Growth of Streptococci in a Glucose Phenolphthalein Broth

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SUMMARY: A liquid medium buffered at pH 9.6 is described which is modified from that of Shattock & Hirsch (1947) by the addition of phenolphthalein and in the method of preparation. Its use in distinguishing streptococcal species was investigated.

Shattock & Hirsch (1947) described a liquid glycine-buffered medium for testing the ability of streptococci to grow at pH 9.6, one of the criteria found useful by Sherman & Stark (1934) and Shattock & Mattick (1943-44) for distinguishing *Streptococcus faecalis* from *Strep. lactis*. This medium was used by Shattock (1949) and by Sharpe & Shattock (1952) in studies on group D streptococci and *Strep. lactis*. It was thought that if a suitable indicator could be added to the medium it might be possible to dispense with the electrometric measurement of pH value, and as streptococci readily produce acid from glucose, growth would be accompanied by a change in colour of the indicator. Phenolphthalein with a pK of 9.7 was selected.

PREPARATION OF MEDIUM

The medium was prepared as described by Shattock & Hirsch (1947) with the modifications discussed above. Only 100 ml. were prepared at a time, sufficient for testing about sixteen strains.

- (1) Stock Lemco broth pH 7.0 + 1 % w/v glucose.
- (2) Glycine buffer: glycine, 0.75 g.; NaCl, 0.585 g., were dissolved in 100 ml. freshly boiled distilled water. To 60 ml. glycine + NaCl were added 40 ml. 0.1 N-NaOH to make solution (2).
- (3) Set of buffer solutions pH 9.5, 9.6, 9.7, 9.8, 9.9. Clark & Lubs borate buffers were used as they were less likely to become contaminated. 20 ml. were prepared at each pH value, to which 0.1 ml. 0.2 % phenolphthalein was added. After mixing, the solutions were poured into bijou bottles (c. 5 ml.) and the caps screwed tightly to exclude air. They remained unchanged for at least 2 weeks.

To 90 ml. solution (1) were added 10 ml. solution (2) and 0.5 ml. phenolphthalein, and the reaction adjusted to pH 9.9 with N-NaOH by matching with the buffers in the bijou bottles, but any standard method for matching pH indicators may be used. No difficulty was experienced in matching the colours but the accuracy obtained was not greater than ± 0.25 pH unit. The medium was kept overnight in a refrigerator and then passed through a Seitz filter. Occasionally precipitation continued after filtration, but this was ignored as it did not affect the results. The sterile medium was distributed into

bijoux bottles leaving as little air space as possible. The bottles were incubated overnight to check sterility and on the next day inoculated from 24 hr. agar or serum agar slope cultures. The colour of the medium at this stage is a definite clear pink. Any bottles in which the pH value has fallen to pH 9.2 or less will appear a pale orange pink colour and can be discarded. This makes possible a check on each individual bottle before inoculation. Uninoculated bottles and known positive and negative organisms were included in each set of tests. Tests were carried out at 37° and 30°. Controls were prepared of the buffered medium adjusted to pH 7.0 with bromthymol blue as indicator, and without indicator. Tubes of glucose peptone water as used for fermentation tests were incubated concurrently in a number of the tests.

Only a small number of strains has been tested; the group D and group N strains maintained in the N.C.T.C., strains of *Strep. lactis* kindly provided by Dr Shattock from her collection, and representative N.C.T.C. strains of the other serological groups of streptococci, and strains of *Aerococcus viridans* (Williams, Hirsch & Cowan, 1953).

RESULTS

The medium was simple to prepare and proved quite satisfactory in use. The reaction after filtration varied between pH 9.5–9.6. Out of twelve batches of medium only an occasional bottle gave a pH value of 9.2 or less, appearing orange-pink instead of a clear definite pink colour. Two *Strep. lactis* strains tested in media at about pH 9.2 were able to grow whereas no growth occurred in the medium at pH 9.5–9.6.

The results were clear-cut with the *Strep. lactis* and *Strep. faecalis* strains tested at 30° and 37°. All the *Strep. faecalis* strains grew with acid production in 24 hr. and none of the *Strep. lactis* in 24 or 48 hr. in repeated tests (Table 1). In the medium at pH 7.0, with or without bromthymol blue and in glucose peptone water, both species grew and produced acid in 24 hr. None of the strains of *Strep. bovis* grew at pH 9.6. For *Strep. durans* this pH is obviously near the critical limits for growth. Shattock & Hirsch (1947) found that nine out of eleven strains grew at pH 9.6. In repeated tests two of our six strains grew

Table 1. *Ability of Streptococcus species to grow in a medium at pH 9.6*

		No. of strains	
		Growing at pH 9.6	Not growing at pH 9.6
Group D	<i>Strep. faecalis</i>	14	0
	<i>Strep. liquefaciens</i>	7	0
	<i>Strep. zymogenes</i>	3	0
	<i>Strep. durans</i>	2	4
	<i>Strep. bovis</i>	0	6
	Unclassified	2	2
Group N	<i>Strep. lactis</i>	0	14
Groups A, B, C, E, G, M	—	0	28
—	<i>Aerococcus viridans</i>	10	0

and produced acid in 24 hr. at pH 9.6, three grew and produced acid in 48 hr. but not in 24 hr., and one strain failed to grow in 3 days. All ten *Aerococcus viridans* strains grew at pH 9.6.

These results agree with those obtained by Shattock & Hirsch. The simplified method of preparation of this medium may contribute to the more general use of this test for growth at pH 9.6.

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(Received 18 May 1953)