A STUDY OF NON-MOTILE AND MOTILE STRAINS OF GROUP D STREPTOCOCCI: BIOCHEMICAL TESTS FOR CHARACTERIZATION OF SPECIES

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SUMMARY

Using resistance to tellurite as the main characteristic for differentiation, 66 strains of enterococci of animal or bird origin were named as follows: *Streptococcus faecalis* (13 from fowls, 2 from pigs and 5 from humans); *Str. faecalis* var. *zymogenes* (2 from fowls, 1 from cattle and 1 from a turkey); *Str. faecalis* var. *liquefaciens* (4 from fowls, 1 from cattle, 1 from humans and 1 from goats); and *Str. faecium* (13 from fowls, 13 from pigs, 3 from cattle, 2 from humans, 2 from sheep and 1 each from a turkey and a budgerigar).

Reduction of tetrazolium and fermentation of sorbitol and arabinose were of use in distinguishing *Str. faecalis* from *Str. faecium* in conjunction with resistance to tellurite but their use for further differentiation of these two species is questionable.

I. INTRODUCTION

The species which Sherman (1937) included in the enterococci were *Str. faecalis, Str. liquefaciens, Str. zymogenes* and *Str. durans.* Subsequently Skadhauge (1950) divided the enterococci into strains resistant to and strains sensitive to 1/2500 potassium tellurite. He placed *Str. faecalis, Str. zymogenes* and *Str. liquefaciens* in the tellurite-resistant group and *Str. faecium* and *Str. durans* in the tellurite-sensitive group. Other characteristics of the tellurite-resistant strains were the ability to produce acid in mannitol and sorbitol and lack of acid production from arabinose and raffinose. Characteristics of the tellurite-sensitive group were acid production from arabinose and no fermentation of sorbitol.

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Barnes (1956) added another criterion, namely the ability to reduce 2, 3, 5-triphenyltetrazolium chloride to the tests for distinguishing between *Str. faecalis* and *Str. faecium*. Morelis and Colobert (1958) considered that *Str. faecalis* and *Str. faecium* were two extreme biotypes of a single species. They considered that the tellurite test was the most important one for differentiating the two biotypes, followed by the fermentation of sorbitol and arabinose, and that the difficulty in reading the result detracted from the value of the tetrazolium test.

Lake, Diebel, and Niven (1957) considered that *Str. faecium* and *Str. durans* differed from *Str. faecalis* on tetrazolium and sorbitol tests, and from each other in that *Str. faecium* produced strong greening on blood agar whereas *Str. durans* produced a narrow zone of beta-haemolysis, and in the fermentation of arabinose and mannitol by *Str. faecium* but not by *Str. durans.*

Other authors have drawn up schemes for differentiating between some or all of these species. Sharpe and Shattock (1951), working with strains isolated from outbreaks of neo-natal diarrhoea in children, found all species of Group D streptococci but did not differentiate *Str. faecium* from the others.

Cooper and Ramadan (1955) used tellurite, thallium acetate and tetrathionate media to isolate strains from human, bovine and sheep sources. They divided the strains into *Str. faecalis* and its varieties, atypical faecalis types I-V, *Str. durans* and *Str. bovis.* They used a number of tests to differentiate the strains but did not include tellurite resistance, reduction of tetrazolium or the fermentation of arabinose.

Barnes, Ingram, and Ingram (1956), who examined strains from specimens obtained from bacon factories, differentiated *Str. faecium* from *Str. faecalis* and designated a group of variant strains as "unclassified type I". These strains usually fermented mannitol, always sucrose but never sorbitol, arabinose or raffinose.

Mieth (1960) described biotypes of *Str. faecalis, Str. faecalis liquefaciens, Str. faecium* and *Str. bovis* and a group of atypical enterococci, using many criteria but not including arabinose or reduction of tetrazolium.

Bartley and Slanetz (1960), working with strains isolated from faeces, sewage and water, differentiated between *Str. faecalis, Str. faecalis* var. *liquefaciens, Str. faecium* and *Str. bovis,* using many tests but not including reduction of tetrazolium. The result for fermentation of sorbitol by *Str. faecium* is given as $\pm$.

