STREPTOCOCCUS S.B.E.: A STREPTOCOCCUS ASSOCIATED WITH SUBACUTE BACTERIAL ENDOCARDITIS

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In two preliminary publications (Loewe, Plummer, Niven, and Sherman, 1946; Niven and White, 1946) a report has been presented outlining the identities of various streptococcus cultures isolated from a large number of cases of subacute bacterial endocarditis. One of the largest groups recorded appeared to represent a hitherto unrecognized variety, or species. This streptococcus, which was recovered from approximately one-third of the patients afflicted with bacterial endocarditis, has several unique physiological characteristics which afford a relatively simple and accurate means for identification. Furthermore, all the strains which have been studied thus far have fallen into one of two serological types. For the sake of convenience this streptococcus is referred to as "*Streptococcus* s.b.e."

The successful use of penicillin-anticoagulant therapy for subacute bacterial endocarditis by Loewe (1945*a*, 1945*b*) has recently received wide attention. For the first time a large proportion of the patients suffering from this disease are being cured. As reported by Loewe, a majority of the cases which do not respond to the treatment are ones from which *Streptococcus* s.b.e. have been recovered.

The purpose of the present publication is to describe in detail the physiological characteristics of *Streptococcus* s.b.e.

SOURCES OF CULTURES

Streptococci recovered from the blood stream or heart vegetation of endocarditis cases have been kindly furnished from seven different hospitals widely distributed over the country. The relative frequency of *Streptococcus* s.b.e. among the cultures from each of these sources was practically the same. To date, 42 cultures of this organism from 36 cases of subacute endocarditis have been studied. In a few instances duplicate cultures were received, one of which had been isolated from the patient's blood or heart vegetation after death, a few weeks after the original blood culture had been taken. In all instances of this kind the duplicate cultures were found to be identical.

PHYSIOLOGICAL CHARACTERISTICS

As the cultures were received, they were examined microscopically for purity; all cultures about which there was any doubt as to purity were reisolated. All cultures were tested for the presence of the enzyme catalase, with entirely negative results. None produced detectable quantities of gas from the fermentation of glucose, and none was found to be bile-soluble.

Several, but not all, cultures of *Streptococcus* s.b.e. were tested on the various Lancefield sera representing groups A through G, with entirely negative results.

The various physiological tests used throughout this study were those described by Sherman (1937), plus a few others which had been developed in this laboratory since that date. Table 1 presents a summary of the general characteristics of this group of streptococci, along with the percentage of cultures deviating from the typical physiological pattern. These cultures were tested as they were received, such work extending over a period of three years.

TABLE 1

Physiological characteristics of Streptococcus s.b.e.

	TYPICAL CHARACTERISTICS	PER CENT HAVING TYPICAL CHARACTERISTICS
Greening of blood agar	+	98
Growth at 10 C	−	100
Growth at 45 C	±	55 (−)
Growth on 40% bile blood agar	+	72
Growth in 6.5% sodium chloride	−	100
Strong reducing action	−	100
Final pH in glucose broth	4.6–5.0	100
Arginine hydrolyzed	+	100
Sodium hippurate hydrolyzed	−	100
Starch hydrolyzed	−	72
Slime synthesis, 5% sucrose broth	+	95
Arabinose	−	100
Xylose	−	100
Maltose	+	100
Lactose	+	100
Sucrose	+	100
Trehalose	+	98
Raffinose	−	67
Inulin	+	81
Glycerol	−	100
Mannitol	−	100
Sorbitol	−	98
Salicin	+	100
Esculin	+	86

Streptococcus s.b.e. is a "viridans" type of streptococcus, generally producing a greening reaction on blood agar. However, one exception was encountered, this culture producing a complete but narrow hemolytic zone after 48 hours' incubation. In conformity with the "viridans" group as defined by Sherman (1937), none of the cultures was capable of initiating growth at 10 C; however, approximately half of them were unable to grow at 45 C.

Of possible significance is the fact that 30 of the 42 cultures were able to grow on blood agar containing 40 per cent bile. The two recognized species of viridans streptococci from human sources, *Streptococcus salivarius* and *Streptococcus mitis*, do not possess this ability.

