THE ENTEROCOCCI AND RELATED STREPTOCOCCI[1]

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Quite out of line with the usual "Presidential Address" I have chosen to speak to you briefly about some of our work of the past few months. These studies have concerned the extension of the Lancefield serological technique to those species or types of streptococci which are usually considered "enterococci," as well as to certain related forms. In doing this I must make use of material which belongs quite as much to others as to me. These investigations have been made possible during a comparatively brief span of time, amid many other duties, only by the untiring industry of my associates, Dr. Floyd R. Smith, now of the University of California, and Mr. Charles F. Niven.

II

"The enterococcus," as this term is commonly used among bacteriologists, has about as much biological meaning as "the bear." The name enterococcus has indeed covered a multitude of sins and has served manifold purposes, not the least being that of a screen behind which the investigator could hide his ignorance of the organisms with which he worked. A few reactions only have been necessary in order to classify an organism as an enterococcus. Such properties as thermal resistance, bile tolerance, reducing action, and the fermentation of mannitol are in general characteristic of enterococci, but by no means peculiar to them. The ability to ferment mannitol has been considered a prime requisite for an enterococcus, but one important species in the group does not attack this substance.

In our own work on streptococci we have applied new criteria

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through which has evolved what we consider to be a true, or at least useful, enterococcus division of the genus *Streptococcus* (Sherman and Stark, 1934; Sherman, Mauer and Stark, 1937; Sherman, Stark and Mauer, 1937; Sherman and Wing, 1937; Sherman, 1937). So defined, the now known types of enterococci are the non-hemolytic *Streptococcus fecalis* and *Streptococcus liquefaciens*, and the hemolytic *Streptococcus zymogenes* and *Streptococcus durans*. Thus far, we have not encountered other streptococci which fully meet our specifications for an enterococcus, although, as will be shown later, at least one type comes close to so doing. However, it is not likely that such a nicely limited and picketed subdivision will long remain unviolated as our knowledge of the streptococci increases.

III

Streptococcus zymogenes was discovered by MacCallum and Hastings (1899), as the causative organism in a case of endocarditis. Although it has been reported from time to time through the years from cases of endocarditis and from human stools, this long-known organism has nevertheless remained little known. For fifteen years, this hemolytic streptococcus masqueraded around the laboratories of the most distinguished medical research institution in the world as a "dairy organism." In the meantime, this same streptococcus had been encountered by workers in dairy laboratories who, identifying it with the organism of MacCallum and Hastings, considered it the private property of the medical bacteriologist. It was upon cultures of this organism, isolated many years before from cheese, that Lancefield (1933) established her group D hemolytic streptococcus. Later, Lancefield and Hare (1935) obtained cultures of group D hemolytic streptococci from the human vagina and related them to the intestinal enterococci. On the basis of cultural studies of a few of Dr. Lancefield's cultures, the group D streptococcus was identified more specifically with *Streptococcus zymogenes* (Sherman, Stark and Mauer, 1937), and it is further shown in a paper now in press (Smith, Niven and Sherman, 1938) that all of the cultures contained in a large collection of *Strepto-*

coccus zymogenes, which had previously been identified by physiological reactions, were found serologically to belong to the Lancefield group D.

Although the Lancefield group D was established on cultures of *Streptococcus zymogenes*, and this is the only streptococcal type which has specifically been identified with that group, it is probable that some of the group D organisms which have been reported have in fact belonged to the hemolytic *Streptococcus durans* type, rather than the prevailing intestinal *Streptococcus zymogenes* which may be considered the most typical form of "hemolytic enterococcus." The unique characteristics which differentiate and widely separate these types from the other hemolytic streptococci have been presented in a number of papers, and need not be reiterated.

IV

An important addition to our knowledge of the serological grouping of streptococci has been made by the finding of Lancefield (1937) that strains of non-hemolytic enterococci, which had been identified as typical cultures of *Streptococcus fecalis* and *Streptococcus liquefaciens* by cultural studies and biochemical tests, belong to her group D. This development was of much interest to us from several points of view, and we have, therefore, sought to extend these observations.

