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Bacteriocin-like activity of group B and group C streptococci of human and of animal origin

By CHRISTINE R. SCHOFIELD and J. R. TAGG
Department of Microbiology, University of Otago, Dunedin, New Zealand

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SUMMARY

An inhibitor 'fingerprinting' technique was used to test 120 group B and 50 group C streptococcus strains for production of inhibitory activity. The incidence of inhibitor production was low. Five of 70 group B streptococci of animal origin and one of 50 from human sources consistently produced inhibitory activity. Six of 20 animal strains and three of 30 human strains of group C streptococci were inhibitor producers. These included two *Streptococcus dysgalactiae*, three *S. equisimilis* and four *S. zooepidemicus*.

The temperature of incubation and species of origin of the blood used in the culture medium were critical determinants of inhibitor production. Investigation of inhibitors for their spectrum of activity, heat stability, sensitivity to trypsin, dialysability and mode of action indicated that five of the group B and seven of the group C streptococci produced inhibitors that had bacteriocin-line properties. Three bovine strains of group B streptococci produced very similar inhibitory activity. A greater variety of bacteriocin-like substances was detected in the group C streptococcus strains. Two of the *S. zooepidemicus* strains were each found to produce more than one inhibitory substance.

INTRODUCTION

Unlike the group A streptococci which appear to have adopted humans almost exclusively as their natural host, streptococci bearing the Lancefield group antigens B or C seem to have successfully adapted to a variety of animal hosts in addition to man (Wilson & Salt, 1978).

Group B streptococci were first recognized because of their association with bovine mastitis, as befits their species designation *Streptococcus agalactiae* (Jelinkova, 1977). However, group B streptococci are also commonly found as residents of the indigenous microbiota of humans, occupying both urinogenital and pharyngeal sites in healthy individuals (Ross, 1978).

Recently, in common with other 'commensal' organisms, group B streptococci have been acting out their innate pathogenic potential in susceptible human hosts with what appears to be an increased frequency and severity; the reasons for this are obscure. This trend has been particularly evident in their association with acute neonatal infections (Parker, 1977; Wilkinson, 1978; Baker, 1980).

One issue that does not yet seem to have been satisfactorily resolved is the nature of the relationship that exists between human and bovine strains of *S. agalactiae*

and the extent to which cross-infection between the two hosts can occur in nature. Some reports have suggested that it is possible to distinguish human and bovine strains on the basis of metabolic and serological differences (Butter & de Moor, 1967; El Ghoroury, 1950*a*, *b*). However, many investigators seem to remain unconvinced that a clear-cut distinction exists (Jelinkova, 1977). In one study, no difference could be found in experimental udder infectivity with either human or bovine strains (Haug, Bakken & Berdal, 1979).

The basis of the typing of group B streptococci has been serological (Jelinkova, 1977) although a phage typing scheme has recently been introduced (Haug, Gudding & Bakken, 1981; Stringer, 1980). Neither of these methods allows the unequivocal discrimination between bovine and human strains. Bacteriocin sensitivity(S)-typing has shown that human strains are generally S-type 120 whereas bovine isolates usually belong to a variety of other S-types (Tagg & Martin, 1980).

The group C streptococci comprise four species (*S. equi*, *S. dysgalactiae*, *S. equisimilis* and *S. zooepidemicus*) that can be differentiated on the basis of physiological tests. Only *S. equi*, the etiological agent of 'strangles', seems highly host specific and in many respects this species appears to be for the *Equidae* the equivalent of what *S. pyogenes* is for humans (Bryans & Moore, 1972). *S. dysgalactiae* is an important cause of mastitis in cattle (Higgs, Neave & Bramley, 1980). *S. equisimilis* is by far the most commonly isolated group C streptococcus from humans (Stamm & Cobbs, 1980) but it has also been isolated in association with a variety of infections in different animals (Wilson & Salt, 1978). *S. zooepidemicus* is a frequent agent of serious epidemic disease in animals (Wilson & Salt, 1978) and is occasionally isolated from humans. The involvement of *S. zooepidemicus* in a milk-borne epidemic of pharyngitis associated with a high incidence of glomerulonephritis is of particular interest (Duca *et al.* 1969).

