INDICATOR ORGANISMS—A REVIEW.
I. TAXONOMY OF THE FECAL STREPTOCOCCI¹

Paul A. Hartman, George W. Reinbold and Devi S. Saraswat²

Departments of Bacteriology and
Dairy and Food Industry
Iowa State University, Ames

ABSTRACT. The terms "enterococci," "fecal streptococci" and "group D streptococci" are discussed and defined. Development of the taxonomy of the fecal streptococci is described. Physiological differences, serology, the chemical basis of serological specificity and some other possible taxonomic approaches are elaborated upon.

INTRODUCTION

Bacterial classification has recently received great impetus from an increasing desire to learn more about the characteristics of various groups of bacteria and the relationships of these bacteria to one another. Efforts to place classification on a quantitative basis (Sokal and Sneath, 1963) and to discover the genetic interrelationships of various microorganisms (Marmur, Falkow and Mandel, 1963) also have contributed to this revival of interest. Although any one discovery or idea may result in the modification of a classification, the resultant classification is always based on the total quantity of information that has accumulated. Classifications are the result of evolutionary processes. For this reason, it is advantageous periodically to view the

¹ Journal Paper No. J-5120 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Projects No. 1050 and 1379. This work was supported in part by U.S. Public Health Service Research Grants EF-00112 from the Division of Environmental Engineering and Food Protection and AI-05219 from the Division of Allergy and Infectious Diseases.

² Rockefeller Foundation Scholar. Present address: Rajasthan College of Agriculture, Udaipur, Rajasthan, India.

classification of a group of microorganisms in retrospect.

During the preparation of a series of reviews on indicator organisms in foods (Hartman, Reinbold and Saraswat, 1965), we decided that a summary of the taxonomy of the fecal streptococci was in order (Buchanan, 1958) to form a firm basis for subsequent discussions of media, methods, distribution and significance of these bacteria. An excellent paper on the group D streptococci by Deibel (1964a) appeared after our review had been substantially completed; however, the approaches differed sufficiently that the two reviews should supplement each other.

Because of their distribution and importance, a considerable quantity of work has been done on the physiological, serological and other characteristics of the fecal streptococci. As a result, the fecal streptococci have become one of the better characterized bacterial groups. Many points have been clarified, yet ambiguities still remain. Most of the early studies have been summarized in comprehensive reviews by Orla-Jensen (1919), Dible (1921), Sherman (1937, 1938) and Frost and Engelbrecht (1940). Skadhauge (1950), Seelemann (1954), Shattock (1955, 1962) and Niven (1963) have more recently described selected aspects of the fecal streptococci. The reader is referred to these reports and the review of Deibel (1964a) for numerous references omitted from the present review.

Definition of Terms

The terms 'enterococci,' 'fecal streptococci,' 'group D streptococci,' 'Streptococcus faecalis' and even 'streptococci' have sometimes been used very loosely and interchangeably to designate various food-borne bacteria. These terms are shown in Table 1. Each term is defined by the species of Streptococcus that it contains. This grouping is similar, but not identical, to the one proposed by Niven (1963).

It is obvious that the term, 'Streptococcus faecalis,' should refer to a specific bacterial species. Furthermore, the term 'streptococci,' is so general that it means very little when used to describe microorganisms isolated from foods. There is no dispute that the serological group D is composed of those streptococci (Table 1) which contain the D antigen. However, the serological group D contains two organisms, S. bovis and S. equinus, that are members of the viridans group (Breed, Murray and Smith, 1957). Slanetz

Table 1. Definition of the terms "enterococci," "group D streptococci" and "fecal streptococci" based on Streptococcus spp. belonging to each group.

Fecal streptococci	
Enterococcus Group	<u>S. faecalis</u> <u>S. faecalis</u> var. <u>liquefaciens</u> ^a <u>S. faecalis</u> var. <u>zymogenes</u> ^a <u>S. faecium</u> ^b <u>S. faecium</u> var. <u>durans</u> ^b
Viridans Group	<u>S. bovis</u> <u>S. equinus</u> <u>S. mitis</u> <u>S. salivarius</u>
Group D	

^a These two varieties are probably not justifiable. See text for discussion.