More than fifty cultures of *Streptococcus fecalis*, which had been identified by physiological studies, have been tested serologically. These cultures were isolated from human feces, milk, ice cream and cheese; included also was one strain obtained in pure culture from a fatal case of endocarditis, sent to us by Dr. E. G. D. Murray, and a culture of a motile streptococcus, isolated from milk by Dr. C. B. van Niel. Without exception, these cultures were found to belong to the Lancefield group D. Likewise, more than twenty cultures of *Streptococcus liquefaciens*, isolated from milk and from plants, have been tested, and all gave good reactions with group D serum. It may therefore be said with fair assurance that *Streptococcus fecalis* and *Streptococcus liquefaciens* are both members of serological group D.

V

For more than ten years we have had a special interest in a hemolytic streptococcus, *Streptococcus durans*, which has been isolated from time to time from dairy products and which, innocently enough, has been at times a serious plague to the industry because of the overzealousness of health officials. The basic characteristics of this organism, which clearly mark it as different from any of the disease-producing hemolytic streptococci, were long ago determined and published. Purely on the basis of physiological characteristics, *Streptococcus durans* was related to the enterococci (Sherman and Wing, 1937), and more recently (Smith and Sherman, 1938) it has been found to be in fact an intestinal organism, apparently ranking second only to *Streptococcus zymogenes* among the prevailing hemolytic streptococci found in human feces.

It is true that if one wished to make the same fine distinctions, based upon the fermentation tests, as some recommend in the establishment of species among the hemolytic streptococci belonging to the Lancefield group A, many "species" would have to be recognized among the enterococci; but when minor fermentative differences are ignored, the three well-known enterococcus species, *Streptococcus fecalis*, *Streptococcus liquefaciens* and *Streptococcus zymogenes*, are very closely related. This relationship appears to be so close that we have contended elsewhere (Sherman, Stark and Mauer, 1937) that these types might well be considered as one species with its respective varieties. *Streptococcus durans*, on the other hand, is not so intimately related to the other known enterococci, and is separated by a sufficient number of correlated characteristics to justify, perhaps, a distinct specific designation. In table 1 are given a few selected characteristics which appear to substantiate this view.

Streptococcus durans has also been serologically identified as belonging to the Lancefield group D, but not without some difficulty. At first, when we had succeeded in making only rather poor group D sera, extracts of *Streptococcus durans* gave negative results, though slight reactions were obtained with some strains. Yet these sera were good enough to identify *Streptococcus zymo-*

genes, Streptococcus fecalis and *Streptococcus liquefaciens*. However, when potent group D sera were obtained, prepared against cultures of *Streptococcus zymogenes*, all cultures of *Streptococcus durans* gave positive reactions. In order to settle unequivocally the serological identity of *Streptococcus durans*, we have sought to make a group D antiserum using this organism as the immunizing antigen. All of these attempts resulted in complete failure until very recently, when we did succeed in obtaining an anti-durans group D serum which reacts with the extracts of the other enterococcus species, as well as with those of *Streptococcus durans*. It can now, therefore, be concluded that *Streptococcus durans*, in common with the other three enterococcus types,

TABLE 1

Some selected characteristics showing the interrelationships of the enterococci

SPECIES	HEMOLYSIS IN BLOOD AGAR	GELATIN LIQUEFIED	STRONG REDUCING ACTION	GLYCEROL FERMENTED	MANNITOL FERMENTED	SORBITOL FERMENTED	SUCROSE FERMENTED
S. fecalis	−	−	+*	±	+	+	±
S. liquefaciens	−	+	+*	+	+	+	+*
S. zymogenes	+	±	+*	+	+	+	+*
S. durans	+	−	−	−	−	−	−*

* Occasional variation from type reaction; extremely rare exceptions not noted.

Streptococcus zymogenes, Streptococcus fecalis and *Streptococcus liquefaciens*, does belong in group D.