There have been very few studies of inhibitor production by group B streptococci (Kramer & Brandis, 1972; Tagg, Dajani & Wannamaker, 1975; Tagg & Bannister, 1979; Tzannetis, Poulaki-Tsontou & Papavassiliou, 1974) and even less is known about the extent and nature of inhibitory activity in the group C streptococci (Tagg & Bannister, 1979). In the present study we have used an inhibitor 'fingerprinting' scheme to examine a collection of group B and group C streptococci to determine whether any significant differences exist in either the incidence or type of bacteriocin-like inhibitors produced by strains of human and animal origin.

MATERIALS AND METHODS

Bacterial strains

The nine standard indicator strains (I1–I9) used for producer(P)-typing and the six standard producer strains (P1–P6) used for S-typing have been described previously (Tagg & Bannister, 1979).

Group B streptococcus strain P3 was used in another study (Tagg, Dajani & Wannamaker, 1975). The other group B streptococcus isolates were obtained by courtesy of Dr D. Martin, National Health Institute, Wellington. These included 49 human isolates, obtained from 21 patients. Some of them were sequential isolates from the same site or were paired isolates from vaginal and rectal swabs.

Of the animal strains, 62 were from the milk of infected cows. In addition, there were isolates from six pigs, a dog and a goat. All of the human strains, twenty-seven of the bovine strains and all but one of the porcine strains were used in preliminary study (Tagg & Martin, 1980). In that study the five porcine strains were inadvertently referred to as bovine in origin.

The 20 animal strains of group C streptococci comprising 8 *S. zooepidemicus* and 4 each of *S. equi*, *S. dysgalactiae* and *S. equisimilis*, were isolated from a variety of infections in horses (13 strains), pigs (4 strains), dogs (2 strains) and a cow (1 strain). They were provided by the Animal Health Laboratory, Lincoln College. Group C streptococcus strain T277 had been reported to be causally related to the development of nephritis in the course of an outbreak of *S. zooepidemicus* infection in humans (Duca *et al.* 1969). This strain and 12 human *S. equisimilis* isolates from throat cultures were kindly supplied by Dr L. W. Wannamaker, University of Minnesota. Another 17 human *S. equisimilis* isolates were obtained from clinical specimens at the Dunedin Public Hospital. The bacterial strains used in the spectrum study were obtained from the culture collection of the Microbiology Department, University of Otago.

Serological typing of the group B streptococci was performed using gel precipitation tests (Wilkinson & Moody, 1969) with antisera specific for types Ia, Ib, Ic, II and III. The group C streptococci were classified into species on the basis of their fermentation patterns in 5-ml volumes of Phenol Red Broth Base (BBL), supplemented with 0·1 ml of filter-sterilized horse serum and containing 1 % (w/v) of carbohydrate (lactose, sorbitol or trehalose) (Wilson & Salt, 1978).

All of the bacterial strains in regular use were stored at 4 °C on blood agar. Stock cultures of the standard indicator and producer strains and of the group B and C inhibitor producers were stored at −70° and in the lyophylized state.

Inhibitor 'fingerprinting'

The basic 'fingerprinting' procedure has been described in detail previously (Tagg & Bannister, 1979). For P-typing, the test strain is grown as a diametric streak culture on human blood agar at 32 °C for 18 h before removing the growth, sterilizing the surface with chloroform and then cross-inoculating the nine standard indicator cultures. Six standard producers (P1–P6) were used for S-typing. Producers P1–P5 were grown as streak cultures for 24 h at 32 °C, whereas P6 was incubated at 37 °C, before scraping, chloroforming and cross-inoculating the test strains. In some experiments the composition of the medium and the test conditions for P-typing were varied to determine the effect on inhibitor production. All nutrient bacteriological media was obtained from Difco Laboratories. Davis agar (1·5 % (w/v)) (Davis Gelatine Ltd., New Zealand) was used to solidify liquid nutrient media. Ovine and equine blood was obtained from Laboratory Services Ltd., Auckland; human blood was from healthy donors and bovine blood was provided by Mr S. Dobinson, Veterinary Surgeon, Dunedin.