^b Streptococcus faecium is not recognized in Bergey's Manual of Determinative Bacteriology, 7th ed. (Breed et al., 1957). In Bergey's Manual, S. faecium var. durans appears as S. durans.

and Bartley (1957) proposed extension of the enterococcus group to include S. bovis and S. equinus, or all organisms belonging to Lancefield's group D (Slanetz and Bartley, 1964). Bartley and Slanetz (1960) thought that all streptococci commonly inhabiting the intestinal tract of animals should be included within the term 'enterococcus.' It would seem wise to us to restrict the term enterococcus to its present usage in Bergey's Manual of Determinative Bacteriology (Breed et al., 1957). Otherwise, the term would require even more extensive broadening to include S. salivarius, which Niven, Smiley and Sherman (1942) found to sometimes compose the predominant streptococcal flora of the human intestine.

Shattock (1955) and Mossell, van Diepen and de Bruin (1957) have suggested that the terms 'enterococci' and 'fecal

streptococci' should be synonymous. Niven (1963) thought that the term 'fecal streptococci' should be limited to either the enterococci or group D streptococci. The philosophy of Cooper and Ramadan (1955) and Kenner, Clark and Kabler (1960) seems most appropriate to us: to include in the fecal streptococci all Streptococcus spp. consistently present in significant numbers in fresh fecal excreta, when adequate methods for detection of all types present are used. Thus, the terms given in Table 1 would have three definite meanings: one, taxonomical (enterococcus and viridans groups); the second, serological (group D); and the third, sanitary significance (fecal streptococci).

Taxonomy

Present day taxonomy of the fecal streptococci has developed from earlier studies by many investigators. Thiercelin (1899) first used the term 'enterococcus' to describe, on a morphological basis, a Gram-positive diplococcus of intestinal origin. Andrewes and Horder (1906) called a group of nonhemolytic streptococci, characteristic of bacteria from the human intestine, 'streptococcus faecalis.' In the opinion of Dible (1921), the two names were synonymous, and he suggested that they be applied to a group of heat-resistant diplococci commonly found in human feces. Orla-Jensen (1919) identified and named two of these heat-resistant diplococci, S. faecium and S. glycerinaceus, without relating them either to the S. faecalis of Andrewes and Horder (1906) or to the 'enterococcus' described by Thiercelin (1899) and other workers. Sherman (1937) used the term 'enterococcus' to designate a group of streptococci including hemolytic, nonhemolytic and gelatin-liquefying types. He thought that the S. faecium and S. glycerinaceus of Orla-Jensen (1919) were both synonymous with the S. faecalis of Andrewes and Horder (1906). Sherman (1937, 1938) regarded the 'enterococcus group' as comprising S. faecalis and its varieties, liquefaciens and zymogenes, and the S. durans of Sherman and Wing (1935, 1937). This was the basis for the classification devised by C. F. Niven, Jr. and collaborators for Bergey's Manual of Determinative Bacteriology (Breed et al., 1957). The enterococcus group is catalase negative (Isaacs and Scouller, 1948; see also Whittenbury, 1965a) and shares the criteria of Sherman (1937); i. e., growth at 10 and 45°C, at pH 9.6, in 6.5% NaCl broth, in 0.1% methylene blue milk, survival at 60°C for 30 min and production of ammonia in peptone broth.

