COMPARISON OF MEDIA WITH AND WITHOUT 'PANMEDE' FOR THE ISOLATION OF *STREPTOBACILLUS MONILIFORMIS* FROM BLOOD CULTURES AND OBSERVATIONS ON THE INHIBITORY EFFECT OF SODIUM POLYANETHOL SULPHONATE

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SUMMARY. Fastidious anaerobe broth and brain-heart infusion cysteine broth supplemented with 'Panmede' (a papain digest of ox liver) 2.5% supported the recovery of five *Streptobacillus moniliformis* strains from simulated blood cultures. Other media tested in parallel—brain heart infusion cysteine broth without 'Panmede' and Brewer's thioglycollate broth—were unreliable. Sodium polyanethol sulphonate (Liquoid) 0.05%, inhibited five isolates of *S. moniliformis*, including isolates from patients with Haverhill Fever. Occasionally, Liquoid 0.025% was also inhibitory and a heavy inoculum of one strain, NCTC11194, was completely inhibited by Liquoid 0.012% in simulated nutrient-broth blood cultures. These results suggest that the choice of media included in each blood-culture set is critical for the optimal isolation of *S. moniliformis*. Brain-heart infusion cysteine broth supplemented with 'Panmede', or commercially available fastidious anaerobe broth, without Liquoid, is recommended.

INTRODUCTION

Optimal blood-culture methods are important for the reliable microbiological diagnosis of Haverhill Fever (*erythema arthriticum epidemicum*), rat bite fever and endocarditis caused by *Streptobacillus moniliformis*. Recently, this organism was isolated from the blood of four patients in a school-based outbreak of Haverhill Fever (Shanson *et al.*, 1983). It was observed with blood cultures collected from three patients that the epidemic strain was isolated from the anaerobic broths, which did not contain sodium polyanethol sulphonate (Liquoid), but parallel aerobic broths, which contained Liquoid 0.05%, gave negative results. Blood-culture broths containing Liquoid gave negative results from the fourth patient, but a repeat blood culture collected on day 27 of the illness, using broth without Liquoid, yielded growth of the epidemic strain. Liquoid neutralises the antibacterial effect of fresh human blood (Von Haebler and Miles, 1938) and is recommended for addition to aerobic blood-culture broths to increase the chances of isolating bacteria (Stokes, 1974). However, it is not recommended for inclusion in anaerobic broths for which an approximately 1 in 17

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dilution of blood in broth has been recommended instead for neutralising the antibacterial effect (Shanson, 1974). The absence of Liquoid from the anaerobic broths may have explained why the anaerobic and not the aerobic blood culture broths yielded growth of the epidemic *S. moniliformis* strain if this strain was inhibited by Liquoid. The question then arose whether *S. moniliformis* can be isolated from some types of anaerobic blood-culture broth more easily than from others. We compared the recovery of *S. moniliformis* from four anaerobic blood-culture media recently tested for the isolation of non-sporing anaerobes (Shanson and Pratt, 1983) and investigated further the inhibitory effect of Liquoid on different strains of this organism.

MATERIALS AND METHODS

Media

The media were stored for up to 2 weeks in the laboratory before use. The four anaerobic blood-culture broths were prepared without Liquoid.

Brain-heart infusion broth with cysteine 0.05%. Brain-heart infusion broth (Bacto 0037-01, Control 657821, Difco) was prepared by adding 37 g of dehydrated powder to 500 ml of distilled water. L-Cysteine (BDH Chemicals Ltd) 0.5 g was dissolved in 5 ml of N HCl and added to 500 ml of broth. The pH was adjusted to 7.4 and the final volume made up to 1 L. This broth was dispensed in 75-ml volumes in medical flat bottles which had perforated metal caps with rubber liners. The medium was autoclaved at 121°C for 15 min.