Properties of the inhibitors

An estimate of the size of inhibitory substances was obtained by assessing the ability of inhibitor molecules to pass through dialysis membranes of M.W. cutoffs 3500 and 12000 (A.H. Thomas Co. Phil. USA) placed between the surface of the

blood agar medium and 0·1 ml drops of the same medium that had been inoculated with the test strain (Tagg & Bannister, 1979). A similar (control) drop was placed directly on the surface of the blood agar medium. After incubation at 32 °C for 18 h., the dialysis sheet and agar drop cultures were removed, the surface of the medium was exposed to chloroform vapor and an appropriate susceptable indicator strain was seeded as a lawn culture.

The heat stability of the inhibitor within blood-agar medium was tested by heating the medium at 80 °C for 45 min in the course of testing the strains by the P-typing procedure. This step was inserted after the removal of producer strain growth and prior to exposure to chloroform.

Trypsin sensitivity was tested at the stage of P-typing following exposure to chloroform. After removing the producer growth, three (5 mm diam) test wells were cut in the agar medium, evenly spaced along the line of the original producer strain growth. Another three wells (controls) were cut into the adjacent uninoculated portion of the medium in a line parallel to that of the first wells. After sealing the base of the wells with a drop of molten agar, 0·1 ml of 2 mg/ml trypsin (1:250, Difco) in 0·2 m TES buffer (Sigma/pH 7·5) was placed in each well and incubated at 37 °C for 2 h. Lawn cultures of three indicator strains were then seeded, each indicator being swabbed so as to cover the vicinity of one test and one control well. Inhibitor inactivation was disclosed by the appearance of a concentric ring of indicator growth bordering the test well and within the zone of inhibited growth.

A preliminary test was conducted to determine whether the inhibitors had a bactericidal or bacteriostatic mode of action. A sterile wire loop was used to sample the surface of the medium within the region of inhibited indicator growth, at the stage of completion of P-typing the inhibitory strains. Care was taken to avoid touching any macroscopically-visible indicator growth. The charged loop was then rubbed onto the surface of blood agar medium which was then incubated. Growth of the indicator strain was taken to be presumptive evidence of a bacteriostatic mode of action. Zero indicator growth suggested a bactericidal effect.

For all investigations of the properties of the inhibitors indicators were selected from I1, I2, I6 and I7, depending upon their susceptibility to the particular inhibitor.

RESULTS

Only six of 120 group B and nine of 50 group C strains were found consistently to produce inhibitory activity against one or more of the nine standard indicator bacteria using the 'fingerprinting' procedure (Table 1). S-typing of the human group B streptococci gave type 120 to 44 of the 50 tested strains (Tagg & Martin, 1980). Seven of the eight non-bovine animal group B strains were S-type 120 and one was S-type 220. By contrast, only three of 62 bovine strains were S-type 120. The other bovine strains and the group C streptococci gave a wide variety of sensitivity patterns (data not shown). The S-type patterns of the 15 producer stains appeared to occur independently of the P-type patterns of these strains (Table 1).

The inhibitory group C streptococci belonged to three of the four known species carrying group C antigen (Table 2). All four inhibitory *S. zooepidemicus* strains were of the lactose positive subtype. Inhibitor production seemed more commonly associated with animal strains. Six of 20 group C and five of 70 group B strains of