Streptococcus faecium, Orla-Jensen (1919), was recognized as a synonym of S. faecalis in the 6th edition of Bergy's Manual (Breed et al., 1948). There is considerable evidence, however, in recent literature to suggest that this species deserves individual recognition. Skadhauge (1950) differentiated S. faecalis from S. faecium by showing that the former organism grows in a medium containing 0.04% potassium tellurite, whereas growth of the latter organism is inhibited. Barnes (1956a) observed that the two species had different reduction and fermentation reactions. She found that, under appropriate conditions, S. faecalis reduced 2,3,5-triphenyl tetrazolium chloride strongly, while S. faecium reduced this compound weakly or not at all. Barnes and Ingram (1955) and Barnes (1956b) reported that S. faecalis usually produces reduction, acid and coagulation in litmus milk in 24 hr, but that S. faecium reduces litmus much less rapidly and that sometimes acid production is the only reaction noted. Lake, Deibel and Niven (1957) reported that S. faecalis and its varieties ferment α -methyl glucoside, citrate, glycerol (anaerobically), melezitose and sorbitol, but do not ferment melibiose and do not require folic acid for growth. Streptococcus faecium and S. durans both gave diametrically different reactions in all these tests. Furthermore, Deibel and co-workers (1958, 1960, 1964) found that S. faecalis and its varieties require lipoic acid to obtain energy for growth from citrate, pyruvate and serine, but not when arginine or malate are utilized as energy sources. Deibel (1960, 1964b) also showed that, although all these organisms hydrolyze arginine, only S. faecalis and its varieties were capable of utilizing arginine as an energy source. London and Appleman (1962) found that, in vigorously aerated glucose-medium cultures, S. faecalis produced acetic acid and acetylmethylcarbinol in a ratio of 1:1, while ratio for S. faecium was 35:1. Hartsell and Caldwell (1961) reported that S. faecalis and its varieties were lysed by a combination of lysozyme and trypsin, but not by lysozyme alone; S. faecium and S. durans were lysed by lysozyme alone; and S. bovis was completely resistant to lysis. Data obtained by using these and other tests (Deibel, Evans and Niven, 1959; Deibel, 1964a) have shown that S. faecium deserves status as a distinct species. Barnes (1964) and Whittenbury (1965a) are of the opinion that tellurite resistance and tetrazolium reduction are key differential tests. Whittenbury (1965a) has described still other differential tests.

Several important reports have appeared in the last 5

years, all indicating that some consolidation and rearrangement of species and varieties within the enterococcus group is desirable. It is interesting that some of the recent suggestions echo sentiments expressed much earlier. For example, Thomson and Thomson, in 1928 (1928-29), mentioned that they had found S. liquefaciens and S. zymogenes to be identical. Similar suggestions have been made by recent workers, among which the report of Deibel, Lake and Niven (1963) is of great importance. The key definitive features described by these workers (see also Niven, 1963 and Deibel, 1964a) are given in Table 2. It is our experience that the set of differential tests proposed by these workers is the most appropriate yet suggested. Only two species would be recognized. One species would be composed of S. faecalis (Andrewes and Horder, 1906; S. glycerinaceus, Orla-Jensen, 1919) and its varieties, liquefaciens (Sternberg, 1892; Long and Hammer, 1936; Mattick, 1949) and zymogenes (McCallum and Hastings, 1899; Mattick, 1949; see also Thomson and Thomson, 1928-29 and Smith, Niven and Sherman, 1938). The second species would be composed of S. faecium (Orla Jensen, 1919) and its variety, durans (Sherman and Wing, 1935, 1937). Thus, S. faecium would be recognized as a species and would absorb S. durans as a variety. An arrangement similar in many respects to that just described was proposed by Horie (1959, 1961).

Further consolidation has been proposed by Colobert and Morelis (1958; see also Morelis and Colobert, 1958 and Colobert, 1962). These workers had reached similar conclusions regarding the varietal status of S. durans. In addition, Colobert and co-workers (*loc. cit.*; see also Colobert and Blondeau, 1962a, 1962b and Defayolle and Colobert, 1962) reached the conclusion that S. faecalis was a collective strain (species), in which there were many diverse biotypes or varieties: durans, faecium, innominatus (a new group, originated by the French workers), liquefaciens, proprium (Skadhauge, 1950), and possibly others, such as the S. faecalis var. glycerinaceus of Severi (1942, Hauduroy et al., 1953) and S. faecium var. mobilis of Langston, Gutierrez and Gouma, 1960; Grandal, 1957a, 1957b). The zymogenes variety was thought not to deserve varietal status, a conclusion similar to that expressed earlier by Thomson and Thomson (1928-29), Evans and Chinn (1947), and Hartsell and Caldwell (1961). Hartman and Rouze (unpublished) also failed to obtain satisfactory differentiation of species and varieties of S. faecalis and S. faecium when 1210 fecal

Table 2. Key features in differentiation of S. faecalis and its varieties, liquefaciens and zymogenes from S. faecium and S. durans^a.

