

Interrelationships Between Viridans Streptococci and Veillonellae Isolated from Dental Abscesses

by

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ABSTRACT

Strains of viridans streptococci which had been isolated concurrently with veillonellae from dental abscesses produced a higher pH and lower Eh when grown in broth than did strains isolated alone. Veillonellae grew well in mixed batch culture with the former strains, producing a high pH and very low Eh whereas they grew poorly alone. It is suggested that some strains of viridans streptococci might similarly facilitate the growth of veillonellae in dental abscesses.

Viridans streptococci and veillonellae have been not infrequently, isolated together from dental abscesses, Feldman and Large (1966), Moore and Russell (1972). These organisms are members of the normal human oral flora and have a complex interrelationship. Streptococci produce lactic acid from carbohydrates thus providing an energy source for veillonellae which cannot utilise carbohydrates, Rogosa, Krichevsky and Bishop (1965). The latter, however cannot grow below a PH of 5.5 — 6.0.

It appears, therefore, that veillonellae although able to utilise an end product of streptococcal metabolism, may not be able to grow in the presence of streptococci due to the accompanying rapid fall in PH. Not all streptococci, however, cause an equally rapid fall in PH and Coulter and Russell (1974) have shown that trace elements like Molybdenum can encourage the growth of some viridans streptococci without simultaneously increasing greatly, the production of lactic acid, Sanyal (1969) has shown that some streptococci rapidly reduce the Eh of an aerated medium to a level which would allow obligatory anaerobes to grow.

It would seem, therefore, that the metabolism of streptococci produce two effects (a) production of Lactic Acid, (b) lowering of electrode potential. Both factors would help the growth of veillonellae. In the case of some strains, the PH is lowered to a level inhibitory to veillonellae. In others however this is not so. In the latter case the growth of veillonellae would remove the lactic acid thus keeping up the PH and enabling streptococci to grow. This mutual enhancement of growth would create the conditions for a true mixed infection and would, perhaps, reflect the conditions that operate in a mixed dental abscess.

Accordingly, in the present study attempts were made to grow *V. parvula* in mixed batch culture with *Strep mitior* or *Strep sanguis* which are common in abscesses and to determine changes in PH and Eh during growth.

METHODS AND MATERIALS :

The organisms were isolated from dental abscesses as described earlier Wickremasinghe and Russell (1976). Four isolates of viridans streptococci and four of veillonellae were obtained from four abscesses each of which contained both organisms. Four further isolates of viridans streptococci were obtained from four abscesses which only contained streptococci. The streptococci were differentiated into species and types by the method of Carlsson (1967) and the veillonellae by their action on hydrogen peroxide Rogosa (1964). Cultures of veillonellae were maintained anaerobically on V15 lactate agar, Rogosa (1956) modified by omitting basic fuchsin and antibiotic. The streptococci were maintained on blood agar plates.

The following media were used:

Blood agar ; brain heart infusion (BHI) (oxid) ; BHI + 1% filter sterilized sucrose ; BHI + 1.2% filter sterilized sodium lactate: V15 lactate agar V17 lactate broth Rogosa (1964).

Measurement of PH and Eh

PH and Eh were measured on a pyc meter using a standard dual glass - reference electrode and an inert platinum electrode (EIL). The observed reading of potential was converted to Eh by the formula $Eh = E_{obs} + E_{ref}$. The Eh system was standardised by the use of saturated quinhydrone solution. E_{ref} . The Eh of the reference electrode was determined from tables, Clark (1928).

Eh of streptococcal cultures:

The eight strains of streptococci were grown on blood agar plates for 24 hrs. at 37° C. Sub-cultures were made into Brain Heart infusion broth (oxid) (BHI) in universal bottles incubated for 10 hrs. at 37° C and then kept overnight at 4° C. One set of 16 bottles was filled with BHI and another 16 with BHI + 1% sucrose; a further set of 8 bottles were filled with BHI + 1.2% sodium lactate. The initial PH and Eh of each broth were noted. One drop of one culture, pre-warmed to room temperature, was then added to each of two bottles of BHI + sucrose. The bottles of BHI + lactate were similarly inoculated with the four cultures of streptococci isolated jointly with veillonellae. All 40 bottles were incubated at 37° C, PH and Eh readings were recorded at 12 hrs. on one of each pair of cultures and at 24 hrs. on the other. Subcultures were made at these times to check for purity.

Eh of veillonella cultures :

The four veillonella cultures were grown on lactate agar for 48 hrs. at 37° C. They were then sub-cultured into V17 lactate broth incubated for 24 hrs. at 37° C then kept overnight at 4° C. One set of 8 bottles was filled with BHI and another set with BHI + lactate. The initial PH and Eh were noted. The cultures were brought to room temperature and two bottles of each medium were inoculated with one drop of a culture. PH and Eh were read at 12 hrs. in one set and at 24 hrs. in the duplicate, sub-cultures being made on each occasion.

Eh in mixed cultures:

The cultures described above, derived from streptococci and veillonellae isolated originally in pairs from a single abscess, were mixed as appropriate in equal proportions. One set of bottles of BHI and another set containing BHI + lactate were inoculated with the mixtures. Measurements of PH and Eh were made as for single cultures.

