

Comparison of Thioglycollate Media, Modified Thioglycollate Media, and GAM for the Cultivation of Non-sporeforming Anaerobes

Yunsop Chong and Samuel Y. Lee

Department of Clinical Pathology, Yonsei University College of Medicine, Seoul, Korea

ABSTRACT

The clinical bacteriology laboratory has to be prepared to isolate and identify anaerobes as the implication of anaerobes in clinical infections is increasing. Although many types of thioglycollate media have been widely used for the enrichment growth of anaerobes, different types are known to have different growth supporting ability. GAM is a recently developed medium, which is said to support a good growth of anaerobes. This study was made to compare GAM and the commonly used thioglycollate medium.

It was found that BTM was superior to FTM, but GAM was showing the heaviest growth after a short incubation time. Hemoglobin powder added to FTM or BTM greatly improved growth of *Bacteroides* without impairing the clarity of the media. Supplementation of FTM with 1/4 strength each of BHI and TSB, and 1000 mg of hemoglobin per liter of medium improved growth of anaerobes. Among all of the tested media, GAM gave the best results for the cultivation of anaerobes including *Bacteroides* and *Fusobacterium*.

INTRODUCTION

The importance of anaerobic bacteria as causative agents of human infection has been well recognized (Senser, 1971; Finegold, 1972; Kosakai, 1972; Sutter, 1972; Holdeman, 1973). Current reports are showing more frequent isolation of these organisms, because of the increased number of patients with lowered defense activity against microorganisms due to various medical and surgical treatments (Tillotson, 1968; Gelb, 1970; Felner, 1971; Finegold, 1971; Bartlett, 1972; Sullivan, 1972).

For the cultivation of anaerobes from clinical specimens, various enrichment broths and selective and non-selective plating media are available commercially. Of these, some of the most frequently used are various thioglycollate media (Blair, 1970; Dowell, 1974). It is shown that not all types of thioglycollate media are suitable for anaerobe isolation (Suzuki, 1969), and it is our experience that growth of some of the non-sporeforming anaerobes is slow and poor in fluid thioglycollate medium.

GAM semisolid medium, developed at the Gifu university, is reported to support good growth of many fastidious anaerobes. In

* This Study was supported by Faculty Research Grant (1974) of Yonsei University College of Medicine.

clinical bacteriology laboratories, it is most important to use the proper medium which will support a faster growth of anaerobes.

This study was made to compare fluid thioglycollate medium, Brewer thioglycollate medium, and GAM semisolid medium for their efficiency in the cultivation of non-sporeforming anaerobes. The effect of modification of FTM and BTM on the growth of anaerobes was also determined.

MATERIALS AND METHODS

Anaerobe strains: VPI strains of non-sporeforming anaerobes (kindly supplied by Professor L.D.S. Smith, Virginia Polytechnic Institute) and YC strains (isolated at Yonsei University College of Medicine Clinical Pathology Department) were used.

Media: The following media were compared: Fluid thioglycollate medium (FTM, Difco, Cat. No. 0256-01, Cont. No. 593809), Brewer thioglycollate medium (BTM, Difco, Cat. No. 0236-01, Cont. No. 575511) and semisolid Gifu University Anaerobe Medium (GAM, Nissui, Lot No. 4 F 1035). Materials used to modify thioglycollate medium were Trypticase soy broth (TSB, BBL, Cat. No. 01-162, Lot No. 201649), Brain heart infusion (BHI, Difco, Cat. No. 0037-01, Cont. No. 591681) and Hemoglobin (Difco, Cat. No. 013602, Cont. No. 525612).

Methods: Test strains kept frozen in milk (Suzuki, 1969) were subcultured in BTM and inocula were prepared by diluting with phosphate buffer saline (Blair, 1970). Autoclave sterilized test media, prepared in test tubes (13×100 mm) of equal optical density in 4 ml amounts, were kept in a 50 C waterbath until inoculations were made. Pasteur pipettes were

used to deliver a drop of inoculum. Viable count of each inoculum was not made, but prior experience showed that the inoculum contain about a few multiples of 10^2 to 10^3 viable bacteria. It was estimated that the subculture media carried over together with the inocula were around 1/8000 of the test media. The inoculated tubes were gently rolled between the hands to disperse the inoculum evenly down to the bottom of the tubes. Incubation was made at 37 C and, to determine degree of growth, daily measurements of optical density were made at 660 nm using a Bausch and Lomb Spectronic 20 instrument.

RESULTS

1. Comparison of FTM and BTM (Table 1, Fig. 1).

P. acnes and *E. limosum* showed rapid and heavy growth in both media. Growths of *Ps. intermedius* were good in both media. However, growth was faster and heavier in BTM. A moderate degree of growth was observed for *Ps. anaerobius*, *Pc. asaccharolyticus*, *Pc. magnus*. *Pc. prevotii* and *V. parvula* in both media, with somewhat better growth in BTM. Growth of *B. fragilis ss fragilis* and *B. fragilis ss thetaiotaomicron* were much faster and heavier in BTM. In FTM they showed moderate growth only after 3 days incubation.