In another paper, Elder and Simmons (1963*a*) have reported 67 strains of Lancefield Group D streptococci isolated from tissues of animal and bird origin, which included 66 strains that had the characteristics of enterococci (Sherman 1937) and one strain of *Str. bovis*.

This paper concerns the further classification of the 66 enterococcal strains.

II. MATERIALS AND METHODS

The strains used were those described previously (Elder and Simmons 1963*a*, 1963*b*).

Resistance to 1/2500 potassium tellurite was determined by the method of Barnes, Ingram, and Ingram (1956).

Tetrazolium reduction was determined by the method described by Elder and Simmons (1963*b*).

Carbohydrate fermentation was determined in 15-ml quantities of peptone water containing 1 per cent. carbohydrate and 1 per cent. Andrade's indicator. The criterion for acid production was a final pH 4·0–6·0 after 7 days' incubation (Elder and Simmons 1963*c*). The pH was determined with a Cambridge pH meter. The carbohydrates used were mannitol, sucrose, raffinose, sorbitol and arabinose (L + isomer).

Gelatin liquefaction was determined using 12 per cent. nutrient gelatin (Difco), which was inoculated by the stab method. The cultures were incubated at 37°C for three days and then kept at room temperature for three weeks before discarding as negative.

The determination of haemolytic activity was done as described by Elder and Simmons (1963*a*).

The litmus milk and tyrosine decarboxylase results used for the calculation of Yule's coefficient of association have been published separately (Elder and Simmons 1963*a*).

III. RESULTS

The results for the 66 strains are given in Tables 1–3, and the salient features are shown graphically in Figures 1 and 2.

TABLE 1

REACTIONS OF FOWL STRAINS

Strain	Haemolysis	Gelatin	Mannitol	Sucrose	Raffinose	Tellurite	Tetrazolium	Sorbitol	Arabinose	Species
1	Alpha	−	+	+	+	+	−	+	+	*Str. faecalis*
2	Alpha	−	+	+	+	+	−	+	+	*Str. faecalis*
3	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
4	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
5	Alpha	−	+	+	−	−	−	+	+	*Str. faecium*
6	Alpha	−	+	+	−	+	−	+	+	*Str. faecalis*
7	Alpha	+	+	+	+	+	−	±	±	*Str. faecalis* var. *liquefaciens*
8	Alpha	−	+	+	+	−	−	±	+	*Str. faecium*
9	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
10	Alpha	−	+	+	−	+	−	−	±	*Str. faecalis*
11	Alpha	−	+	+	+	+	+	+	+	*Str. faecalis*
12	Alpha	−	+	+	−	+	+	+	−	*Str. faecalis*
13	Alpha	−	+	+	−	−	−	−	±	*Str. faecium*
14	Alpha	−	+	+	−	−	−	+	+	*Str. faecium*
15	Alpha	−	+	+	+	+	+	+	+	*Str. faecalis*
16	Alpha	−	+	+	−	−	−	+	+	*Str. faecium*
17	Alpha	−	+	+	−	+	+	+	+	*Str. faecalis*
18	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
19	Alpha	−	+	+	−	−	−	−	+	*Str. faecium*
20	Alpha	+	+	+	+	+	+	+	+	*Str. faecalis* var. *liquefaciens*
22	Alpha	−	+	+	−	+	+	+	±	*Str. faecalis*
23	Alpha	−	+	+	+	+	−	+	+	*Str. faecalis*
24	Alpha	−	+	+	+	+	+	+	−	*Str. faecalis*
25	Alpha	−	+	+	+	+	+	+	+	*Str. faecalis*
26	Alpha	−	+	+	−	−	−	−	±	*Str. faecium*
27	Beta	−	+	−	−	+	+	+	−	*Str. faecalis* var. *zymogenes*
28	Beta	−	+	+	−	+	+	+	−	*Str. faecalis* var. *zymogenes*
29	Alpha	−	+	+	+	−	+	+	+	*Str. faecium*
30	Alpha	−	+	+	+	+	+	+	+	*Str. faecalis*
31	Alpha	+	+	+	−	+	+	+	−	*Str. faecalis* var. *liquefaciens*
32	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
33	Alpha	+	+	+	+	+	−	+	+	*Str. faecalis* var. *liquefaciens*