RESULTS:

The organisms isolated were coded and identified as follows :-

Streptococcus isolated alone:

Strain	177 —Strep.	sanguis	type	'b'
Strain	275 —Strep.	sanguis	type	'c'
Strain	220 —Strep.	mitior	type	'd'
Strain	274 —Strep.	mitior	type	'f'

Streptococcus and Veillonella isolated together:

276	—Strep.	sanguis	type	'b'	V. alcalescens
73/249	—Strep.	mitior	type	'a'	V. alcalescens
321	—Strep.	mitior	type	'h'	V. parvula
370a	—Strep.	mitior	(unclassifiable),		V. parvula.

TABLE 1.

pH and Eh in cultures of Streptococci in BHI

Average of two experiments

Initial pH = 7.2; initial Eh = 386 mv.

	Culture No.	pH	12hrs Eh, mv	PH	24hrs Eh, mv
Isolated singly	San 177	6.0	438	5.9	467
	San 275	5.9	431	5.8	466
	Mit 220	5.9	455	5.9	471
	Mit 274	5.8	449	5.8	459
Isolated with veillonella	San 276	6.3	446	6.1	459
	Mit 73/249	7.1	384	6.9	391
	Mit 321	7.0	364	6.7	398
	Mit 370a	7.1	418	5.8	440

San = Strep. sanguis

Mit = Strep. mitior

TABLE 2.

pH and Eh of cultures of
streptococci in BHI + sucrose

Average of two experiments
Initial pH = 7.2; initial Eh = 386 mv.

Culture No.		12hrs		24hrs	
		pH	Eh, mv	pH	Eh, mv
Isolated singly	San 177	5.4	487	4.4	519
	San 275	4.3	496	4.4	543
	Mit 220	6.2	433	4.4	512
	Mit 274	6.3	452	4.2	540
Isolated with veillonella	San 276	6.5	442	4.6	509
	Mit 73/249	7.1	387	6.7	362
	Mit 321	7.1	373	6.8	390
	Mit 370a	7.1	422	4.4	483

The effect of streptococci on pH and Eh of BHI and BHI + sucrose is shown in Tables 1 and 2 respectively. It may be seen that in both media those strains isolated with veillonellae were associated with a slight fall or only a modest rise in Eh after 12 hrs. whereas those isolated alone showed a substantial rise. Even after 24 hrs. in general a lower rise in Eh was found with the former strains than the latter. Streptococci isolated together with veillonellae caused no appreciable fall in pH in either medium; those isolated alone caused a rapid fall. Even after 24 hrs. pH remained comparatively high in cultures of the former isolates in both media.

Cultures of streptococci originally isolated together with veillonellae gave the results in Table 3, when grown in BHI + lactate. After 12 hrs. pH remained high and Eh fell appreciably but by 24 hrs. the situation was reversed.

TABLE 3.

pH and Eh of cultures of streptococci in BHI + lactate

Average of two experiments

Initial pH = 7.1; initial Eh = 192 mv.

Culture No.		12hrs		24hrs	
		pH	Eh, mv	pH	Eh, mv
Isolated with veillonella	San 276	6.9	113	5.7	293
	Mit 73/249	7.1	-8.1	6.1	286
	Mit 321	7.0	6.9	6.8	305
	Mit 370a	7.0	21	5.5	258

When the strains of streptococci and veillonellae isolated together were inoculated together into BHI + lactate the results shown in Table 4 were obtained. In contrast to the situation when the streptococci were grown alone, mixed growth caused a rapid fall in Eh by 12 hrs. which was maintained at 24 hrs. pH remained high in all cases. Veillonellae alone grow poorly or not at all in BHI and BHI + lactate, but grew well in mixed culture.

TABLE 4.

pH and Eh of cultures of streptococci and veillonellae grown together in BHI + lactate

Average of two experiments

Initial pH = 7.1; initial Eh = 192 mv

Culture No.*	12hrs		24hrs	
	pH	Eh, mv	pH	Eh, mv
San/alc 276	7.1	-68	5.9	-135
Mit/alc 73/249	6.9	-128	6.4	-297
Mit/parv 321	6.7	-274	6.0	-240
Mit/parv 370a	7.1	-143	6.0	-137

* Code no. of both streptococcus and veillonella.

alc = *V. alcalescens*; parv = *V. parvula*.

DISCUSSION:

Strep. mutans, *Strep. salivarius* and Group IV viridans streptococci are known to produce a lower terminal pH in broth than *Strep. mitior* or *Strep. sanguis* Carlsson (1967). Parker & Snyder (1961) and Coulter & Russell (1974) found that veillonellae could not grow in mixed batch culture with either *Strep. salivarius* or *Strep. mutans*. Subsequently, the latter authors (1975) showed that *V. parvula* could grow in continuous culture with *Strep. sanguis* provided *Corynebacterium hofmanni* was also present the medium then having a low Eh and high pH. These conditions did not obtain in the absence of the corynebacterium. In the present work those streptococci isolated together with veillonellae produced a higher pH than other streptococci when grown in BHI or BHI + sucrose while Eh was lower. In some cases after 24 hrs. Eh was lower than in the uninoculated medium. When these strains of streptococci were grown in mixed culture with veillonellae in BHI + lactate there was a marked fall in Eh and pH did not drop below 5.6. It is also significant that the veillonellae grew poorly or not at all when inoculated alone into BHI or BHI + lactate.

It appears therefore that some strains of *Strep. mitior* and *Strep. sanguis* can produce a sufficiently low Eh and high PH in batch culture to permit veillonellae to grow concurrently. If these conditions are similar to these in dental abscesses it could account for the co-existence of these organisms in such lesions.

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