Growth of *B. melaninogenicus* was not detected after a 1 week incubation time in FTM, while growth appeared in BTM after 5 days of incubation. Only light growth of *F. necrophorum* was observed in FTM after a 1 week incubation time, but in BTM the growth was rapid and moderately heavy. Growth of *F. nucleatum* was very poor in FTM, showing

Table 1. Comparison of FTM for their efficiency in anaerobe cultivation (O.D. at 660 nm).

Incubation time (day)	FTM					BTM				
	1	2	3	5	7	1	2	3	5	7
Anaerobe										
<i>P. acnes</i> . VPI 0207**	0.01	0.02	0.42	0.89	0.89	0.01	0.03	0.26	1.00	1.00
<i>E. limosum</i> . VPI 0260	0.01	0.05	0.77	0.89	0.85	0.01	0.20	0.59	1.16	1.16
<i>Ps. anaerobius</i> . YC 74100915**	0.02	0.08	0.16	0.25	0.24	0.01	0.05	0.09	0.26	0.32
<i>Ps. intermedius</i> . VPI 5939A	0.31	0.47	0.51	0.55	0.57	0.75	0.89	0.96	0.96	1.07
<i>Pc. asaccharolyticus</i> . VPI 2608	0.01	0.06	0.06	0.05	0.05	0.02	0.17	0.19	0.21	0.21
<i>Pc. magnus</i> . VPI 6217	0.03	0.06	0.06	0.06	0.07	0.17	0.23	0.28	0.28	0.28
<i>Pc. prevotii</i> . VPI 7100	0.00	0.02	0.04	0.05	0.05	0.06	0.06	0.06	0.07	0.07
<i>V. parvula</i> . VPI 5788-1	0.00	0.01	0.01	0.02	0.02	0.09	0.11	0.11	0.10	0.10
<i>B. fragilis ss fragilis</i> . VPI 6859B	0.00	0.00	0.08	0.24	0.28	0.04	0.41	0.52	0.50	0.50
<i>B. fragilis ss thetaiotaomicron</i> . VPI 6180A	0.00	0.01	0.08	0.30	0.30	0.19	0.50	0.50	0.44	0.44
<i>B. melaninogenicus</i> . VPI 7795	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.08
<i>F. necrophorum</i> . YC 74110664	0.00	0.00	0.00	0.00	0.27	0.19	0.47	0.47	0.44	0.34
<i>F. nucleatum</i> . VPI 4355	0.00	0.00	0.00	0.00	0.00*	0.02	0.04	0.27	0.43	0.42

* Some granules developed at the upper portion of the broth which was not detectable with the photometer due to location of light path.

** VPI: Virginia Polytechnic Institute strain.

YC: Yonsei University College of Medicine Clinical Pathology strain.

Table 2. Comparison of BTM and GAM for their efficiency in anaerobe cultivation (O.D. at 660 nm).

Incubation time (day)	BTM					GAM				
	1	2	3	5	7	1	2	3	5	7
Anaerobe										
<i>P. acnes</i> . VPI 0207	0.04	0.26	0.85	0.85	0.82	0.03	0.64	0.66	0.54	0.54
<i>E. limosum</i> . VPI 0260	0.05	0.20	0.59	1.16	1.16	0.17	0.47	0.51	0.55	0.57
<i>Ps. anaerobius</i> . YC 74100915	0.01	0.05	0.09	0.26	0.32	0.50	0.51	0.48	0.46	0.43
<i>Ps. intermedius</i> . VPI 5939A	0.75	0.89	0.96	0.96	1.07	0.80	0.80	0.82	0.89	0.92
<i>Pc. asaccharolyticus</i> . VPI 2608	0.07	0.12	0.13	0.13	0.10	0.01	0.14	0.16	0.17	0.22
<i>Pc. magnus</i> . VPI 6217	0.08	0.14	0.16	0.16	0.12	0.07	0.32	0.30	0.28	0.28
<i>Pc. prevotii</i> . VPI 7100	0.01	0.06	0.06	0.07	0.05	0.00	0.16	0.17	0.17	0.17
<i>V. parvula</i> . VPI 5788-1	0.12	0.10	0.09	0.08	0.07	0.12	0.14	0.13	0.11	0.09
<i>B. fragilis ss fragilis</i> . VPI 6859B	0.04	0.41	0.52	0.50	0.50	1.10	1.30	1.40	1.30	1.30
<i>B. fragilis ss thetaiotaomicron</i> . VPI 6180A	0.19	0.50	0.50	0.44	0.44	0.92	1.30	1.30	1.30	1.22
<i>B. melaninogenicus</i> . VPI 7795	0.00	0.00	0.00	0.08	0.08	0.02	0.54	0.85	0.70	0.51
<i>F. necrophorum</i> . YC 74110664	0.19	0.47	0.47	0.44	0.34	0.31	0.68	0.62	0.54	0.48
<i>F. nucleatum</i> . VPI 4355	0.01	0.03	0.14	0.46	0.43	0.13	0.89	1.10	0.75	0.72

only some granules without uniform turbidity. However, rapid and moderately heavy growth was observed in BTM.