Nineteen of the 32 fowl strains were resistant to tellurite, 13 reduced tetrazolium, 4 liquefied gelatin and 2 produced beta-haemolysis. All strains had strong fermentative ability; all fermented mannitol, 31 sucrose, 18 raffinose, 28 sorbitol, and 25 arabinose. Thirteen strains were considered to be *Str. faecalis,* 4 *Str. faecalis* var. *liquefaciens,* 2 *Str. faecalis* var. *zymogenes, and* 13 *Str. faecium.*

Two of the 15 pig strains were resistant to tellurite, 4 reduced tetrazolium and none of them produced beta-haemolysis or liquefied gelatin. Nine fermented mannitol, 14 sucrose, 8 raffinose, 12 sorbitol, and 10 arabinose. Two strains were considered to be *Str. faecalis* and 13 *Str. faecium.*

TABLE 2

REACTIONS OF PIG AND CATTLE STRAINS

Strain	Haemolysis	Gelatin	Mannitol	Sucrose	Raffinose	Tellurite	Tetrazolium	Sorbitol	Arabinose	Species
Pig										
41	Alpha	−	−	+	+	−	−	−	+	*Str. faecium*
42	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
43	Alpha	−	−	+	−	−	−	−	−	*Str. faecium*
44	Alpha	−	−	+	+	−	+	+	+	*Str. faecium*
45	Alpha	−	+	+	−	+	−	+	−	*Str. faecalis*
46	Alpha	−	+	+	−	+	+	+	−	*Str. faecalis*
47	Alpha	−	+	+	−	−	−	+	+	*Str. faecium*
48	Alpha	−	+	+	−	−	−	+	+	*Str. faecium*
49	Alpha	−	−	+	+	−	−	+	−	*Str. faecium*
50	Alpha	−	−	+	+	−	−	+	+	*Str. faecium*
51	Alpha	−	+	+	−	−	+	+	+	*Str. faecium*
52	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
53	Alpha	−	+	+	−	−	−	+	+	*Str. faecium*
54	Alpha	−	+	+	+	−	+	+	+	*Str. faecium*
55	Alpha	−	−	−	+	−	−	−	−	*Str. faecium*
Cattle										
60	Beta	−	+	−	−	+	+	+	−	*Str. faecalis* var. *zymogenes*
61	Alpha	−	+	+	+	−	−	+	−	*Str. faecium*
62	Alpha	−	+	+	+	−	+	+	+	*Str. faecium*
63	Alpha	−	−	+	+	−	−	−	−	*Str. faecium*
65	Alpha	+	+	+	−	+	+	+	−	*Str. faecalis* var. *liquefaciens*

TABLE 3

REACTIONS OF HUMAN, SHEEP, GOAT, TURKEY AND BUDGERIGAR STRAINS

Strain	Animal	Haemolysis	Gelatin	Mannitol	Sucrose	Raffinose	Tellurite	Tetrazolium	Sorbitol	Arabinose	Species
70	Human	Alpha	−	+	+	+	+	+	+	−	*Str. faecalis*
71	Human	Alpha	−	+	+	−	+	+	+	−	*Str. faecalis*
72	Human	Alpha	−	+	+	+	+	+	+	−	*Str. faecalis*
73	Human	Alpha	−	+	+	−	−	−	+	+	*Str. faecium*
74	Human	Alpha	−	+	+	+	+	+	+	−	*Str. faecalis*
75	Human	Alpha	−	−	+	+	−	+	+	+	*Str. faecium*
76	Human	Alpha	+	+	+	+	+	+	+	−	*Str. faecalis* var. *liquefaciens*
77	Human	Alpha	−	+	+	+	+	+	+	+	*Str. faecalis*
80	Sheep	Alpha	−	+	+	+	−	−	+	+	*Str. faecium*
81	Sheep	Alpha	−	+	+	+	−	−	−	+	*Str. faecium*
84	Goat	Alpha	+	+	+	−	+	+	+	−	*Str. faecalis* var. *liquefaciens*
87	Turkey	Alpha	+	−	+	+	−	−	+	+	*Str. faecium*
88	Budgerigar	Beta	+	+	−	−	+	+	+	−	*Str. faecalis* var. *zymogenes*
89	Budgerigar	Alpha	−	+	−	−	−	−	+	+	*Str. faecium*