None of the strains was able to grow in a broth containing 6.5 per cent sodium

chloride, nor did any show strong reducing action in litmus milk. These tests, along with the temperature limits of growth, would adequately differentiate *Streptococcus* s.b.e. from the enterococci.

None of the cultures was able to hydrolyze sodium hippurate. Twelve strains hydrolyzed starch. The final pH produced in a 1 per cent glucose broth varied between 4.6 and 5.0, with an average of 4.8.

A test of primary importance for the identification of *Streptococcus* s.b.e. is the hydrolysis of arginine. The established species of the viridans group of streptococci, namely, *Streptococcus bovis*, *Streptococcus equinus*, *Streptococcus thermophilus*, and *Streptococcus salivarius*, characteristically do not attack this amino acid. All *Streptococcus* s.b.e. cultures in our collection were able to do so. The most likely "species" to cause confusion in this respect is that heterogeneous group of streptococci occurring in the normal human throat classified as *Streptococcus mitis*. About one-third of these organisms hydrolyze arginine (Sherman, Niven, and Smiley, 1943). Of the 45 *Streptococcus mitis* cultures we have studied from endocarditis, 12 hydrolyzed arginine, but other physiological tests offered adequate differentiation between these strains and *Streptococcus* s.b.e.

It has been reported (White, 1944; Niven, Kiziuta, and White, 1946) that *Streptococcus* s.b.e. cultures have the ability to synthesize large amounts of a dextran in broth containing 5 per cent sucrose, but not on sucrose agar. All but two of the cultures tested possessed this ability. The sucrose cultures presented striking appearances, becoming extremely viscous after 24 hours' incubation, and many cultures actually became solidified upon further incubation. This simple test has proved to be of great assistance in identifying *Streptococcus* s.b.e. It is not a perfect one, however. Two cultures of *Streptococcus mitis* and seven *Streptococcus bovis* strains isolated from subacute endocarditis have also been found to synthesize a polysaccharide from sucrose in broth culture, some of these appearing very much like the *Streptococcus* s.b.e. strains.

Recently Hehre and Neill (1946) have reported that certain cultures of streptococci associated with subacute bacterial endocarditis synthesized large amounts of a dextran in sucrose broth, or on sucrose agar plates incubated anaerobically. From the description given by these authors, there would appear to be no doubt that the streptococci studied by them are identical with *Streptococcus* s.b.e.

Among the fermentation tests employed, two seemed to be of significance and aid in identifying *Streptococcus* s.b.e. The majority (34) of these organisms fermented inulin, whereas a minority (14) fermented raffinose. The ability to ferment inulin but not raffinose is a unique combination of fermentation characteristics among the recognized species of streptococci. Even though appreciable variation was experienced in the fermentation of these substances, they were of great help in recognizing this variety of streptococcus. If a streptococcus from endocarditis was found to ferment inulin but not raffinose, it invariably proved to be *Streptococcus* s.b.e.

STREPTOCOCCUS S.B.E. FROM OTHER SOURCES

Since the great majority of the streptococci from subacute bacterial endocarditis reported in the literature have been found to be of the viridans type, it

has been generally assumed that the primary source of these organisms is the mouth and throat. In line with this assumption, efforts were made to isolate *Streptococcus* s.b.e. from the normal human throat. Swabs were made around the tonsillar areas of 20 healthy individuals. Also, throat swabs from one dog and one cat were included in the experiment. The swabs were rinsed in a nutrient broth and plated immediately by the loop dilution technique on 5 per cent sucrose agar. The plates were incubated at 37 C for 24 hours. In order to avoid *Streptococcus salivarius* only those colonies showing no evidence of marked slime formation were isolated. Of the 680 streptococcus cultures isolated from these plates not a single one was found to be *Streptococcus* s.b.e.

In another attempt advantage was taken of the fact that most cultures of *Streptococcus* s.b.e. are much more tolerant to bile than the ordinary streptococci commonly found in the human throat. Swabs from the throats of ten healthy individuals were lightly streaked over the surface of blood agar containing 30 per cent bile. After incubation for 24 hours the surface growth was washed off with sterile broth and plated immediately by loop dilution on blood agar. Colonies were picked which showed greening reactions. Of the 42 streptococcus cultures isolated, not one was *Streptococcus* s.b.e.