2. Comparison of BTM and GAM (Table 2, Fig. 2).

P. acnes, *E. limosum* and *Ps. intermedius* showed heavy growth in both media. *Pc. asaccharolyticus*, *Pc. magnus*, *Pc. prevotii* and *V. parvula* showed moderate growth with slightly heavier growth in GAM.

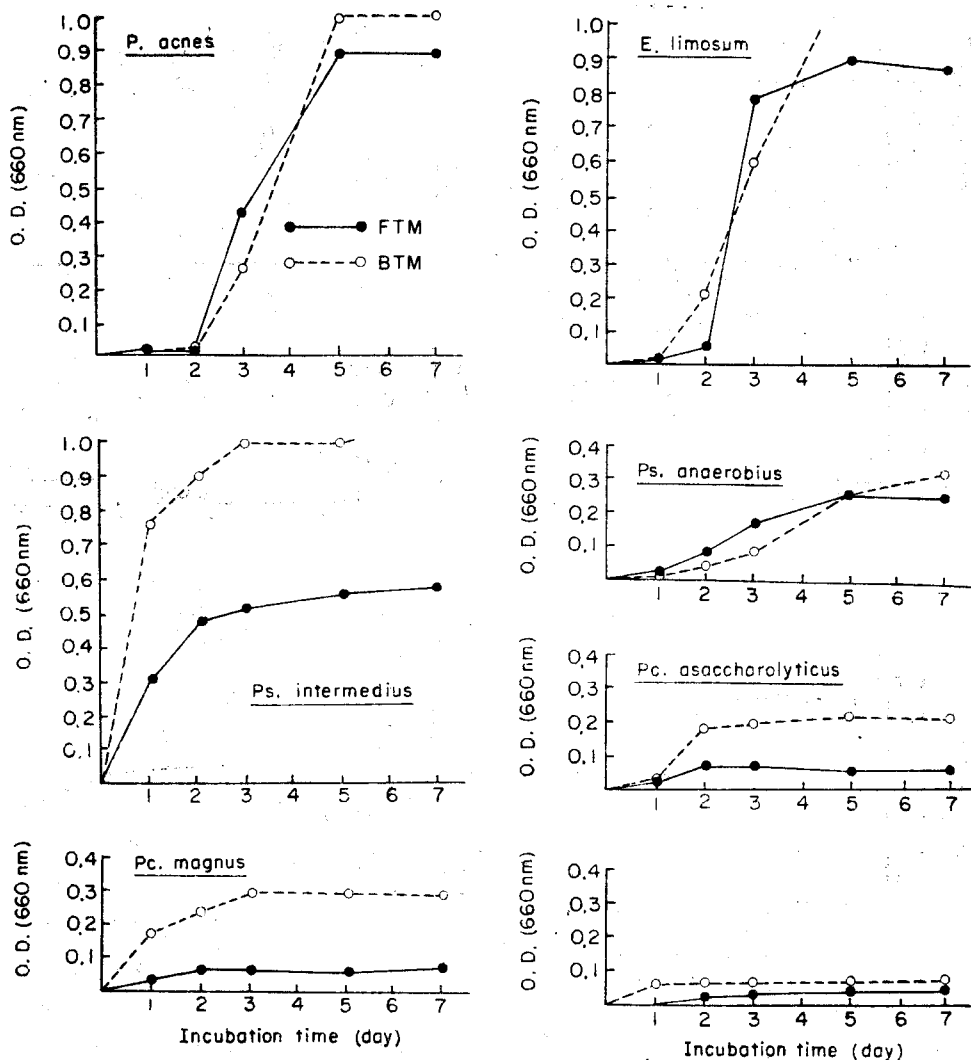


Fig. 1-1. Comparison of FTM and BTM for their efficiency in anaerobe cultivation.

anaerobius was showing good growth in both media, but in GAM faster and heavier growth was observed. The growths of *B. fragilis ss fragilis* and *B. fragilis ss thetaiotaomicron* were very heavy in both media, but the growths were much faster in GAM. While *B. melaninogenicus* showed only light growth in BTM only after 5 days incubation time, the growth in GAM was rapid and heavy. Grow-

ths of *F. necrophorum* and *F. nucleatum* were heavy in both media, but in GAM the growth was much faster.