Feature	<u>S. faecalis</u>	<u>S. faecium</u>
Fermentation:		
Arabinose	-	+ (<u>durans</u> -)
Citrate	+	Usually - ^b
Glycerol (anaerobic)	Usually + ^b	Usually -
Gluconate	Usually +	Usually -
Mannitol	+	+ (<u>durans</u> -)
Melezitose	Usually +	Usually -
Melibiose	Usually -	Usually +
Sucrose	+	+ (<u>durans</u> usually -)
Tetrazolium reduced	Usually +	Usually -
Growth, 0.04% tellurite	+	Usually -
Folic acid required	-	Usually +
Gelatinase, tube method	- (<u>liquefaciens</u> +)	-
Strong greening on blood agar pour plates	- (<u>zymogenes</u> β -hemolytic)	Usually + (some <u>durans</u> β -hemolytic)

^a Data from Deibel et al. (1963). See footnotes (a) and (b) of Table 1.

^b The term, "usually," means that exceptions to the reaction given have been observed.

streptococci from diverse animal and plant sources were examined by a battery of 42 different tests. Many strains, especially those from vegetation (see also Mundt, 1963 and references cited therein) and foods, possessed sets of characteristics that placed them between established groups. Their results indicated that, if any mutually exclusive set of features was used to delineate a species of enterococcus, variants could be isolated that would fit into neither group (see also Krantz and Dunne, 1965).

Evidently, enterococci may be readily differentiated into well-defined 'species' if all organisms that do not meet the Sherman criteria in entirety are first eliminated from further study and if subsequent tests are limited to a few 'important' features. Under these circumstances, intermediates

are rarely detected. Thus, Kereluk (1959) studied 307 isolates of enterococci isolated from frozen meat pies. All fulfilled the Sherman criteria. (This is an indication that the isolation procedure used was highly selective). He separated S. faecalis and S. faecium strains by use of an identification scheme similar to that proposed by Barnes (1956a) and concluded that S. faecium was a separate and easily distinguishable species of enterococcus deserving independent status. Differentiation of enterococci into well-defined species becomes more difficult when isolates that do not fulfill all the Sherman criteria are subjected to further study. Thus, Kenner *et al.* (1960) placed such strains in an 'enterococcus biotype' group and Mieth (1960, 1961) placed such strains in an 'atypical enterococcus' group. Burkwall and Hartman (1964) also had to designate an 'atypical enterococcus' group to accommodate many of the strains isolated in their study. (See also Ferraro, 1960.) It is apparent, therefore, that some flexibility of the description of the enterococci is desirable to permit inclusion of many isolates that would not fit into a species when very rigid criteria are used. Caution in this regard also has been expressed by Deibel (1964a). One method of introducing some flexibility would be to specify that a certain number or percentage of features (i. e., 8 or 9 or 10, or 80 or 90%) must be fulfilled for an organism to be designated an enterococcus. Such flexibility will probably be necessary if the criteria suggested by Deibel *et al.* (1963; Deibel, 1964a) are established for routine speciation, because intermediates exist in nature (Deibel, Lake and Niven, 1959; Barnes, 1964). Identification is complicated further by the failure of some recently isolated strains to yield typical reactions until several transfers have been made in vitro (Mannweiler, 1955; Hartman and Rouze, unpublished). The reason for these anomalous results is unclear, but a possible explanation is offered by the work of Whittenbury (1963).

There is much less controversy that S. bovis (Orla-Jensen, 1919) and S. equinus, S. mitis and S. salivarius (all described by Andrewes and Horder, 1906) are separate species. (See also Engelbrecht, 1935 and Seelemann and Obiger, 1964). There may eventually be consolidation within these species also because of the many similarities of S. bovis and S. equinus on the one hand and S. mitis and S. salivarius on the other hand. Most strains of these bacteria grow at 45°C, but do not share any of the other criteria of Sherman (1937) for enterococci. Streptococcus bovis fer-