Brain-heart infusion broth with cysteine 0.05% plus Panmede 2.5% was prepared as above but Panmede, a papain digest of ox liver (Paines and Byrne, Greenford, Middlesex) was added. Panmede 50 g was added to 50 ml of distilled water and mixed; 10N NaOH (40%) 4.6 ml was added and the solution made up to 100 ml with distilled water. To 1 L of the brain-heart infusion broth containing cysteine 0.05% was added 50 ml of the 50% Panmede solution. The pH was adjusted to 7.8 with 10N HCl and the broth was dispensed in 75-ml volumes in medical flat bottles. The medium was then autoclaved at 121°C for 15 min. The pH of the medium was reduced by autoclaving. The autoclaved broth was checked to ensure that the working pH was 7.4.

Fastidious anaerobe broth with thymidine (Lab M, Salford) was obtained as commercially prepared flat bottles of broth, ready to use, containing 75 ml.

Brewer's thioglycollate broth (Southern Group Laboratories, Code no. 0586C) was obtained as commercially prepared bottles, ready to use, containing 80 ml.

Nutrient broth (Oxoid no. 2) for Liquoid broth experiments, in 10-ml volumes in McCartney bottles, was prepared without Liquoid and with doubling concentrations of Liquoid from 0.012% to 0.5%, by a method similar to that recommended in the Association of Clinical Pathologist's broadsheet (Stokes, 1974).

Blood and blood agar containing Liquoid. Defibrinated horse blood (Gibco Europe), 5 ml, was added to each anaerobic or nutrient broth just before inoculation with *S. moniliformis*. Blood-agar plates were prepared with 19 ml of Columbia agar containing 33% defibrinated horse blood and 1 ml of Liquoid (Roche) solution so that plates contained doubling concentrations of Liquoid from 0.006 to 0.8%.

Serum nutrient broth was used to prepare each culture of *S. moniliformis* before inoculation of blood broths or agar plates. Nutrient broth was prepared with Oxoid special peptone 2%, sodium chloride 2%, liver digest 0.5%, yeast extract 0.5%, and 'Lab-Lemco' powder 0.5%, at pH 7.35. Sterile horse serum (Tissue Culture Services) was added to sterilised nutrient broth to give a concentration of 10%.

Bacteria

Five test strains of *S. moniliformis* were used: clinical laboratory isolates 1, 2 and 3 from the bacteraemic cases in the 1983 Haverhill Fever outbreak, and NCTC strains 10773 and 11194 originally isolated from sporadic cases of rat bite fever and Haverhill Fever (National Collection of Type Cultures, Public Health Laboratory, Colindale).

The inoculum was calculated by making surface viable counts of dilutions of a 48-h culture in serum nutrient broth. After preliminary tests, 0.1 ml of the dilution likely to contain the required number of cfu (tables I and II) was inoculated into the test anaerobic blood broths by puncturing each cap with a separate needle attached to a disposable 1.0-ml syringe. The inoculum was checked on each occasion by surface viable counts on fresh blood-agar plates incubated for 48 h in a moist aerobic atmosphere with CO₂ 10% added.

Comparison of anaerobic broths

Each medium was tested in parallel in triplicate during each experiment. After inoculation the bottles were incubated at 37°C. The broths were subcultured with a Pasteur pipette; 0.1 ml of broth was spread across the surface of a freshly prepared 5% horse-blood-agar plate. Subcultures were made after incubation of bottles for 1, 2, 4 and 10 days; the blood-agar plates were incubated aerobically in a CO₂ incubator for 48 h. The broths were also examined macroscopically daily.

Liquid broth experiments

The method of inoculation of the nutrient broths was similar to that for testing anaerobic broths except that the caps of the bottles were removed and 1 drop from a 50-dropper pipette (0.02 ml) was the inoculum. Duplicate bottles of each nutrient broth with the different concentrations of Liquid were tested in parallel with bottles of nutrient broth without Liquid.