Table 1. *Inhibitor 'fingerprinting' of streptococcus strains*

Strain no.	Production (P)-type pattern: Inhibition* of indicator									Sensitivity (S)-type pattern: Inhibition* of test strain by producer strain					
	I1	I2	I3	I4	I5	I6	I7	I8	I9	P1	P2	P3	P4	P5	P6
Group C *streptococcus*															
T1	+	+	−	−	+	−	+	(+)	−	−	+	+	+	+	+
3617	−	−	−	−	−	−	+	−	−	+	+	(+)	+	+	−
T145	+	+	−	−	(+)	+	+	(+)	−	+	+	+	+	+	+
T147	−	(+)	−	−	−	−	(+)	−	−	+	+	+	−	+	(−)
4003	+	+	−	+	(−)	+	+	(+)	+	(+)	+	+	+	+	(+)
T277	+	−	−	−	−	−	+	+	+	−	−	−	−	+	−
2681	+	−	−	(+)	+	(+)	+	+	(+)	−	−	−	−	+	−
4881	−	+	−	−	+	−	+	+	−	−	−	+	−	+	−
3151	+	(+)	−	−	−	+	(+)	−	−	−	−	−	−	+	−
Group B *streptococcus*															
P3	+	+	(+)	−	+	+	+	+	−	−	−	−	−	+	−
120	+	(+)	−	+	−	+	(+)	−	(+)	−	+	+	−	+	−
121	+	+	−	(+)	−	(−)	+	−	(+)	−	+	−	−	+	−
134	−	−	−	−	−	−	+	−	−	+	(+)	+	−	+	−
176	+	(+)	−	+	−	(−)	+	−	(+)	−	+	−	−	+	−
194	−	−	−	−	−	−	+	−	−	−	+	+	−	+	+

* +, present; −, absent; ( ), weak activity may sometimes occur.

Table 2. *Specifications of inhibitor-producing streptococcus strains*

Strain no.	Species designation	Source
Group C streptococcus		
T1	*S. dysgalactiae*	Mastitis – bovine
3617	*S. dysgalactiae*	Mastitis – bovine
T145	*S. equisimilis*	Pharyngitis – human
T147	*S. equisimilis*	Pharyngitis – human
4003	*S. equisimilis*	Abortion – equine
T277	*S. zooepidemicus*	Nephritis – human
2681	*S. zooepidemicus*	Abortion – equine
4881	*S. zooepidemicus*	Abortion – equine
3151	*S. zooepidemicus*	Cervical swab – equine
Group B streptococcus		
P3	*S. agalactiae* type II	Pharyngitis – human
120	*S. agalactiae* nontypable	Mastitis – bovine
121	*S. agalactiae* type II	Mastitis – bovine
134	*S. agalactiae* nontypable	Mastitis – bovine
176	*S. agalactiae* nontypable	Mastitis – bovine
194	*S. agalactiae* nontypable	Mastitis – bovine

animal origin were inhibitor producers whereas only three of 30 group C and one of 50 group B strains from humans were inhibitory.

The spectrum of inhibitory activity of the producers against a variety of bacterial strains was examined (Table 3). None showed activity against Gram-negative bacteria. *S. zooepidemicus* strains T277 and 2681 gave similar spectra. *S. zooepidemicus* strain 4881 was widely active but was particularly notable for its strong inhibition of all the tested strains of *S. mutans* and *S. sanguis*. Three of the group B strains (120, 121 and 176) gave very similar inhibitory spectra. The human strain P3 was the most widely inhibitory of all the producers tested in this study.

Inhibitor production was tested under a variety of incubation conditions (Table 4). For group C strains production was generally poor on blood-free media and with the exception of strains 4881 and 4003 it seemed that the species of origin of the blood was an important determinant of inhibitor production. Three of the strains (3617, T147 and 3151), representing three of the group C species failed to produce inhibitors when blood was absent from the test medium. For *S. zooepidemicus* strains T277 and 2681 inhibitor production appeared to be enhanced by sheep and suppressed by equine blood. *S. zooepidemicus* strain 3151 seemed to produce two inhibitors, one of which was increased and the other decreased by bovine blood. For the group B streptococci, there appeared to be blood dependency for inhibitor production only in strains 194 and 134.

The temperature of incubation was also found to be an important determinant of inhibitory activity. Inhibitor production by the group C producers was more commonly favoured by elevated temperature whereas the group B strains generally produced better at a lower temperature of incubation.