ments lactose and produces acid in litmus milk, whereas S. equinus characteristically does not ferment lactose and produces no apparent reaction in litmus milk. Streptococcus bovis hydrolyzes starch (Dunican and Seeley, 1962), is insensitive to lysozyme systems (Hartsell and Caldwell, 1961) and is the only streptococcal species reported to be capable of using ammonium salts as a sole source of nitrogen for growth (Prescott, Williams and Ragland, 1959; Wolin, Manning and Nelson, 1959). Smith and Shattock (1962) believed that S. bovis and S. equinus were distinct species; however, as pointed out by Deibel (1964a), further studies on these two species are necessary to more clearly establish intraspecies boundaries. Streptococcus mitis and S. salivarius (Safford, Sherman and Hodge, 1937) differ from S. bovis in that they are insensitive to 40% bile and do not hydrolyze starch. (However, amylase-negative strains of S. bovis have been noted—see, for example, Mieth, 1960). Streptococcus salivarius and S. mitis are differentiated (Sherman, Niven and Smiley, 1943) on the basis of hemolysis of blood, fermentation of inulin and production of mucoid colonies on 5% sucrose agar by S. salivarius. Streptococcus mitis lacks these three properties.

Williams, Hirsch and Cowan (1953) described certain cocci isolated from the air. These bacteria were placed in a new genus, Aerococcus; however, their physiological properties resemble closely those of the viridans streptococci. The taxonomic relationships of Aerococcus, as well as members of another genus, Pediococcus, to the fecal streptococci are in need of clarification. (See also Whittenbury, 1965b.)

Serology

The serological differentiation of fecal streptococci from other streptococci is based on the grouping scheme originated by Lancefield (1933). It soon became evident that S. faecalis belonged to the serological group D (Lancefield and Hare, 1935; Hare and Maxted, 1935; Shattock and Mattick, 1943). Streptococcus faecalis var. zymogenes also was found to possess group D antigen (Houston, 1936; Sherman, Maurer and Stark, 1937; Smith et al., 1938), as did S. faecalis var. liquefaciens and S. durans (Sherman, 1938; Graham and Bartley, 1939). When better differentiation between S. faecalis and S. faecium was made (Skadhauge, 1950), the latter microorganism also was found to belong to group D (Skadhauge, 1950; Sharpe and Shattock, 1952).

Conclusive demonstration of group D antigen in S. bovis was more difficult; only about half of Sherman's (1938) cultures of S. bovis reacted definitely with potent group D antiserum, and about one-third gave negative results. (See also Shattock and Mattick, 1943.) When improved methods were used for the preparation of antisera and of extracts for precipitin tests, S. bovis was found to possess group D antigen (Shattock, 1949a; Seelemann and Carstens, 1951). The D antigen was produced by all strains examined, but was not always extractable with hydrochloric acid, although broken cells always yielded D antigen. Streptococcus equinus was more refractive to serological grouping than S. bovis. Sherman (1938) had noted that some of his strains of S. equinus reacted weakly with group D antisera; however, not until recently was this species definitely placed into group D (Smith and Shattock, 1962; Fuller and Newland, 1963).

The other fecal streptococci evidently do not possess group D antigen. Streptococcus salivarius was found by Sherman, Niven and Smiley (1943; see also Shattock, 1949b) to be devoid of group D antigen. Members of this species were subsequently found to possess group K antigen (Williams, 1956; Stewart and McKeever, 1963). Not all strains of S. salivarius (as determined on a physiological basis) react with group K antiserum, so additional studies will be necessary to elucidate further antigenic properties of this species. Furthermore, the resemblance of some strains of S. salivarius to certain group D streptococci is so striking (Hartman and Rouze, unpublished data) that it might be profitable to search again for group D antigen in selected strains of this species, using refined techniques. Such combinations of antigens are not unprecedented: Sharpe (1952) noted that a common type-antigen was present in both a strain of S. lactis and a strain of S. faecium which would allow them to react with both group D and group N antisera. (See also Lancefield, 1941; Perry and Briggs, 1955; Perry Newland and Briggs, 1958). In addition, Smith and Shattock (1964) observed that group Q streptococci (Guthof, 1955) possessed both group Q and group D antigens. As far as we are aware, S. mitis does not produce group D specific antigen (Sherman et al., 1943), but re-examination of members of this species, and even of Aerococcus spp. (Williams et al., 1953) might be warranted now that improved techniques have been developed.