RESULTS

Comparison of medium containing 'Panmede' with other anaerobic media

None of the anaerobic blood broths appeared to be positive by macroscopic inspection 24 h after inoculation but subculture yielded growth of all three strains tested of *S. moniliformis* from the brain-heart infusion cysteine broths supplemented with Panmede and from the fastidious anaerobe broth (table I). The difference between the total number of broths positive after incubation for 24 h with either the Panmede medium or the fastidious anaerobe broth (12 out of 15 tested) and the Brewer's thioglycollate (6 out of 15) was statistically significant ($p=0.03$). After incubation for 2–3 days, all except one of the Panmede broths and all of the fastidious anaerobe broths appeared positive by macroscopic inspection; numerous characteristic 'puff ball' colonies were present in the broths. In contrast, only six Brewer's thioglycollate broths appeared positive macroscopically after incubation for 2–3 days and none of the brain-heart infusion cysteine broths without Panmede appeared positive. The difference between the number of brain-heart infusion cysteine broths without Panmede that yielded growth of *S. moniliformis* during incubation for 4 days, none of 12 tested, and the number of positive broths of the same medium supplemented with Panmede 2.5%, 14 of 15 tested, was highly significant ($p<0.001$).

Effect of adding 'Liquid' to broths and blood agar

Liquid 0.05% added to simulated nutrient-broth blood cultures inhibited *S. moniliformis*—strain NCTC11194, strain NCTC10773 tested with the 2×10^4 cfu inoculum, and the clinical laboratory isolate no. 1 tested with the 20 cfu inoculum—during incubation of the broths for 4 days (table II). The difference between the number of positive broths without Liquid (14 of 14 tested) and the number with Liquid 0.05% added (6 of 14 tested) was highly significant ($p<0.001$). Strain

TABLE I
Recovery of *S. moniliformis* from simulated blood cultures with different anaerobic media

Strain and inoculum	Bottle no.	Density of growth* on subcultures after incubation of broths for															
		1 day				2 days				4 days				10 days			
		BCP	BC	FAB	TH	BCP	BC	FAB	TH	BCP	BC	FAB	TH	BCP	BC	FAB	TH
NCTC11194 35 cfu	1	+	—	+	—	++	—	++	—	++	—	++	++	++	—	++	++
	2	++	—	+	—	++	—	++	—	++	—	++	++	++	—	++	++
	3	+	—	+	—	++	—	++	—	++	—	++	++	++	—	++	++
NCTC10773 15 cfu	1	+	—	+	—	++	—	++	—	++	—	++	—	++	—	++	—
	2	+	—	+	—	++	—	++	—	++	—	++	—	++	—	++	—
	3	+	—	+	—	++	—	++	—	++	—	++	—	++	—	++	—
Clinical laboratory isolate no. 1 5 cfu	1	—	—	—	—	+	—	+	—	++	—	++	+	++	—	++	+
	2	—	—	—	—	—	—	+	—	—	—	++	—	—	—	++	—
	3	—	—	—	—	+	—	+	—	—	—	++	—	++	—	++	—
28 cfu	1	++	...	++	+	++	+	++	+	++	+	++	+	...	...	...	...
	2	++	...	++	+	++	+	++	+	++	+	++	+	...	...	...	...
	3	++	...	++	+	++	+	++	+	++	+	++	+	...	...	...	...
60 cfu	1	+	—	+	+	++	—	++	++	++	—	++	++	++	—	++	++
	2	++	—	+	+	++	—	++	++	++	—	++	++	++	—	++	++
	3	++	—	+	+	++	—	++	++	++	—	++	++	++	—	++	++
Total number of positive broths	12	0	12	12	6	14	0	15	6	14	0	15	10	11	0	12	7
Total number of broths tested	15	12	15	15	15	15	12	15	15	15	12	15	15	12	12	12	12

BCP = Brain heart infusion cysteine broth with added 'Panmede'; BC = Brain heart infusion cysteine broth; FAB = Fastidious anaerobe broth; TH = Thioglycollate broth from Southern Group Laboratories.
* ++ = Profuse growth on blood agar; ++ = moderate growth; + = scanty growth; — = no growth; ... = not tested.