It has been shown that all of the four now recognized species of the enterococcus group, as this group has been defined by us, belong to the Lancefield group D. More than two hundred cultures representing these four types, which had previously been identified by means of cultural tests, have been serologically identified as group D organisms without a single miss. This looks easy, and it was in fact easy; but it was easy only because we had, so to speak, first caught our rabbits. The cultures had been identified by careful study and the application of a broad series of tests. With none of the previous standards which have been used for the identification of enterococci would such a correlation have been possible.

VI

Streptococcus lactis is a bacterium about which I can speak with much enthusiasm, considerable affection, a bit of pride, and even some heat. This, I feel sure, is the organism which a distinguished governor of a progressive state, preëminent for its dairy industry, had in mind when, upon welcoming the members of this Society to the State Capitol, he addressed us on the powers and magic of "This Bacteria."

My own interest in streptococci has been superficial, largely taxonomic in nature, aimed chiefly at the differentiation and identification of those species, of little general interest, with which we have had to deal in our work. If there is anything of originality which has been applied in these expeditions of mechanical moss gathering, it is contained in the work done many years ago (Sherman and Albus, 1918) which established for the first time the precise identity of *Streptococcus lactis*. The definition then arrived at has stood thus far. But the similarity of *Streptococcus lactis* and *Streptococcus fecalis* in certain characteristics was noted years earlier, and *Streptococcus lactis* was not recognized as an independent species in the better-known early classifications of the streptococci. Although differences between these organisms have been pointed out from time to time in papers from our laboratory, most of the supposedly more authoritative compilations on the streptococci have not admitted *Streptococcus lactis* as a specific type. It is frequently dignified by some such reference as "the so-called *Streptococcus lactis* of the dairy workers." The "dairy workers," on the other hand, have had full confidence in the integrity of their organism as a distinct biological entity, and, as is usually the case, it is those who have worked intimately with the subject who know what they are talking about.

The finding that *Streptococcus fecalis* is a member of the Lancefield group D was indeed grist for our mill. We have naturally had an interest in testing *Streptococcus lactis* extracts against group D antisera. A substantial number of cultures have been so tested and not one has given the slightest reaction. *Streptococcus lactis* is, therefore, as clearly differentiated from *Streptococcus fecalis* serologically as it is physiologically.

In this connection we have made a few attempts, not thorough ones, to produce an anti-lactis group serum using *Streptococcus lactis* as the immunizing antigen. We have been rewarded only with sera which are type-specific but not group-specific. This, perhaps, is the expected finding in view of the works of Hitchcock (1924) and of Lancefield (1925a, b) on the non-hemolytic streptococci. But our few trials do not justify a conclusion that *Streptococcus lactis* contains no group antigen; only that it does not belong to serological group D, of which *Streptococcus fecalis* is a member.

At the risk of boredom and needless repetition, the characters which differentiate *Streptococcus lactis* from *Streptococcus fecalis* are given in table 2.

TABLE 2

Some selected characteristics of Streptococcus fecalis and Streptococcus lactis

SPECIES	LANCEFIELD GROUP D	GROWTH AT 45°C.	GROWTH IN 6.5 PER CENT NaCl	GROWTH AT pH 9.6	SORBITOL FERMENTED	GLYCEROL FERMENTED	MANNITOL FERMENTED
S. fecalis	+	+	+	+	+	±	+
S. lactis	−	−	−	−	−	−	±

This would appear to provide ample grounds upon which to base even a conservative "species" rating among bacteria.

VII

Among the clearly defined types of intestinal streptococci is *Streptococcus bovis*. This organism was first differentiated as a definite species by Orla-Jensen (1919) and its description has been further amplified by a few subsequent investigators (Ayers and Mudge, 1923; Sherman and Stark, 1931). It is the predominating streptococcus in the bovine mouth and intestine, but it also occurs in smaller numbers in the human intestine. *Streptococcus bovis* is more closely related to the so-called viridans streptococci and only rather remotely related to the true enterococci. One would have to have small regard indeed for physi-

ological characteristics, to classify *Streptococcus bovis* in the same group with the enterococcus streptococci. This is shown in table 3.