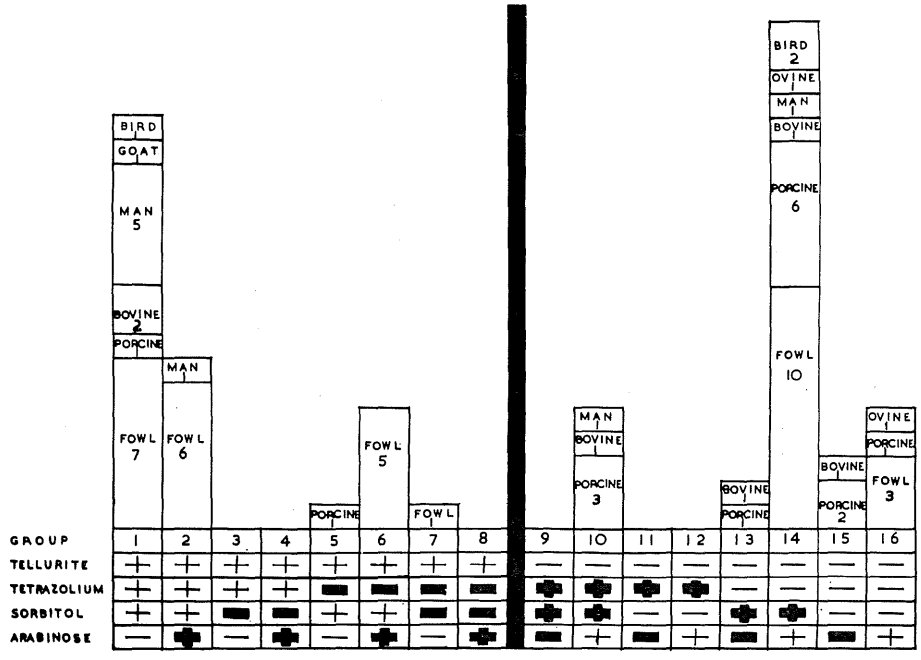

Fig. 1.—Distribution of Queensland strains based on results obtained for resistance to tellurite, reduction of tetrazolium and fermentation of sorbitol and arabinose. Heavy black symbols represent tests deviating from characteristics of either Group 1 or Group 16.

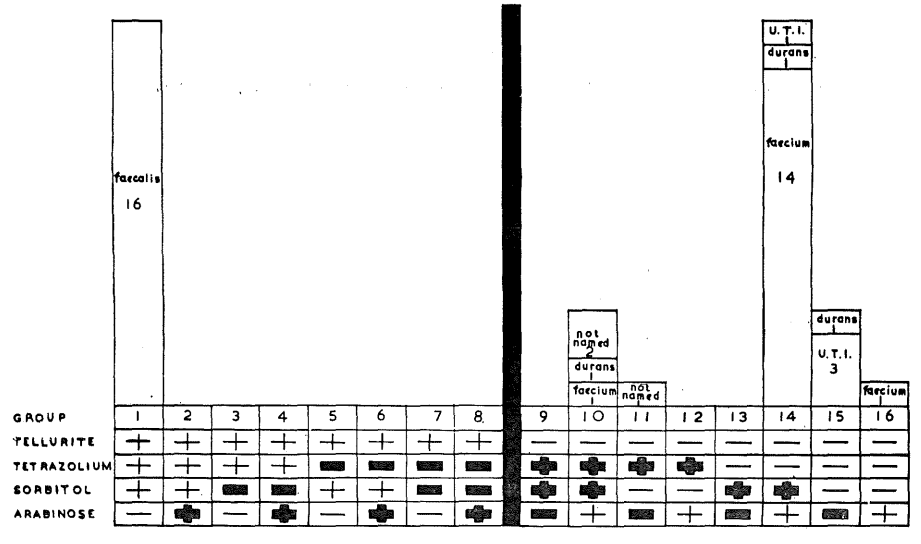

Fig. 2.—Distribution of overseas strains based on results obtained for resistance to tellurite, reduction of tetrazolium and fermentation of sorbitol and arabinose. Heavy black symbols represent tests deviating from characteristics of either Group 1 or Group 16.