Using preparations of type-specific antisera, Sharpe and Shattock (1952) designated 24 serological types within the

group D streptococci. Sharpe's (1952) serological type of S. faecium became number 25; 14 new types described by Sharpe and Fewins (1960) were added to the previous list, making a total of 39 serological types in group D. Further revision of the typing scheme was suggested by Sharpe (1964), based on the recommendation of Lancefield (Williams and Wilson, 1963). Many different serological types of S. bovis also exist (Medrek and Barnes, 1962a). Stewart and McKeever (1963) have subdivided the group K, containing S. salivarius. Therefore, it is apparent that many serological subgroups can be obtained within the fecal streptococci. In 1949, Shattock (1949b) predicted that there were more serological types than had been reported within the group D at that time. The three broad divisions proposed by her within the group D on physiological grounds (viz., S. faecalis and its varieties; S. faecium and S. durans; and S. bovis and S. equinus) have been substantiated by later physiological and serological studies. Her arrangement of species is markedly similar to the one recently proposed by Deibel *et al.* (1963).

Additional serological studies on the group D streptococci have been made by Jones and Shattock (1960), Papavassiliou (1962), Barnes (1964) and others. Their findings have generally supported the conclusions drawn by Shattock (1949); refer to these papers for details of these investigations. Deibel (1964a) has precisely summarized the development of methodology in serological analyses of group D streptococci; papers by Kunter (1963) and Bleiweis, Karakawa and Krause (1964) are the only contributions in this area that have appeared subsequent to Deibel's review. Details of methodology will not be elaborated upon in the present review except to mention that Medrek and Barnes (1962a) reported that some group D streptococci, when grown under certain conditions, did not yield hydrochloric acid extracts which would react serologically with group D antisera. This phenomenon had also been observed by other workers. Cultural conditions conducive to production of the group D antigen were elucidated by Medrek and Barnes (1962b).

Chemical Basis of Serological Specificity

Lancefield (see Niven *et al.*, 1964) suggested that there was sufficient information now available to place serological classification of enterococci on a chemical basis (Table 3).

Table 3. Chemistry of the antigenic components of Lancefield's serological types.^a

Group	Group Specific Antigens	Chief Component
A	Cell wall polysaccharide	Rhamnose + glucosamine
B	Cell wall polysaccharide	Rhamnose + glucosamine
C	Cell wall polysaccharide	Rhamnose + galactosamine
D	Intracellular ^b teichoic acid	Glucosyl- glycerophosphate
G	Cell wall polysaccharide	Rhamnose + galactosamine + galactose
N	Intracellular ^b teichoic acid	Galactosyl- glycerophosphate

^a After Lancefield (1964; see Niven *et al.*, 1964).

^b "Intracellular" means immediately exterior to the cell membrane (Smith and Shattock, 1964).

This suggestion was founded on studies made by several investigators in the last decade on location of the group D antigen in the cell and the antigenicity and chemical composition of cellular fractions.

Interest in the exact location of group D antigen was stimulated greatly when Elliott (1959, 1960) and Jones and Shattock (1960) could not demonstrate the presence of the antigen in cell-wall fractions of group D streptococci. The group-antigen also was not present in L-phase variants of group D streptococci (Hijmans, 1962), although it was later found that L-phase variants secreted group D antigen into the medium (Smith and Shattock, 1964). However, when the cell walls of normal cells were removed enzymatically, group D antigen was released into solution (Smith and Shattock, 1964). The site of location of the majority of group D antigen, therefore, seems to be at the surface of the protoplast, immediately beneath the cell wall. At least part of the group D antigen is retained by the protoplast membrane upon dissolution of the cellular structure (Slade and Shock-

man, 1963; Shockman and Slade, 1964); repeated washing of the membrane fraction resulted in progressive release of the antigen. Group D antigen has a similar location in group Q cells (Smith and Shattock, 1964). The group Q antigen, however, is located principally in the cell-wall fraction, as are the 'type' antigens of group D bacteria (Smith and Shattock, 1964). Thus, the group D (and, incidentally, group N—Elliott, 1963) streptococci possess intracellular teichoic acids (group antigens) just beneath the cell wall, while in the other groups the group antigens are located in the cell wall. In the group D, the 'type' antigens are located in the cell walls. Sharpe (1965) suggested a serological typing scheme for all group D streptococci, based on the presence of chemically defined type-specific antigens.