However, *Streptococcus bovis* does have some characteristics in common with the enterococci, such as the ability to grow at high temperatures, thermal resistance, and bile tolerance. In addition, a substantial proportion of strains of *Streptococcus bovis* ferment mannitol, though the majority do not attack this substance. A number of investigators have classified organisms of the *Streptococcus bovis* type as enterococci.

For the present work we had at our disposal a collection of more than one hundred strains of *Streptococcus bovis* which had

TABLE 3

Some selected characteristics of the enterococci and of Streptococcus bovis

SPECIES	GROWTH AT 10°C.	GROWTH IN 6.5 PER CENT NaCl	GROWTH IN 0.1 PER CENT METHYLENE BLUE	GROWTH AT pH 9.6	NH_3 PRODUCED FROM PEPTONE	STARCH HYDROLYZED	RAFFINOSE FERMENTED	INULIN FERMENTED
Enterococci	+	+	+	+	+	−	±*	−
S. bovis	−	−	−	−	−	+*	+	±*

* Some strains of *S. bovis* do not hydrolyze starch; the majority of the enterococci do not ferment raffinose; the majority of strains of *S. bovis* ferment inulin.

been isolated and carefully studied by Mrs. Pauline Stark. This collection included strains from the bovine mouth, bovine feces, beet pulp, normal human feces, and some additional strains which had been isolated from cases of ulcerative colitis and identified as the "Bargen streptococcus." Extracts of all of these cultures were tested with potent group D antiserum.

About one-half of the *Streptococcus bovis* cultures gave definite reactions with group D serum; about one-third gave absolutely negative results; while the remaining cultures gave slight "plus-minus" tests which, however, could not be confused with a definitely positive reaction. Among the cultures from each of the various sources enumerated above were some which gave posi-

tive reactions and others which were negative with group D serum.

The exact meaning of these reactions between *Streptococcus bovis* and antiserum prepared against group D streptococci would probably best be left for interpretation by a competent immunologist. Even if we were competent to judge, our work is not sufficient to justify an opinion as to whether this is merely a cross reaction or whether the enterococci and *Streptococcus bovis* have in fact a common antigen. We have, however, performed some simple absorption tests which may be worth mentioning. When absoption was attempted with whole cells of *Streptococcus bovis* some technical difficulties, probably not insurmountable, were met, so we resorted to absorption with the unconcentrated extracts of the cells. This involves a dilution of the serum, but it was sufficiently potent to give good reactions after being so diluted. Serum which had been absorbed with *Streptococcus zymogenes* extracts gave no reactions either with *Streptococcus zymogenes* or *Streptococcus bovis*. Although we were not able, within the allowable limits of dilution, to remove completely the antibodies of the serum when extracts of *Streptococcus bovis* were used, the titer appeared to be reduced to about the same degree for each of these organisms, only very faint reactions being obtained.

In order properly to elucidate the serological relationship of *Streptococcus bovis* to the enterococci, it would be desirable to have anti-bovis serum. We have made two attempts to produce anti-bovis group sera without success, though good type sera were obtained. Influenced by the results of earlier work on the non-hemolytic streptococci, we may have discontinued the experiments too soon when the expected result was obtained.

In connection with the results obtained with *Streptococcus bovis*, it is pertinent to mention a few tests which have been made with other viridans streptococci with group D antiserum. The *Streptococcus equinus* of the intestine of the horse shows some points of relationship to *Streptococcus bovis*. A number of cultures of *Streptococcus equinus* have been tested with group D serum but none gave a positive reaction, though half of them

gave very slight precipitates. Cultures of *Streptococcus salivarius* from the human throat gave only negative reactions. *Streptococcus thermophilus*, which has in common with the enterococci high thermal resistance, but which could not be considered otherwise closely related, was also tested with group D serum and all cultures gave completely negative results.

VIII

We have in our possession six cultures of a streptococcus which does not belong to the Lancefield group D, but which would easily qualify as an enterococcus according to any previous definition of that group. It almost meets our own specifications; in fact, it straddles our fence, and is clearly more closely related to the true enterococci than to any other group of known streptococci. The streptococci show no more respect for the barriers which we have constructed than they do for those erected by others; they refuse to remain neatly placed in their respective cages.