TABLE II
Effect of adding Liquoid to nutrient broth on recovery of *S. moniliformis* from simulated blood cultures

Strain and inoculum	Bottle no.	Density of growth* on subcultures after incubation of nutrient broths with the given concentration of Liquoid for													
		1 day				4 days				7 days					
		0	0.012	0.025	0.05	0	0.012	0.025	0.05	0	0.012	0.025	0.05	0.05	0.05
NCTC11194 7 × 10 ³ cfu	1	+	+	—	—	+	—	—	—	+	—	—	—	—	—
	2	+	+	—	—	+	—	—	—	+	—	—	—	—	—
	1	+	+	—	—	+	—	—	—	+	—	—	—	—	—
	2	+	+	—	—	+	—	—	—	+	—	—	—	—	—
NCTC10773 2 × 10 ⁵ cfu	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	—	+	+	+	—	+	+	+	—	+	+
	2	+	+	+	—	+	+	+	—	+	+	+	—	+	+
Clinical laboratory isolate no. 1 3 × 10 ⁴ cfu	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3 × 10 ³ cfu	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	—	+	+	+	—	+	+	+	—	+	—
	2	+	+	+	—	+	+	+	—	+	+	+	—	+	—
20 cfu	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total number of positive broths		14	10	8	6	14	10	10	6	14	10	10	6	8	8
Total number of broths tested		14	14	14	14	14	14	14	14	14	14	14	14	14	14

Results of subcultures after 10 and 21 days were the same as for those after 7 days.
* See footnote to table I.

NCTC11194 was the most susceptible of the three strains; the 7×10^3 cfu inoculum was completely inhibited by Liquoid 0.012% even after incubation for 1 week.

In a separate simulated blood culture experiment the effect of adding Liquoid 0.05% to fastidious anaerobe broth was investigated with *c.* 30 cfu of the clinical laboratory isolate no. 1 as inoculum. In this experiment, 5 ml of fresh human blood was added to each bottle containing 75 ml of fastidious anaerobe broth just before inoculation. All six bottles of broth without Liquoid yielded growth of the organism after incubation for 2 days, but no growth occurred in the six parallel bottles with Liquoid 0.05% added, even after incubation for 3 weeks.

All three clinical isolates and both NCTC strains were inhibited by 0.05% Liquoid in 33% blood agar during incubation for 72 h.

DISCUSSION

The results of this study show that the choice of blood-culture medium is of critical importance for the optimal isolation of *S. moniliformis*. This organism was isolated during the first 2 days of incubation from nearly all the bottles of fastidious anaerobe broth and brain-heart infusion cysteine broth supplemented with Panmede 2.5%. In contrast, the Brewer's thioglycollate broth and brain-heart infusion cysteine broth without Panmede failed to support the early growth of small inocula.

The study also confirmed previous observations that the strain of *S. moniliformis* isolated from the blood of cases in the recent British Haverhill Fever outbreak was inhibited by Liquoid 0.05% (Shanson *et al.*, 1983). Inhibition by Liquoid is probably a frequent characteristic of *S. moniliformis* because the strains obtained from the National Collection of Type Cultures were also inhibited by Liquoid, either 0.01 or 0.05%, and there is a previous report of inhibition by Liquoid of a strain isolated from the blood of a sporadic case of Haverhill Fever (Lambe *et al.*, 1973). The number of bacteria inoculated into the media in each experiment is uncertain because the pleomorphic streptobacillus is characteristically filamentous. Nonetheless, the experiment with Liquoid showed, as expected, that the degree of inhibition of *S. moniliformis* by Liquoid was greatest when small inocula were used. This is clinically relevant because it is likely that a low grade bacteraemia may sometimes be present at the time blood cultures are collected.

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