3. Effect of hemoglobin (Table 3, Fig. 3).

The effect of various amount of hemoglobin supplementation to FTM and to BTM on the growth of *Bacteroides* were determined. The result with FTM showed that the greater the

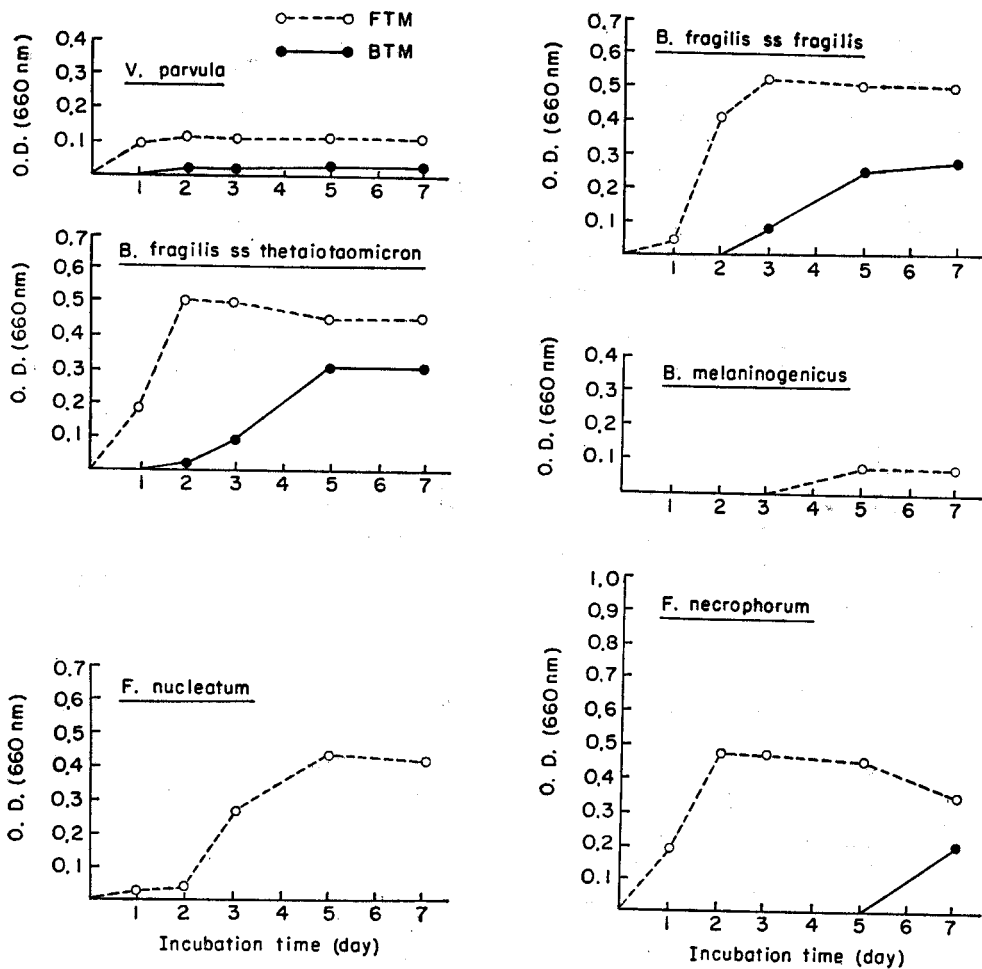


Fig. 1-2. Comparison of FTM and BTM for their efficiency in anaerobe cultivation.

amount of added hemoglobin the faster and heavier were the growths of *B. fragilis ss fragilis* and *B. fragilis ss thetaiotaomicron*. *B. melaninogenicus* did not grow even in the modified FTM.

Growth promoting effect of hemoglobin was also observed in BTM. Rapidity and degree of growth were again proportional to the amount of added hemoglobin. In BTM which is supplemented with 100 mg and over of he-

moglobin per liter of medium, growth of *B. melaninogenicus* was also observed. The amounts of hemoglobin which show definite improvement of growth were 1000 mg for FTM and 10 mg and over for BTM.

4. Comparison of modified FTM, modified BTM, and GAM (Table 4, Fig. 4).

FTM, and BTM, each supplemented with 1000 mg per liter of hemoglobin (FH and BH),