Other factors to be examined included the period of incubation and the effect of incubation under anaerobic conditions. Strains P3, T1, 4003, T277 and 3151 gave good yields of inhibitor within 12 h of incubation, whereas release of inhibitor from other strains seemed to continue up until 18–24 h of incubation. All of the strains appeared to give at least comparable inhibitor production when incubated

Table 3. *Range of activity of inhibitory streptococci*

Indicator strain	No. of strains sensitive to producer strains‡														
	T1	3617	T145	T147	4003	T277	2681	4881	3151	P3	120	121	134	176	194
Streptococcus															
Group A (10)*	9	2	3	2	10	8	8	10	2	10	7	5	1	6	3
Group B (10)	0	0	0	0	5	3	2	7	0	8	2	2	0	2	0
Group C (10)	0	0	0	1	10	6	7	8	0	7	4	2	0	2	0
Group D (10)	0	0	1	0	0	1	1	1	0	3	0	0	0	0	0
Group E (3)	0	0	0	0	3	3	3	0	0	2	3	3	0	3	0
Group F (2)	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Group G (10)	1	0	1	1	10	9	9	7	0	3	10	9	0	9	0
S. salivarius (5)	1	0	1	1	3	4	4	1	0	5	1	0	0	0	0
S. mutans (5)	0	0	0	0	1	0	0	5	0	1	0	0	0	0	0
S. sanguis (3)	1	0	1	1	0	1	1	3	0	3	0	0	0	0	0
S. pneumoniae (1)	0	1	1	1	1	1	1	0	0	1	1	1	0	1	1
Lactobacillus sp. (2)	0	0	0	0	1	2	2	1	0	2	0	0	0	0	0
Actinomyces viscosus (4)	1	0	1	0	1	1	1	0	1	1	2	2	0	2	0
Staphylococcus aureus (4)	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Corynebacterium sp. (4)	0	0	0	0	2	1	1	1	0	2	1	0	0	0	0
Bacillus sp. (4)	1	1	0	0	1	3	3	0	0	3	1	1	0	1	0
Micrococcus luteus (1)	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Gram negatives† (10)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Number of strains tested is given in parenthesis.

† Including strains of *Escherichia* (3), *Pseudomonas* (2), *Salmonella* (2), *Proteus* (1), *Enterobacter* (1), and *Serratia* (1).

‡ The producer strains were incubated using the standard P-typing conditions.

Table 4. *The effect of cultural conditions on inhibitory activity*

	Amount of inhibition* by producer strain when standard procedure modified								
	Blood supplement to CBAB				Blood free media‡			Incubation at	
Strain no.	Bovine	Equine	Ovine	Nil	TSA	THA	BHIA	25 °C	35 °C
Group C streptococcus									
T1	++	++	+	−	+	−	−	+	+++
3617	+++	+	+++	−	−	−	−	−	+++
T145	+	+	+++	−	−	+	−	+	+++
T147	+++	++	++	−	−	−	−	++	+++
4003	++	++	++	++	−	+	+	+	+
T277	++	−	+++	+	+	+	++	−	+++
2681	++	−	+++	−	−	−	+	++	+++
4881	++	++	++	+	+	++	++	++	++
3151	++†	++	++	−	−	−	−	+	+++
Group B streptococcus									
P3	+	+	++	−	+	+	++	+	+++
120	++	++	+++	+	++	++	++	+++	+
121	++	++	+++	−	++	++	++	+++	+
134	+++	++	++	−	−	−	−	+++	++
176	++	++	+++	−	++	++	++	+++	+
194	+	+	++	−	−	−	−	+++	++

* The amount of inhibitory activity is qualitatively estimated relative to the amount of activity (++) observed under standard conditions; +++, increased activity (wider zones or increased spectrum); +, reduced activity; −, no activity.

† Activity against I2 and I7 increased; activity against I1 and I6 decreased.

‡ TSA, tryptic soy agar; THA, Todd-Hewitt agar; BHIA, Brain heart infusion agar.