Two of the 5 cattle strains were resistant to tellurite, 3 reduced tetrazolium, 1 produced beta-haemolysis, and 1 liquefied gelatin. Four fermented mannitol, 4 sucrose, 3 raffinose, 4 sorbitol, and 1 arabinose. One strain was considered to be *Str. faecalis* var. *zymogenes,* 1 *Str. faecalis* var. *liquefaciens,* and 3 *Str. faecium.*

Six of the 8 human strains were resistant to tellurite, 7 reduced tetrazolium, none produced beta-haemolysis, and 1 liquefied gelatin. Seven fermented mannitol, all sucrose, 6 raffinose, all sorbitol, and 3 arabinose. Five strains were considered to be *Str. faecalis,* 1 *Str. faecalis* var. *liquefaciens,* and 2 *Str. faecium.*

There were 2 sheep strains and 1 goat strain. The sheep strains were both sensitive to tellurite, did not reduce tetrazolium and did not liquefy gelatin. They fermented mannitol, sucrose, raffinose and arabinose, one fermented sorbitol and the other did not. They were both considered to be *Str. faecium.* The goat strain was resistant to tellurite, reduced tetrazolium, did not produce beta-haemolysis but liquefied gelatin. It fermented mannitol, sucrose and sorbitol but did not ferment raffinose or arabinose. It was considered to be *Str. faecalis* var. *liquefaciens.*

There were two budgerigar strains and one turkey strain. One budgerigar strain was resistant to tellurite, reduced tetrazolium, produced beta-haemolysis and liquefied gelatin. It fermented mannitol and sorbitol but did not ferment sucrose, raffinose or arabinose. It was considered to be *Str. faecalis* var. *zymogenes.* The other budgerigar strain was sensitive to tellurite but did not reduce tetrazolium, produce beta-haemolysis or liquefy gelatin. It fermented mannitol, sorbitol and arabinose but did not ferment sucrose or raffinose. It was considered to be *Str. faecium.* The turkey strain was sensitive to tellurite, did not reduce tetrazolium or produce beta-haemolysis but did not liquefy gelatin. It fermented sucrose, raffinose, sorbitol and arabinose but did not ferment mannitol. It was considered to be *Str. faecium.*

Table 4 shows the Yule Coefficient of Association (Q) between the reactions tellurite resistance, tetrazolium reduction, fermentation of sorbitol, arabinose, raffinose, sucrose and mannitol, liquefaction of gelatin and haemolytic activity, taken two at a time. The value of Q between tellurite resistance and production of tyrosine decarboxylase was + 0·676, and between tellurite resistance and reduction of litmus milk + 0·373.

There is a strong association between tellurite and tetrazolium, gelatin and sorbitol and a strong disassociation between tellurite and arabinose. There is a strong disassociation between tetrazolium and raffinose which is not reflected between tellurite and raffinose; also sorbitol and arabinose are not strongly associated.

TABLE 4

YULE'S COEFFICIENT OF ASSOCIATION (Q) FOR TEST REACTIONS OF THE 66 STRAINS (REACTION IN COLUMN A WITH REACTIONS IN COLUMNS B, C AND D