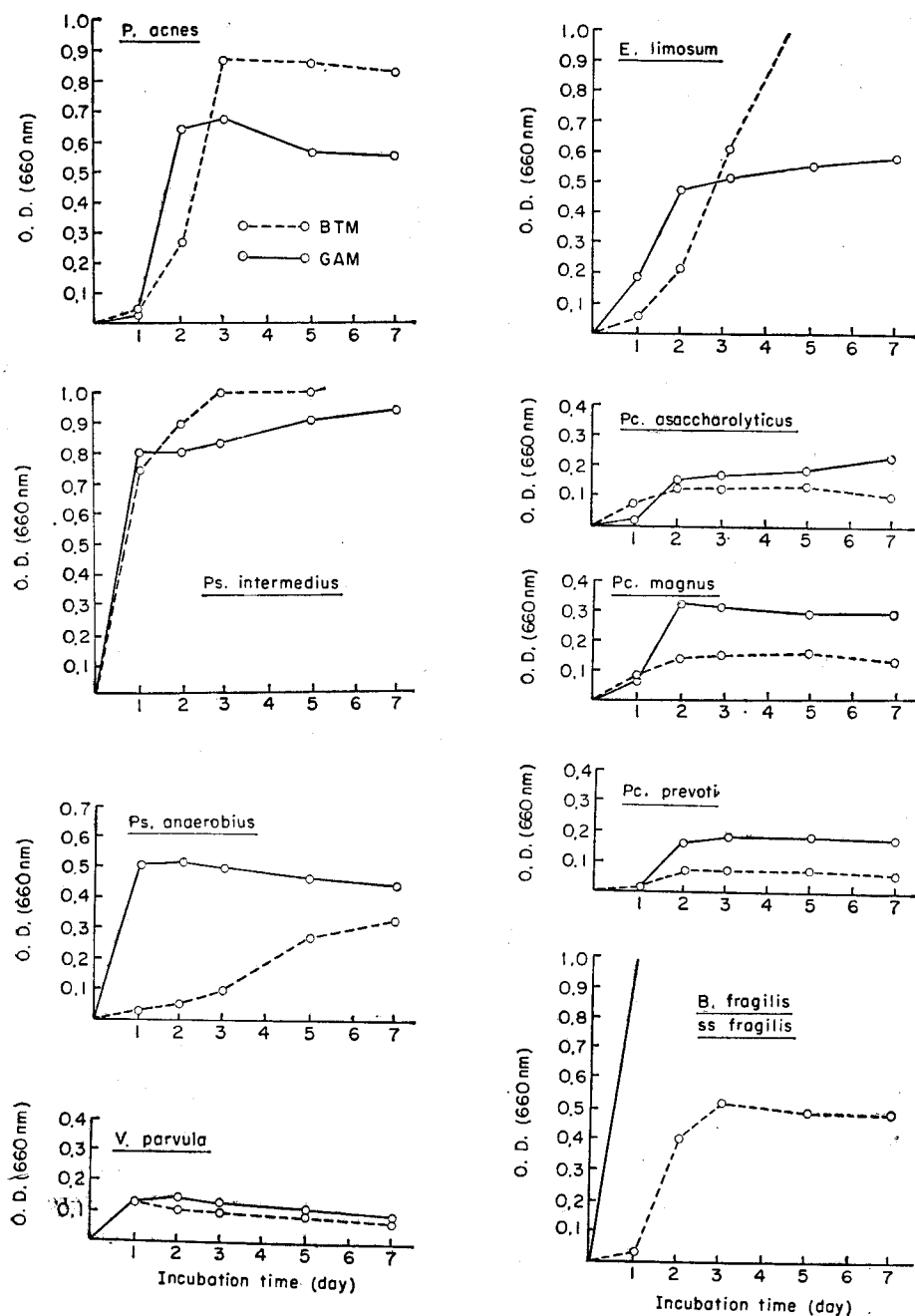


Fig. 2-1. Comparison of BTM and GAM for their efficiency in anaerobe cultivation.

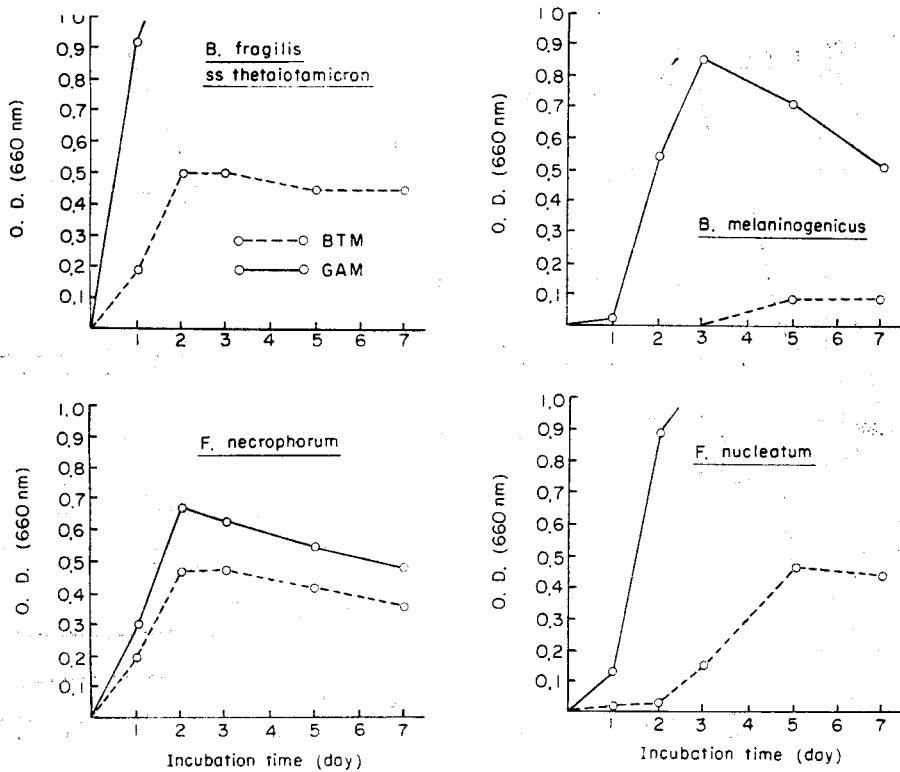


Fig. 2-2. Comparison of BTM and GAM for their efficiency in anaerobe cultivation.

