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An experimental comparison of Thiol broth with Brewer's thioglycollate for anaerobic blood cultures

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SYNOPSIS In a series of simulated blood culture experiments, small inocula of eight different strains of *Bacteroides* and five strains of anaerobic cocci were added to Difco Thiol broth and Southern Group Brewer's thioglycollate. Both methods enabled all of the strains to be isolated after one to three days' incubation, with the exception of *Bacteroides melaninogenicus*, and most strains to survive after one week. *B. melaninogenicus* grew more quickly in Difco Thiol broth than in Southern Group Brewer's whereas three strains of anaerobic cocci were isolated first from Southern Group Brewer's. Difco Thiol broth appears to be a satisfactory alternative to Southern Group Brewer's for the isolation of non-sporing anaerobes likely to be found in the blood.

For anaerobic blood cultures, thioglycollate broths have previously been found to give earlier growth of non-sporing anaerobes than cooked meat broths (Forgan-Smith and Darrell, 1974; Shanson, 1974). Southern Group Brewer's thioglycollate appeared to be the only type which allowed reasonable survival of the organisms and was also found to be the best of the media commonly used in Britain (Shanson, 1974). Some American workers use Difco Thiol broth, under vacuum and with carbon dioxide already added, for anaerobic blood cultures (Washington, 1971), and it was decided to compare this medium experimentally with Southern Group Brewer's for the growth of small inocula of non-sporing anaerobes in the presence of fresh human blood. Liquoid was omitted from both types of medium since some strains of anaerobic cocci are inhibited (Hoare, 1939) especially when it is added to thioglycollate (Shanson, 1974).

Materials and Methods

BACTERIA

Bacteroides fragilis NCTC 9343, *Sphaerophorus necrophorus* NCTC 10575, *Bacteroides necrophorus* NCTC 7155, *Bacteroides melaninogenicus* NCTC 9337, anaerobic coccus NCTC 9801, and anaerobic coccus NCTC 9803 were obtained from the National Collection of Type Cultures. The other strains were freshly isolated from routine wound swabs on blood agar and included two strains of *B. fragilis*, two strains of *B. melaninogenicus*, and three strains of anaerobic cocci.

MEDIA

Southern Group Brewer's thioglycollate (0586 C), 80 ml, without liquoid.

Difco Thiol broth (0355-37-8), 50 ml, under vacuum and with carbon dioxide, without liquoid, prepared by the manufacturers.

Cooked meat medium (0530) in bottle with perforated cap from Southern Group Laboratories.

BLOOD

Fresh human blood was obtained from healthy volunteers. After collection the blood was introduced directly into the blood culture bottles; 5 ml was obtained from each donor not more than 30 minutes before each test.

INOCULATION

The inoculum was calculated by making viable counts of 72-hour cooked meat cultures of the strains to be tested by the Miles and Misra method. Laked blood was added to the cooked meat broth in order to obtain better growth of *B. melaninogenicus* but was not required for the other species. After preliminary tests 0·5 ml of the dilution likely to contain the required number of viable organisms

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Organism	Inoculum[1]	Difco Thiol				Southern Group Brewer's			
		24 hr	72 hr	96 hr	1 week	24 hr	72 hr	96 hr	1 week
B. fragilis									
NCTC 9343	27	+	+	+	+	+	+	+	+
Lab 1802	40	+	+	+	+	+	+	+	+
Lab 1902	7	+	+	+	+	+	+	+	+
B. necrophorus									
NCTC 1755	46	0	+	+	+	+	+	+	+
S. necrophorus									
NCTC 10575	3	0	+	+	+	+	+	+	+
B. melaninogenicus									
NCTC 9337	20	0	+	+	+	−	0	+	+
Lab 0558 1	41	±	+	+	+	0	0	+	+
2	25	0	+	+	+	0	0	0	+
Lab 5656	27	0	+	+	+	0	0	±	+
Anaerobic cocci									
NCTC 9801	58	0	+	+	+	+	+	+	+
NCTC 9803	29	+	+	+	+	+	+	+	+
Lab 4450	34	+	+	+	+	+	+	+	0
Lab 1855	82	0	+	+	0	+	+	+	0
Lab 2690	44	0	+	+	+	+	+	+	+

Table *Results of subculture of the blood broths*

[1]Viable particles per bottle:
+ growth in all three bottles
± growth in two of three bottles
0 no growth, or growth in only one of three bottles

was inoculated into the test blood-broths by puncturing each cap with a separate needle attached to a disposable 1 ml syringe. The actual inoculum was checked on each occasion by concurrent viable counts of the dilution inoculated using blood agar incubated in anaerobic jars containing 10% carbon dioxide. Colonies were counted after 48 hours' incubation.

METHOD FOR EACH EXPERIMENT

Blood from each donor was distributed so that possible differences between their blood would not affect the comparison between the methods, and 5 ml blood was added to each bottle. Each medium was tested in triplicate. After inoculation the bottles were incubated without the use of an anaerobic jar, at 35°C, with their caps screwed on tightly. In some tests further sets of Southern Group Brewer's were also incubated with their tops vented by a needle containing a sterile cotton wool plug or with their caps loose, or with the tops vented by a plugged needle in air plus 10% carbon dioxide to discover whether the addition of carbon dioxide was beneficial even though exposure to air would also be increased.

Bottles were subcultured onto blood agar after one, three, four, and seven days' incubation. The blood agar plates were incubated in anaerobic jars containing 10% carbon dioxide for up to four days, anaerobiosis being checked with an external indicator.

Results

These are summarized in the Table. No difference was found between the two methods for the early growth and survival of *B. fragilis*. Three strains of *B. melaninogenicus* were all more rapidly isolated from Difco Thiol broth. Southern Group Brewer's appeared to facilitate the better isolation of anaerobic cocci, after 24 hours' incubation.

When vented, in either air or carbon dioxide, growth as judged by the blood agar subculture plate was reduced to about one-third that found on subculture plates from parallel Brewer's which had been incubated with the caps tight, on the first positive subculture. This phenomenon was observed with two strains of *B. fragilis*, one strain of *B. melaninogenicus*, and two strains of anaerobic cocci.

Discussion

Many anaerobes do not survive in USP thioglycollate (Shanson, 1974). This effect was also noted by Forgan-Smith and Darrell (1974), who suggested that a good cooked meat method be used together with USP thioglycollate for each routine blood culture. However, it is probable that some anaerobes which do not survive after one week in USP thioglycollate will not grow in the cooked meat, since thioglycollate methods were found to be superior to cooked meat methods for the isolation of anaerobes.

On experimental grounds a reliable system which requires only one anaerobic bottle in each routine blood culture set is provided for by using Southern Group Brewer's thioglycollate (Shanson, 1974). From the results presented in this paper it would appear that Difco Thiol broth represents a suitable alternative to Southern Group Brewer's for use as a routine anaerobic blood culture bottle. In any case where an anaerobic bacteraemia is strongly suspected it might prove beneficial to inoculate blood into both Southern Group Brewer's and Difco Thiol broth, since there are certain anaerobes which can most speedily be isolated by one method rather than the other. As might be expected, Southern Group Brewer's appears to give the best results when the caps are tight and not vented, the introduction of air with the carbon dioxide in these bottles being undesirable. The effect of adding carbon dioxide to anaerobic liquid media other than Thiol broth, without venting or loosening the caps, has yet to be determined.

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