Effect of adding cysteine to brain-heart infusion broth on the isolation of *Bacteroides fragilis* from experimental blood cultures

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SUMMARY Adding 0.05% cysteine to brain-heart infusion broth greatly improved the recovery of three strains of *Bacteroides fragilis* from simulated blood cultures. No bacteroides were isolated after 24 hours' incubation in brain-heart infusion broth without added cysteine, and this was therefore a poor medium for the isolation of *B. fragilis*. Results with brain-heart infusion broth containing 0.05% cysteine were similar to those obtained with commercially prepared Thiol and Brewer's thioglycollate media.

The choice of anaerobic medium is critical for the successful isolation of *Bacteroides fragilis* from the blood. There have been several reports on anaerobic blood culture media in recent years based on the results of simulated blood culture experiments. Thioglycollate media were superior to cooked-meat media for the early growth of bacteroides.^{1,2} In a series of experiments in 1973 with media in common use Brewer's thioglycollate (Southern Group Laboratories) 80 ml gave the best overall results.¹ This medium was recommended in the Association of Clinical Pathologists *Broadsheet* on blood culture techniques.³ There was little difference between Brewer's thioglycollate medium and Thiol medium 50 ml under vacuum with added carbon dioxide for isolating *B. fragilis*,⁴ but in a subsequent study Thiol gave better results than thioglycollate media.⁵ Recently, Collee *et al.*⁶ reported that Thiol medium was inferior to Brewer's thioglycollate medium for isolating bacteroides. Their best results were with a mixture of brain-heart infusion broth and cooked-meat broth. They found brain-heart infusion broth without added cooked meat a poor medium for isolating *B. fragilis*.

The purpose of the present study was to investigate the effect on the isolation of *B. fragilis* from simulated blood cultures of adding cysteine, a known reducing substance, to brain-heart infusion broth reconstituted from a commercial dehydrated powder. We also compared it with Brewer's thioglycollate medium (Southern Group Laboratories) and Thiol

medium under vacuum with added carbon dioxide (Difco).

Material and methods

BLOOD CULTURE MEDIA

(1) Brain-heart infusion broth (Bacto 0037-01, control 657821, Difco) prepared by dissolving 37 g powder in 500 ml distilled water, adjusting the pH to 7.4, and making up to 1 l. The broth was dispensed in 50-ml volumes and autoclaved at 121°C for 15 minutes.

(2) Brain-heart infusion broth (Difco) with cysteine 0.05%. This was prepared in a similar way to (1) but with the addition of L-cysteine (BDH Chemicals Ltd). L-Cysteine 0.5 g was dissolved in 5 ml normal hydrochloric acid and the 5 ml solution added to 500 ml broth, pH adjusted to 7.4, and made up to 1 l with more broth. The brain-heart infusion broth containing cysteine was dispensed in 50-ml volumes and autoclaved at 121°C for 15 minutes.

(3) Thiol broth (Difco, 0355-37-8) 50 ml under vacuum and with carbon dioxide prepared by the manufacturers.

(4) Brewer's thioglycollate 80 ml (Southern Group Laboratories, 0586C).

BLOOD

Defibrinated horse blood 5 ml (Tissue Culture Services) added to each broth just before the bacteroides were added.

BACTERIA

Three strains of *B. fragilis* freshly isolated on blood agar from the wound swabs of different patients with abdominal sepsis.

INOCULATION

The inoculum was calculated by making viable counts of dilutions of 48-hour cooked-meat cultures of the strains to be tested by the Miles and Misra method. After preliminary tests 0.25 ml of the dilution likely to contain the required number of viable organisms 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

Each medium was tested either in duplicate or in triplicate. After inoculation the bottles were incubated without the use of an anaerobic jar at 35°C. Bottles were subcultured on to blood agar after one, two, and five days' incubation using syringes and needles for the subcultures. The blood-agar plates were incubated in anaerobic jars containing 10% carbon dioxide for up to 48 hours.

Results

The addition of cysteine 0.05% to the brain-heart infusion broth enormously improved the recovery of the *B. fragilis* strains from the blood broths even after only 24 hours' incubation (Table). No bacteroides were isolated from the brain-heart infusion broth without added cysteine after two days' incubation but after five days' incubation *B. fragilis* was isolated from 11 of the 21 bottles. There were the same number of bacteroides isolations after two and

five days' incubation from the brain-heart infusion broths containing 0.05% cysteine as there were after 24 hours' incubation.

The brain-heart infusion broth with 0.05% cysteine, the Thiol, and the Brewer's thioglycollate media all yielded growth of bacteroides from 19 out of 21 bottles after 24 hours', 48 hours', and 5 days' incubation.

Discussion

Brain-heart infusion broth without additives was a poor medium for the isolation of *B. fragilis*. This confirms the observation of Collee *et al.*⁶ However, the simple addition of 0.05% cysteine to the brain-heart infusion broth greatly improved the isolation of the bacteroides strains from the broth after only 24 hours' incubation of the simulated blood cultures. Laboratories that rely on brain-heart infusion broth for the isolation of *B. fragilis* from blood cultures should consider the routine addition of cysteine to the medium.

Interestingly, the results obtained with the brain-heart infusion broth containing 0.05% cysteine were identical with those obtained with the much more expensive Thiol medium under vacuum with added carbon dioxide and the Brewer's thioglycollate medium.

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Results of subculture of brain-heart infusion (BHI) blood broths after 24 hours' incubation

Culture No.	Organism	Inoculum*	BHI and cysteine 0.05%		BHI	
			No. bottles inoculated	No. bottles positive	No. bottles inoculated	No. bottles positive
1	<i>B. fragilis</i> 8560	50	2	2	2	0
2	<i>B. fragilis</i> 8560	20	3	2	3	0
3	<i>B. fragilis</i> 8560	130	2	2	2	0
4	<i>B. fragilis</i> 112	70	2	2	2	0
5	<i>B. fragilis</i> 112	44	3	2	3	0
6	<i>B. fragilis</i> 112	58	2	2	2	0
7	<i>B. fragilis</i> 113	60	2	2	2	0
8	<i>B. fragilis</i> 113	85	3	3	3	0
9	<i>B. fragilis</i> 113	36	2	2	2	0
Totals			21	19	21	0

*Viable particles added per bottle.

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* Collee JG, Duerden BI, Brown R. Recovery of anaerobic bacteria from small inocula: a model for blood culture studies. *J Clin Pathol* 1977;30:609-14.

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