Table 5. *Properties* of inhibitors*

Stain no.	Molecular weight	Inactivation by Trypsin	Inactivation by Heating	Mode of action
Group C streptococcus				
T1	> 12000	Yes	No	Bacteriocidal
3617	< 3500	No	No	Bacteriostatic
T145	> 12000	Yes	No	Bacteriostatic
T147	< 3500	No	No	Bacteriostatic
4003	> 3500, < 12000	Yes	No	Bacteriocidal
T277	> 3500, < 12000	Yes	No	Bacteriocidal
2681	> 3500, < 12000	Yes	No	Bacteriocidal
4881†	> 12000	Yes	No	Bacteriocidal
3151‡	a > 12000	Yes	Yes	Bacteriocidal
	b < 3500	No	No	Bacteriostatic
Group B streptococcus				
P3	> 12000	Yes	No	Bacteriocidal
120	> 3500, < 12000	Yes	No	Bacteriocidal
121	> 3500, < 12000	Yes	No	Bacteriocidal
134	< 3500	No	No	Bacteriostatic
176	> 3500, < 12000	Yes	No	Bacteriocidal
194	> 3500, < 12000	Yes	No	Bacteriocidal

* Tested as produced in human blood agar at the optimal temperature of incubation for inhibitor production by each producer.

† This strain also produced a heat labile, trypsin sensitive bacteriostatic inhibitor of *S. mutans* with apparent molecular weight > 12000.

‡ This strain produced two inhibitors; a was active on I1 and I6, b was active on I2 and I7.

anaerobically. For group B strains 120, 121 and 176 production was substantially enhanced by anaerobic incubation.

Crude preparations of inhibitors produced by group B strains P3, 120, 121 and 176 and group C strains 4003, T277, 2681 and 4881 could be obtained by collecting the eluants following freezing and thawing of lawn cultures of producer strains that had been grown under conditions optimal for inhibitor production. Crude inhibitor from group B strain 194 could be recovered from the supernatants of tryptic soy broth cultures. Inhibitor-containing extracts could not be obtained from any of the other strains either by elution from agar cultures or from cultures grown in Todd Hewitt broth, tryptic soy broth or brain heart infusion.

The properties of the inhibitory substances as produced in blood agar medium are reported in Table 5. As was indicated above, strain 3151 appeared to produce two inhibitors. These are referred to as a and b. Inhibitor a was nondialysable; trypsin and heat sensitive and was bactericidal for indicators I1 and I6. Inhibitor b had a low molecular weight, was stable to trypsin and heat and was bacteriostatic for indicators I2 and I7. The production of inhibitor b had been shown (above) to be enhanced by bovine blood. Two other group C strains (3617 and T147) and one group B (strain 134) also appeared to produce inhibitors with properties similar to inhibitor b. When calcium carbonate (1 % w/v) was incorporated within the test medium, production of this inhibitory activity was prevented. Addition of glucose (0·5 % w/v) enhanced activity.

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Strain 4881 also seemed to produce at least two distinguishable inhibitors. In addition to producing a high molecular weight, trypsin sensitive, heat stable, bacteriocidal inhibitor of group A streptococcus strains I2 and I7, this strain was also found to produce a heat labile, trypsin sensitive, bacteriostatic inhibitor active against *S. mutans*.

DISCUSSION

In the present study, application of an inhibitor 'fingerprinting' technique indicated that 5% of 120 group B and 18% of 50 group C streptococci were inhibitor producers. The apparent incidence of inhibitory organisms detected in any such study is necessarily dependent upon both the choice of incubation conditions and the selection of indicator strains. In this study we elected to use for screening purposes a set of nine indicator bacteria and particular incubation conditions that had previously been found to be appropriate for the detection and differentiation between a wide range of bacteriocin-like inhibitors produced by beta-haemolytic streptococci (Tagg & Bannister, 1979). Having identified inhibitory strains by use of these 'standard' conditions, the effect on production of variations in incubation conditions was evaluated prior to attempting the preliminary characterization of the inhibitory substances.