The cultures of this streptococcus, isolated some years ago from human feces, caught the ever alert eye of Mrs. Stark, who recognized them as being quite unusual, and preserved them. They resemble *Streptococcus fecalis*, but diverge from it in some characteristics which seemed sufficiently significant so that we did not so classify them when making a study of that species a few years ago.

The experience with this enterococcus-like organism, that with *Streptococcus lactis*, and the perplexing results obtained with *Streptococcus bovis*, make it amply clear that had we started with the usual hazy conception of the nature of enterococci, this serological excursion could have resulted only in utter confusion, so far as the non-hemolytic organisms studied are concerned. But with more rigid physiological criteria, the cultural and serological findings dovetail very nicely indeed. From sources where, so far as published records reveal, an "enterococcus" is very superficially defined, reports have come to the effect that all enterococci belong to one serological group. Unless critical standards are used in the definition of an enterococcus, such

claims can be accurate so far as, and only so far as, habitat and statistical probability operate in their favor. True enough, when the source of the isolations is limited to the human intestine, chance alone might cause a very high percentage of hits; but the conclusion, under the conditions indicated, must remain essentially without substance. They must first catch their rabbits.

IX

In summary, the results of our attempts to extend the Lancefield serological technique, with especial reference to group D, to the enterococci and certain other streptococci, are presented in table 4.

In the study of streptococci a wedge, quite artificial in my opinion, has been driven deeper and deeper between the hemolytic and non-hemolytic species. Even the methods of study long ago began to diverge. Most of those interested in the hemolytic forms have long tended to limit their studies to a few reactions, apparently satisfied that the proper cultural tests had been found. Among the workers on the non-hemolytic streptococci were a few restless souls who kept increasing the number of tests used in the hope of ultimately finding some better ones. It is fair to say that before the advent of the Lancefield technique, more progress had been made in the classification of the non-hemolytic streptococci than in classifying the hemolytic types. Now, of course, the situation is quite reversed. Only time can tell just what sort of rude awakening is in store for us when methods comparable in accuracy to the Lancefield precipitin technique are developed for the non-hemolytic streptococci. From the results of our efforts to correlate the physiological and serological findings with the enterococci, I am encouraged to believe that sound progress has been made in the differentiation of at least some of the non-hemolytic streptococci.

The crying need in bacteriology is for new, not standard, methods. Standardization is ultimately desirable, but it is a vicious mirage during the formative stage of development. Bacterial taxonomy will not be put on a sound basis until better methods are employed; and I know of no way to arrive at new and im-

proved techniques without the employment of many tests. Again, it is necessary, at the present time at least, to make use of many reactions which appear to be without value, since no one can predict which ones will prove useful when applied to new organisms. I have mentioned the case of the enterococcus-like streptococcus which does not belong to the Lancefield group D. This streptococcus differs from *Streptococcus fecalis* in five physiological characteristics. Regardless of what one may choose to consider a "species," a difference in five characters is ample to

TABLE 4

The enterococci and certain non-hemolytic streptococci in relation to the Lancefield group D

SPECIES	DIVISION	GROUP D
S. fecalis	Enterococcus	+
S. liquefaciens	Enterococcus	+
S. zymogenes	Enterococcus	+
S. durans	Enterococcus	+
Unclassified	Intermediate	−
S. lactis	Lactic	−
S. cremoris	Lactic	−
S. bovis	Viridans	±
S. equinus	Viridans	−
S. salivarius	Viridans	−
S. thermophilus	Viridans	−

tell us that two bacteria are not the same. Of these five differences, however, only one is revealed by a test now commonly used in the study of streptococci. I mentioned earlier that the identity of *Streptococcus zymogenes* was not recognized over a period of time when it had attracted the attention of a number of workers on different problems. It is amusing to think that this organism would probably have been connected, in the first place, with what was already known, had some one had the wit to apply enough of the simple art of studying bacteria to determine that many of the cultures liquefied gelatin.

This picture of one of the needs of bacteriology is not attractive; it means much tedious and uninspiring labor; it is, nevertheless, the path which must be followed if order is to issue from our efforts to classify bacteria.

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