A	B ($Q > 0{\cdot}700$)		C ($Q < 0{\cdot}700$)		D ($Q = \pm 1$)	
Reaction	Reaction	Q	Reaction	Q	Reaction	Q
Tellurite + 31* − 35	Tetrazolium Gelatin Sorbitol Arabinose	+ ·866 + ·844 + ·798 − ·785	Sucrose Raffinose	− ·277 − ·277	Mannitol Haemolysis	+ 1 (no Tell. +ve. Mann. −ve) + 1 (no Tell. −ve Haem. +ve)
Tetrazolium + 29 − 37	Tellurite Raffinose	+ ·866 − ·874	Mannitol Gelatin Arabinose Sucrose	+ ·518 + ·494 − ·681 − ·338	Haemolysis Sorbitol	+ 1 (no Tetr. −ve Haem. +ve) + 1 (no Tetr. +ve Sorb. −ve)
Sorbitol + 57 − 9	Mannitol Tellurite	+ ·825 + ·798	Sucrose Arabinose Raffinose	+ ·247 + ·231 + ·231	Haemolysis Gelatin Tetrazolium	+ 1 (no Sorb. −ve Haem. +ve) + 1 (no Sorb. −ve Gel. +ve) + 1 (no Tetr. +ve Sorb. −ve)
Arabinose + 43 − 23	Sucrose Tellurite	+ ·797 − ·785	Raffinose Mannitol Sorbitol Tetrazolium Gelatin	+ ·526 + ·231 + ·231 − ·681 − ·461	Haemolysis	− 1 (no Arab. +ve Haem. +ve)
Haemolysis + 4 − 62	Sucrose	− ·978	Gelatin	+ ·385	Mannitol Tetrazolium Sorbitol Tellurite Arabinose Raffinose	+ 1 (no Haem. +ve Mann. −ve) + 1 (no Haem. +ve Tetr. −ve) + 1 (no Haem. +ve Sorb. −ve) + 1 (no Haem. +ve Tell. −ve) − 1 (no Haem. +ve Arab. +ve) − 1 (no Haem. +ve Raff. +ve)

Gelatin + 9 − 57	Tellurite	+ ·844	Tetrazolium Haemolysis Mannitol Raffinose Arabinose Sucrose	+ ·494 + ·385 + ·133 − ·476 − ·461 − ·247	Sorbitol	+ 1 (no Gel. +ve Sorb. −ve)
Mannitol + 57 − 9	Sorbitol Raffinose	+ ·825 − ·756	Tetrazolium Sucrose Arabinose Gelatin	+ ·518 + ·247 + ·231 + ·133	Tellurite Haemolysis	+ 1 (no Mann. −ve Tell. +ve) + 1 (no Mann. −ve Haem. +ve)
Sucrose + 61 − 5	Arabinose Raffinose Haemolysis	+ ·747 + ·721 − ·978	Sorbitol Mannitol Tetrazolium Tellurite Gelatin	+ ·247 + ·247 − ·338 − ·277 − ·247		
Raffinose + 38 − 28	Sucrose Tetrazolium Mannitol	+ ·721 − ·874 − ·756	Arabinose Sorbitol Gelatin Tellurite	+ ·526 + ·231 − ·476 − ·227	Haemolysis	− 1 (no Raff. +ve Haem. +ve)

* Number of strains positive and negative for reaction in column A

Figure 1 shows the distribution of strains over the various combinations of the four characters of tellurite resistance, tetrazolium reduction, sorbitol and arabinose fermentation. For purposes of comparison, Figure 2 gives the same results for 43 strains obtained from overseas workers, most of which were designated as to species.

IV. DISCUSSION

Although Orla-Jensen (1919) divided the enterococci into three species—*Str. faecium, Str. glycerinaceus* and *Str. liquefaciens*—on carbohydrate fermentation tests, Skadhauge (1950) pointed out the value of resistance to potassium tellurite to distinguish *Str. faecium* from the other species. As tellurite resistance is relatively easy to determine and the results are rarely open to misinterpretation, this test was chosen by us to divide the strains into either *Str. faecalis,* including varieties *liquefaciens* and *zymogenes,* or *Str. faecium.*

Strongly associated with tellurite resistance was tetrazolium reduction (Q + ·866), gelatin liquefaction (Q + ·844) and acid production from sorbitol (Q + ·788). Arabinose fermentation is strongly disassociated with tellurite resistance (Q — ·785).

Production of tyrosine decarboxylase and tellurite resistance are not sufficiently associated (Q + ·676) to use tyrosine decarboxylase activity as a criterion for placing an organism into either *Str. faecalis* or *Str. faecium* taxa, if the character of tellurite resistance is taken as the major criterion; similarly with litmus milk (Q + ·373).

