. Krieger, and K. C. Gross.** 1977. The species of viridans streptococci associated with microbial endocarditis: incidence and antimicrobial susceptibility. Trans. Am. Clin. Climatol. Assoc. **89**:36-48.
15. **Roberts, R. B., A. G. Krieger, N. L. Schiller, and K. C. Gross.** 1979. Viridans streptococcal endocarditis: the role of various species, including pyridoxal-dependent streptococci. Rev. Infect. Dis. **1**:955-965.
16. **Sherman, S. P., and J. A. Washington II.** 1978. Pyridoxine inhibition of a symbiotic streptococcus. Am. J. Clin. Pathol. **70**:689-690.
17. **Werner, A. S., G. C. Cobbs, D. Kaye, and E. W. Hook.** 1967. Studies on the bacteremia of bacterial endocarditis. J. Am. Med. Assoc. **202**:199-203.