The low apparent incidence of inhibitory group B streptococci means that the present P-typing scheme is unlikely to be of epidemiological value as a strain marker within this serogroup. On the other hand, S-typing has been shown to offer considerable potential both for the differentiation between strains of human and bovine origin and for the labelling of bovine isolates (Tagg & Martin, 1980). It seems that there is a high correlation between S-type 120 and strains of human origin. Group B isolates from animals other than cows were also generally S-type 120, perhaps indicating that these could have been acquired by the animals from human sources. A similar suggestion was made by Butter & de Moor (1967), who, on the basis of biotyping, found that 'human varieties' of group B streptococci were predominantly isolated from non-bovine animal sources.

There is very little information available regarding the characterization of bacteriocin-like substances produced by *S. agalactiae* (Kramer & Brandis, 1972; Tagg, Dajani & Wannamaker, 1975). The properties of the bacteriocin produced by strain P3 have been recorded previously (Tagg, Dajani & Wannamake, 1975; Tagg & Bannister, 1979) and the strain was included in the present study only for purposes of comparison with newly identified inhibitors.

Three bovine isolates (strains 120, 121, 176) seemed to produce very similar inhibitors, on the basis of studies of their inhibitory spectra, production conditions and physiochemical properties. Inhibitor production by these strains was markedly enhanced by anaerobic incubation, under which conditions the inhibitory activity (P-type) was similar to that produced by M-type 4 T-pattern 4 group A streptococcus strains (Johnson, Tagg & Wannamaker, 1969). None of these group B strains had detectable M-type 4 protein (Tagg, unpublished results).

Group B streptococcus strain 194 produced small amounts of an inhibitor which had the bacteriocin-like properties of high molecular weight (> 3500), trypsin sensitivity and narrow spectrum of bactericidal activity. By contrast, the inhibitor produced by strain 134 was dialysable, heat stable, trypsin resistant and bacter-

iostatic in action. These properties are more consistent with those expected of a low molecular weight metabolite. Elimination of the inhibitory effect on media buffered with calcium carbonate and increased inhibition on glucose supplemented media indicates that inhibitory levels of lactic acid may have been produced, as has been described in oral *Actinomyces* strains (Tompkins & Tagg, 1981). Alternatively, it is possible that the inhibitor could be acid-dependent for its production and that it is a low molecular weight compound similar to the peptide antibiotics described for staphylococci and some other gram-positive bacteria (Tagg, Dajani & Wannamaker, 1976; Hsu & Wiseman, 1971; Noble, Lloyd & Appiah, 1980).

The incidence of inhibitory group C streptococci was higher than that found with group B strains and as with the group B streptococci, inhibitor production seemed more common among animal strains. *S. dysgalactiae* strain T1 produced an inhibitor which had a typical bacteriocin-like profile of properties. By contrast, *S. dysgalactiae* strain 3617 and also *S. equisimilis* strain T147 seemed to produce inhibitory effects similar to that detected in group B strain 134. *S. equisimilis* strains T145 and 4003 both produced non-dialysable, trypsin-sensitive inhibitors. The inhibitor from strain T145 was a little unusual in that it appeared to be bacteriostatic in action under the test conditions.

S. zooepidemicus strains T277 (human) and 2681 (equine) had similar inhibitory activity, the production of which was enhanced by the presence of sheep blood and eliminated by equine blood. The production of certain inhibitors by group A streptococcus strains has also been reported to be strongly influenced by the species of origin of the blood added to the test medium (Tagg & Bannister, 1979).

The other two inhibitory *S. zooepidemicus* strains each appeared to be producing more than a single inhibitory substance. Strain 4881 produced one inhibitor which was bacteriocidal for group A streptococcus indicator strains I2, I5, I7, I8 and another substance which was bacteriostatic for all of five tested strains of *S. mutans*. Inhibitory activity by a haemolytic streptococcus directed against *S. mutans* strains is most unusual. *S. zooepidemicus* strain 3151 seemed to produce both an acid-type effect against strains I2 and I7 and a bacteriocin-like substance active against strains I1 and I6.

It is possible that some of the other bacteriocin-like effects described in this study may also represent the combined action of several different inhibitory substances. More complete analysis will be dependent upon the successful recovery of the active principles in soluble form as a prelude to purification and characterization.

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