There was a strong association between beta-haemolysis and tellurite resistance (Q + 1·0) and also the other characteristics of the *Str. faecalis* group. Beta-haemolysis has been considered an important criterion in the classification of enterococci and the name "zymogenes" has been used, with both specific and varietal rank. Hence Strains 27, 28 and 60 may be considered *Str. faecalis* var. *zymogenes.* Strain 88 also has the characteristics of *Str. faecalis* but in contrast with the other beta-haemolytic strains also liquefies gelatin. According to Skadhauge (1950), in Sherman's classification only Strain 88 would be a true *"zymogenes"* and the other three strains would be named *Str. faecalis* var. *hemolyticus.*

There appears to be general agreement that gelatin-liquefying strains of enterococci may be designated "liquefaciens," using this term either as a specific or a varietal epithet. It is possible therefore to consider Strain 88 as *Str. faecalis* var. *liquefaciens* or *Str. faecalis* var. *zymogenes.* The non-haemolytic gelatin-liquefying strains (Strains 7, 20, 31, 33, 76 and 84) do not form such a compact group, as only two characteristics—tellurite resistance and sorbitol fermentation —have a strong association with gelatin liquefaction. All of the nine strains liquefying gelatin except Strain 87 were tellurite-resistant. If the tellurite

resistance test is considered to be the definitive test for separating *Str. faecalis* from *Str. faecium* it would be desirable to use *"liquefaciens"* as a varietal epithet and recognize both *Str. faecalis* and *Str. faecium* as possessing varieties with this character. In this case, Strains 7, 20, 31, 33, 65, 76, 84 and 88 would be named *Str. faecalis* var. *liquefaciens* and Strain 87 *Str. faecium* var. *liquefaciens.*

Beta-haemolysis has also been claimed characteristic of *Str. durans* by some workers (Sherman 1937; Skadhauge 1950; and Lake, Diebel, and Niven 1957), whereas Shattock (1945) came to the conclusion after examination of strains obtained from other workers, as well as 30 strains isolated from spray-dried milk powder and pasteurized milk, that *Str. durans* should not be restricted only to those strains showing beta-haemolysis. Barnes, Ingram, and Ingram (1956) designated a group of strains isolated from pigs as unclassified type 1. These strains were tellurite and tetrazolium-negative and therefore are of the "faecium" rather than the "faecalis" type. They differentiated the unclassified type 1 strains from *Str. faecium* by their failure to ferment arabinose. The results of examining three named strains of the unclassified type 1 group and three named strains of *Str. durans* are given in Table 5. Although the number of strains is too small to justify any definite conclusions, it would appear that there is a strong similarity between alpha-haemolytic strains of *Str. durans* and the unclassified type 1 group, and consequently with *Str. faecium* as suggested by Lake, Diebel, and Niven (1957). No tellurite-sensitive strains were beta-haemolytic; if there had been any they could be called *Str. faecium* var. *durans,* rather than *Str. durans.* Whether it is possible to distinguish unclassified type 1 strains from *Str. faecium* strains is debatable and depends largely on whether the investigator restricts *Str. faecium* only to those strains fermenting mannitol, sucrose and arabinose, or whether *Str. faecium* is considered to have results varying from this.

Mieth (1960) subdivided the streptococci on physiological characteristics but did not include tetrazolium reduction or fermentation of arabinose. There is therefore a problem of which tests to choose, and more important the problem of the reproducibility of the tests selected. It could be expected that from reports in the literature (Shattock 1945; Sharpe 1948; Sharpe and Shattock 1952; Barnes, Ingram, and Ingram 1956) that inability to ferment raffinose would be characteristic of *Str. faecalis* and *Str. faecium.* However, of the 33 fowl strains (Table 1) examined, 18 were positive, 8 of 15 porcine strains, 3 of 4 bovine, 6 of 8 human, both sheep strains and one turkey strain (Tables 2 and 3). As these results were unexpected, various indicators, brands of carbohydrates and other modifications of the media were tried (Elder and Simmons 1963*c*). In addition, a number of strains were sent to the Bacteriology Department of the University of Queensland, where similar results were obtained. Other workers have reported fermentation of raffinose (Bartley and Slanetz 1960; Mieth 1960; Hugh 1959). Also, Langston, Gutierrez, and Bouma (1960) stated that the majority of their strains fermented raffinose. Sorbitol and arabinose are included in the results given in Figure 1, but whether subdivision of the streptococci into groups on these characters is justified will depend on the examination of many more strains.