In a third attempt throat swabs from 19 healthy individuals were plated on a blood agar containing 0.02 per cent sodium azide in order to aid in eliminating the growth of most of the organisms in the human throat other than those of the genus *Streptococcus*. After 48 hours' incubation 98 colonies, not of the indifferent type, were isolated. None of these proved to be *Streptococcus* s.b.e. Therefore, if *Streptococcus* s.b.e. exists in the normal human throat, the numbers present must be very small indeed.

Probably quite by accident, a culture of *Streptococcus* s.b.e. was isolated from the irrigations of a chronically infected maxillary sinus. This culture was identified by both physiological and serological methods. Washings from the infected sinuses of eight other individuals, however, yielded only *Streptococcus pyogenes*.

Another culture of *Streptococcus* s.b.e. has been isolated from an extracted tooth. This culture, which was sent to us by Dr. Loewe, was recovered from a patient suffering from subacute bacterial endocarditis caused by *Streptococcus* s.b.e. Other than these two cultures, strains of *Streptococcus* s.b.e. have not been isolated from any other source than the blood stream or heart of endocarditis patients.

NUTRITIVE REQUIREMENTS

Streptococcus s.b.e. grows somewhat more slowly than most streptococci on ordinary laboratory media. Another annoying feature of this organism is that it tends to die out rapidly in liquid media, requiring frequent transfers when broth cultures are used.

This organism, however, does not appear to be very fastidious in its nutritive requirements. Eighteen of the 20 cultures tested produced satisfactory growth upon serial transfer in a casein hydrolyzate medium, plus all the known B

vitamins. Two strains were studied in some detail as to their vitamin requirements. For both strains nicotinic acid, riboflavin, biotin, pyridoxine, and pantothenic acid were found to be essential for growth.

DISCUSSION

As a possible means of preventing the entry of *Streptococcus* s.b.e. into the blood stream with subsequent infection of the heart valves, it would be of great interest to determine the usual habitat of this organism. The results of the present investigation would seem to indicate that this group of streptococci is not a normal inhabitant of the human throat. Of the 820 streptococcus cultures isolated by selective procedures from the human throat not a single member of this group was found. In connection with independent investigations in this laboratory a very large number of viridans streptococci have been studied from several different sources. None of these streptococci has been found to be *Streptococcus* s.b.e. with the exception of the culture from an infected sinus. The *Streptococcus* s.b.e. culture received from Dr. Loewe which was isolated from an extracted tooth of a patient afflicted with endocarditis might be of some significance.

The fact that most strains of *Streptococcus* s.b.e. are relatively tolerant to bile would suggest that these organisms could survive, and possibly grow, in the human intestine. No direct attempts were made to isolate *Streptococcus* s.b.e. from this source.

Lamanna (1944) has studied a streptococcus strain from subacute bacterial endocarditis in some detail as to its morphological variations. An explanation was offered for the diphtheroid appearance of this strain when grown under certain conditions. This culture was received from Dr. Lamanna and found to be a typical strain of *Streptococcus* s.b.e.

Although there exists some degree of physiological variation among the different strains of *Streptococcus* s.b.e., no single culture was encountered which could not be recognized by its physiological pattern alone. In every instance its identity was confirmed by serological methods. Because of its unique display of physiological characteristics, the ease with which it can be characterized serologically, and its apparent medical significance, it would seem fitting to attach to this group of streptococci a specific name. White (1944) has proposed *Streptococcus sanguis*, which would appear to be an appropriate descriptive name. From the biological standpoint the group appears to be as homogeneous and distinct as many of the well-established species in the genus *Streptococcus*. Only by a study of a much larger collection of this group will the validity of a species name be ascertained.

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SUMMARY

A description of a hitherto unrecognized group of streptococci associated with subacute bacterial endocarditis is given. The streptococcus is of the viridans type, having a unique combination of physiological characteristics, including the ability to synthesize large amounts of a polysaccharide in a sucrose broth, to hydrolyze arginine, and to ferment inulin but not raffinose. The species name *Streptococcus sanguis*, suggested by White (1944), seems appropriate.

This streptococcus was recovered from approximately one-third of the cases studied.

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