Chemically, the cell exterior of group D streptococci has been noted to contain glucose and galactose in addition to the usual components (Salton, 1953; Cummins and Harris, 1956; Abrams, 1958; Ikawa, 1959; Armstrong *et al.*, 1959; Slade and Slamp, 1962). The glucose may have been a contaminant rather than a normal cell-wall constituent (Curtis and Krause, 1964), although later studies (Bleiweis and Krause, 1965) indicate that glucose is a component of the cell walls of group D streptococci. Group D antigen preparations were found to contain glucose and α -glycerophosphate in polymerized form (Wicken, Elliott and Baddiley, 1963; Wicken and Baddiley, 1963). The differences discussed are shown in Table 3. Other aspects of streptococcal cell-wall structure and synthesis have been reviewed by Perkins (1963) and Krause (1963), while McCarty (1964) and Willers and co-workers (1964) have recently described serological and chemical studies on soluble antigens of streptococci.

Other Possible Taxonomic Approaches

A survey of enterococcins was made recently by Brock, Peacher and Pierson (1963). These workers concluded that bacteriocinogeny would be of little value in 'delineating the grand design of the enterococcus group,' although some relationships were found between the type of bacteriocine produced and the assigned name of the production strain. Furthermore, bacteriocine production was not an admirably stable characteristic of the enterococci; mutants of *S. faecalis* var. *zymogenes* were obtained which had lost or regained bacteriocinogenic properties concomittantly with loss and gain of hemolytic properties (Brock and Davie,

1963). Deibel et al. (1963; Deibel, 1964a) have discussed hemolytic capacity as a taxonomic tool for differentiation of the enterococci.

Most of the streptococcal bacteriophage studies appearing through 1960 have been reviewed by Groman (1961) and will not be repeated here. Recent attempts to clarify the interrelationships of the enterococci by bacteriophage typing have met with some encouraging results (Brock, 1964). This method of delineating subgroups within the enterococci has promise for the future. Certain other fecal streptococci also contain prophages (Ciucă et al., 1960; Brailsford et al., 1964), but phage-host interrelationships in species other than S. faecalis and S. faecium have not been studied extensively.

Transduction of enterococci has not been accomplished (Brock, 1964), so experiments such as those conducted by Allen, Sandine and Elliker (1963) on S. lactis (see also Sandine et al., 1962) cannot yet be carried out with the fecal streptococci. Possibly a genetic marker other than streptomycin resistance should be used. Transformation has not been reported to occur within the enterococci (Perry and Slade, 1962, 1963), except possibly at a very low frequency (Pakula, 1961). This genetic approach is yielding valuable information regarding certain other streptococci and closely related genera (see also Ravin and De Sa, 1964; Perry and Slade, 1964). The discovery that genetic exchange within some enterococci may be mediated by sexduction (Raycroft and Zimmerman, 1964) opens up another avenue for future study. Likewise, various studies on the utilization of base combinations and fractions of cellular DNA (c.f., Marmur et al., 1963) will aid in elucidating the interrelationships of the enterococci and other fecal streptococci.

In Conclusion

Taxonomy of the fecal streptococci, particularly the enterococci, has been dynamic. Several changes have resulted in changed specific epithets for certain strains. At other times, the name assigned to a microorganism has been changed as more information is obtained about it. Gunsalus, Niven and Sherman (1944), for example, discovered that three strains of "S. lactis R," an organism used for the assay of folic acid, possessed group D antigen and was, in fact, a strain of S. faecalis. Now, by using the criteria listed in Table 2, the organism would be called S. faecium

(Brock, Mosser and Peacher, 1963) or S. faecium var. durans (Deibel et al., 1963; Wyatt and Hartmann, unpublished data). The name of another, more infamous, strain of enterococcus has also been changed several times. Linden, Turner and Thom (1926) isolated a "S. lactis type" of bacterium from cheese that purportedly had caused an outbreak of food poisoning. This organism was found to be S. faecalis by Sherman, Smiley and Niven (1943), but is now considered to be quite typical of S. faecium (Deibel and Silliker, 1963). Changes such as these may be confusing to many investigators, but we hope that each change represents progress in taxonomy of this important group of bacteria.

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