TABLE 5

REACTIONS OF NAMED STRAINS OF *Str. durans* AND UNCLASSIFIED TYPE 1 (BARNES)

Species	No.	Haemolysis	Mannitol	Sucrose	Growth 45°	Resistance to Tellurite	Reduction of Tetrazolium	Sorbitol	Arabinose	Growth at pH 9·6	Survival 60°C for 30 min	Growth 6·5% NaCl
Str. durans ..	N16	Alpha	−	+	−	−	−	+	+	−	+	+
	H2	Beta	+	+	+	−	+	+	+	+	+	+
	C3	Alpha	−	−	−	−	−	−	−	−	+	−
Unclassified Type 1	CH12	Alpha	+	+	+	−	−	−	−			
	P/20/5	Alpha	+	+	+	−	−	+	+			
	P/16/5	Alpha	−	+	+	−	−	−	−			

Str. faecalis has been used as an indicator of human contamination in processed pig products (Barnes and Ingram 1955; Barnes, Ingram, and Ingram 1956), and as an indicator of sewage contamination of water by Bartley and Slanetz (1960). Our results show that *Str. faecalis* and its varieties were present in pigs and *Str. faecium* in human faeces. These results agree with those of Buttiaux (1958) and Morelis and Colobert (1958).

REFERENCES

BARNES, E. M. (1956).—*J. Gen. Microbiol.* 14:57.

BARNES, E. M., and INGRAM, M. (1955).—*Ann. Inst. Pasteur Lille* 7:115-9.

BARNES, E. M., INGRAM, M., and INGRAM, G. C. (1956).—*J. Appl. Bact.* 19:204.

BARTLEY, C. H., and SLANETZ, L. W. (1960).—*Amer. J. Publ. Hlth.* 50:1545.

BUTTIAUX, R. (1958).—*Ann. Inst. Pasteur* 94:778.

COOPER, K. E., and RAMADAN, F. M. (1955).—*J. Gen. Microbiol.* 12:180.

ELDER, JEAN K., and SIMMONS, G. C. (1963*a*).—*Qd J. Agric. Sci.* 20:257.

ELDER, JEAN K., and SIMMONS, G. C. (1963*b*).—*Qd J. Agric. Sci.* 20:263.

ELDER, JEAN K., and SIMMONS, G. C. (1963*c*).—*Qd J. Agric. Sci.* 20:271.

HUGH, R. (1959).—*Canad J. Microbiol* 5:351.

LAKE, D. E., DIEBEL, R. H., and NIVEN, C. F. (1957).—*Bact. Proc.* (Soc. Amer. Bact. 57th General Meeting, Michigan), p. 13.

LANGSTON, C. W., GUTIERREZ, J., and BOUMA, C. (1960).—*J. Bact.* 80:714.

MIETH, H. (1960).—*Zbl. Bakt. I. Orig.* 179:456.

MORELIS, P., and COLOBERT, L. (1958).—*Ann. Inst. Pasteur.* 95:667.

SHARPE, M. E. (1948).—*Proc. Soc. Appl. Bact.*, p. 13.

SHARPE, M. E., and SHATTOCK, P. M. F. (1952).—*J. Gen. Microbiol.* 6:150.

SHATTOCK, P. M. F. (1945).—*Proc. Soc. Appl. Bact.*, p. 18.

SHERMAN, J. M. (1937).—*Bact. Rev.* 1:1.

SKADHAUGE, K. (1950).—"Studies on the Enterococci with Special Reference to the Serological Properties." (Einar Munksgaards Forlag: Copenhagen.)

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