Table 3. Effect of hemoglobin supplementation on the growth of *Bacteroides* (O.D. at 660 nm).

Anaerobe	Incubation (day)	FTM						BTM					
		Hemoglobin (mg/l)						Hemoglobin (mg/l)					
		None	0.1	1.0	10	100	1000	None	0.1	1.0	10	100	1000
<i>B. fragilis ss fragilis</i> VPI 6859B	1	0.04	0.02	0.01	0.07	0.08	0.13	0.13	0.22	0.35	0.62	0.75	0.82
	2	0.16	0.19	0.14	0.22	0.31	0.62	0.40	0.51	0.54	0.85	0.89	1.05
	3	0.24	0.30	0.24	0.29	0.35	0.72	0.41	0.48	0.57	0.77	0.96	1.05
<i>B. fragilis ss thetaiotaomicron</i> . VPI 6180A	1	0.03	0.01	0.01	0.03	0.04	0.14	0.20	0.30	0.37	0.62	0.57	0.54
	2	0.16	0.15	0.05	0.19	0.34	0.60	0.42	0.77	0.75	1.05	1.05	1.05
	3	0.28	0.20	0.14	0.32	0.34	0.68	0.38	0.68	0.64	0.92	1.22	1.30
<i>B. melaninogenicus</i> VPI 7795	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.24

Comparison of Media for Anaerobes

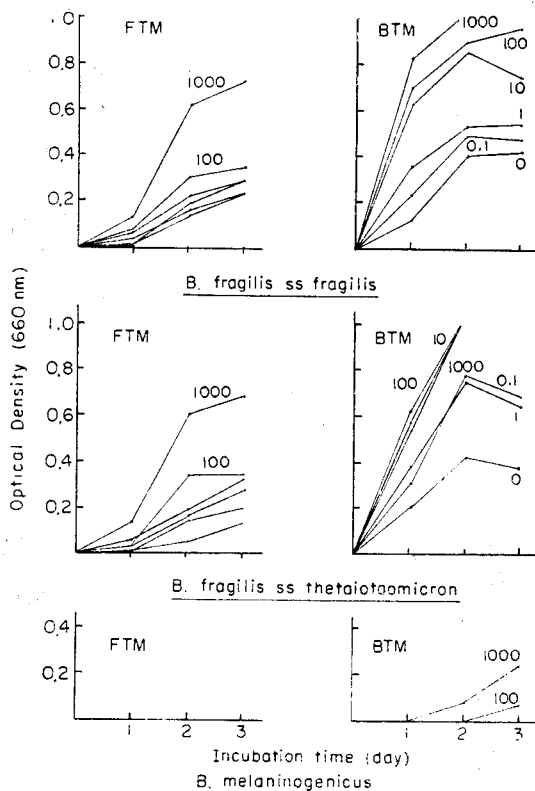


Fig. 3. Effect of hemoglobin supplementation on the growth of Bacteroides (Figure: hemoglobin in mg per liter of medium)

Table 4. Comparison of modified thioglycollate media and GAM for their efficiency in anaerobe cultivation (O.D. at 660 nm).

Anaerobe	Incubation (day)	FTM Hb*	FTM BHI TSB* Hb	BTM Hb*	GAM
<i>B. fragilis ss fragilis</i> VPI 6859B	1	0.13	0.80	0.82	1.00
	2	0.62	1.00	1.05	1.05
	3	0.72	1.05	1.05	1.05
<i>B. fragilis ss thetaiotaomicron</i> VPI 6180A	1	0.14	0.68	0.54	0.89
	2	0.60	1.10	1.05	0.96
	3	0.68	1.16	1.30	0.96
<i>B. melaninogenicus</i> VPI 7795	1	0.00	0.01	0.00	0.02
	2	0.00	0.34	0.08	0.54
	3	0.00	0.59	0.24	0.85
<i>F. necrophorum</i> YC 74110664	1	0.00	0.02	0.04	0.31
	2	0.00	0.52	0.52	0.68
	3	0.00	0.64	0.66	0.68

* Hb: 1000 mg per liter.

BHI, TSB: 1/4 strength each.

FTM modified by supplementing 1/4 strength each of BHI and TSB, and with 1000 mg hemoglobin per liter of medium (FBTH), and GAM were compared for their efficiency in anaerobe cultivation. The results with *B. fragilis ss fragilis* and *B. fragilis ss thetaiotaomicron* have showed that FH were inferior to other media. In BH and in FBTH growths were faster and heavier than in FH. The fastest and heaviest growth was observed in GAM.

B. melaninogenicus did not show growth in FH during the 3 day incubation period, but in BH some growth was observed. Growth in FBTH was heavy and that in GAM was heaviest. Growth of *F. necrophorum* was not observed in FH after 3 days incubation. In BH and in FBTH quite heavy growths were observed. The growth was heaviest in GAM.

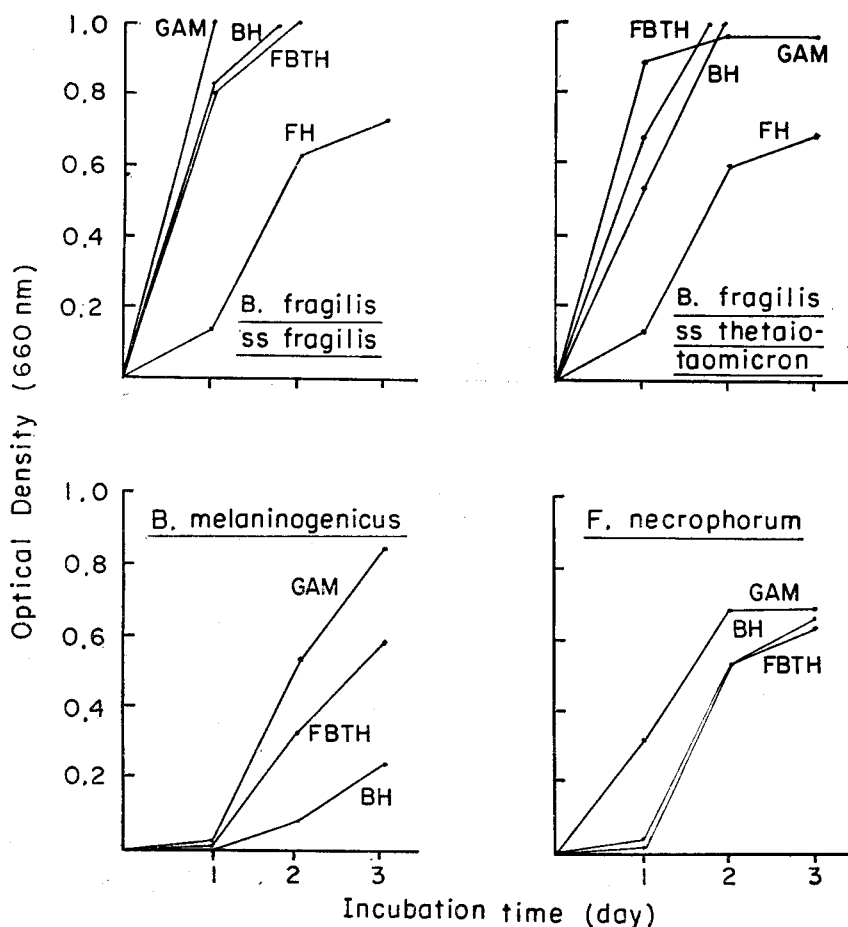


Fig. 6. Comparison of modified thioglycollate media and GAM for their efficiency in *Bacteroides* and *Fusobacterium* cultivation.

DISCUSSION

Thioglycollate medium has been used widely in clinical bacteriology for the enrichment growth of anaerobes as well as aerobes (Blair, 1970). The medium is easy to prepare, may be used for some time after preparation and is incubated in air to culture anaerobes. Because of the clarity of the medium, even a light growth of bacteria can be detected easily.

The medium supports a good growth of va-

rious anaerobes and this is especially true with *Clostridium*. However, the majority of anaerobes isolated from clinical specimens are other than *Clostridium* and some of them are very fastidious in nutrition requirements (Kosakai, 1968).

It is known that some types of thioglycollate media support a better growth of anaerobes. GAM is a new enrichment medium. In this laboratory, thioglycollate medium has been used and it was thought necessary to compare the two media before deciding whe-

ther to continue to use thioglycollate medium or switch to the new medium. Because GAM is not available commercially in this area, attempts were made to modify thioglycollate medium by simply adding other media rather than a small amount each of various ingredients.

1. This experiment with the common non-sporeforming anaerobes showed that in general FTM was inferior to BTM in rapidity and degree of growth (Table 1, Fig. 1). FTM supported rapid and heavy growth of *P. acnes*, *E. limosum* and *Ps. intermedius*. The medium was not very satisfactory for the rapid cultivation of *Peptococcus* and *Veillonella*. *Veillonella* is known to require lactate for better growth (Holdeman, 1973).

Growth of *Ps. anaerobius*, *B. fragilis* ss *fragilis* and *B. fragilis* ss *thetaiotaomicron* were slow, but moderately heavy growth was obtainable. The medium was not suitable for the cultivation of *F. necrophorum*, *F. nucleatum* and *B. melaninogenicus*. *B. melaninogenicus* is known to require hemin and vitamin K for growth (Gibbons, 1960).

In general BTM showed faster growths. Particularly the growth of *Bacteroides* and *Fusobacterium* were remarkably heavy in BTM. Even *B. melaninogenicus* showed growth in the medium.

2. When BTM was compared with GAM semisolid medium, a much faster and heavier growth was observed in the later (Table 2, Fig. 2). It was remarkable that in GAM, *B. fragilis* showed heavy growth only after 24 hours incubation and *B. melaninogenicus* after 2 days incubation. Also the growth of *F. nucleatum* was much faster in GAM. The result showed that GAM was the best among the three tested media for the cultivation of

the test organisms.

3. For the cultivation of *Bacteroides* it is customary to supplement culture medium with 5 µg of hemin per ml of medium. Hemoglobin powder, which is used to prepare chocolate agar, is usually found in laboratories and this material was tested for growth promoting effect (Table 3, Fig. 3). The test result showed that growth of *Bacteroides* was greatly improved with the addition of hemoglobin. Depending on the base medium, amounts of 10~1000 mg per liter improved growth definitely. *B. melaninogenicus* did not grow in the hemoglobin supplemented FTM, but in the hemoglobin supplemented BTM slow growth was observed. FTM seemed unsuitable for *B. melaninogenicus* cultivation even after hemoglobin supplementation.

4. It is natural to assume that the better growths of anaerobes in GAM than in FTM may well be due to some of the ingredients found only in the former. A test was done previously, to test if FTM could be improved by supplementing with a few commonly available media, instead of with many individual ingredients which is tedious to do. By supplementing FTM with 1/4 strength each of BHI and TSB, faster and heavier growth of *P. acnes*, *Ps. anaerobius*, *Pc. asaccharolyticus* and *B. fragilis* were observed. However, the improvement of growth of *B. fragilis* was not great. If hemoglobin was added to the modified medium the growth was greatly improved (unpublished data).

In this test (Table 4, Fig. 4) GAM and the three modified thioglycollate media were compared using *Bacteroides* and *Fusobacterium*. It was found that FH was the least satisfactory. FBTH and BH were similar and supported good growth. The best result was obser-

ved with GAM.

The authors wish to express their sincere appreciation to Professor L. D. S. Smith for his providing many VPI strains of anaerobes, to Professor Kazuo Ueno for his supplying GAM and to Miss Soung Ok Kim for her untiring assistance.

REFERENCES

- Bartlett, J. G., and Finegold, S. M.: *Anaerobic pulmonary infections. Medicine*, 51:413-450, 1972.
- Blair, J. E., Lennette, E. H., and Truant, J. P.: *Manual of clinical microbiology*, Am. Soc. Microbiol. Baltimore, William and Wilkins, 1970.
- Dowell, Jr., V. R. and Hawkins, T. M.: *Laboratory methods in anaerobic bacteriology*. Atlanta, USDHEW, 1974.
- Felner, J. M., and Dowell, Jr., V. R.: "*Bacteroides*" bacteremia. *Am. J. Med.*, 50:787-796, 1971.
- Finegold, S. M., Marsh, V. H., and Bartlett, J. G.: *Anaerobic infections in the compromised host. Proc. Internat. Conf. on nosocomial infections. Am. Hosp. Assoc., Chicago*, 1971.
- Finegold, S. M., Rosenblatt, J. E., Sutter, V. L. and Attebery, H. R.: *Scope monograph on anaerobic infections*. Kalamazoo, Upjohn Co., 1972.
- Gelb, A. F., and Seligman, S. J.: *Bacteroidaceae bacteremia. Effect of aged and focus of infection upon clinical courses. JAMA*, 212:1038, 1970.
- Gibbons, R. J. and Macdonald J. B.: *Hemin and vitamin K compound as required factor for the cultivation of certain strains of B. melaninogenicus. J. Bact.* 80:164-170, 1960.
- Holdeman, L. V., and Moore, W. E. C.: *Anaerobe laboratory manual*. Blacksburg, Virginia polytechnic institute, 1973.
- Kosakai, N., and Suzuki, S.: *Anaerobes in clinical medicine. 1st ed.*, Tokyo, Igaku Shoin, 1968.
- Kosakai, N.: *Isolation of anaerobic bacteria. Naika*, 30:617-620, 1972.
- Sencer, D. J.: *Emerging disease of man and animals. Ann. Rev. Microbiol.*, 25:466-467, 1971.
- Sullivan, L. M., Sutter, V. L., Carter, W. T., Attebery, H. R. and Finegold, S. M.: *Bacteremia after genitourinary tract manipulation: Bacteriological aspects and evaluation of various blood culture systems. Applied Microbiol.*, 23:1101-1106, 1972.
- Sutter, V. L., Attebery, H. R., Rosenblatt, J. E., Bricknell, K. S., and Finegold, S. M.: *Anaerobic bacteriology manual*. Los Angeles, School of Med., UCLA, 1972.
- Suzuki, S. and Ueno, K.: *Anaerobic bacteriology. 1st ed.*, Tokyo, Igaku Shoin, 1969.