STREPTOCOCCUS SALIVARIUS AND OTHER NON-HEMOLYTIC STREPTOCOCCI OF THE HUMAN THROAT

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The non-hemolytic streptococci of the human throat form, simultaneously, one of the oldest and least known groups of the genus. These familiar but ill-defined organisms are usually referred to as the "salivarius group," as "*Streptococcus viridans*" and "indifferent streptococci" according to the degree of greening produced in blood agar, or simply as the "mouth streptococcus."

In their early classical work Andrewes and Horder (1906) applied the names of *Streptococcus salivarius* and *Streptococcus mitis* to these organisms, *Streptococcus salivarius* being marked by its ability to curdle milk, *usually* to reduce neutral red, and *frequently* by the fermentation of raffinose and inulin, whereas these properties were *usually* lacked by *Streptococcus mitis*. The distinction was therefore a statistical one without a sharp boundary and they suggested that *Streptococcus mitis* might be considered only as a "variant by defect" of *Streptococcus salivarius*. Although Andrewes and Horder were inclined to consider *Streptococcus salivarius* "in its most typical form" as a distinct entity, in many cultures its separation rested only on the "tenuous milk reaction."

Safford, Sherman and Hodge (1937) carried the description of *Streptococcus salivarius* much further, showing that the typical cultures formed a very homogeneous group marked by the fermentation of raffinose and inulin, little or no greening in blood agar, vigorous acid production as shown by final pH values of 4.4 to 4.0 in glucose broth and the prompt coagulation of milk, together with many other correlating characteristics. However, as they did not feel justified in drawing the line rigidly on the basis of inulin fermentation, there was a gradual gradation from what they considered the "typical" *Streptococcus salivarius* through the entire group; the differentiation therefore remaining a statistical one without a sharp boundary line. In such compilations as that by Sherman (1937), in which all of the non-hemolytic streptococci of the human throat are considered as *Streptococcus salivarius*, the resulting "species" is a poorly defined and somewhat heterogeneous group.

Oerskov (1930) and Oerskov and Poulsen (1931) reported that certain non-hemolytic streptococci, when grown on sucrose or raffinose agar, produce a polysaccharide which results in the formation of large mucoid colonies, a property long known to be possessed by the heterofermentative streptococcal organisms of the genus *Leuconostoc* or *Betacoccus*. As they did not establish the homofermentative nature of their organisms, and as isolations were made from milk and animal sources as well as the human throat, it is probable that the doubt concerning the nature of the organisms dealt with prevented a proper appreciation of Oerskov's important discovery. The heterofermentative beta-

cocci were well known to be commonly found in vegetable products, milk, butter and cheese, and Orla-Jensen (1919) had reported them as also occurring in the alimentary tracts of herbivora.

In previous papers (Niven, Smiley and Sherman, 1941a, 1941b) it has been reported that a number of typical cultures of *Streptococcus salivarius* synthesize a large amount of a soluble levan from sucrose and raffinose, some strains producing in addition a smaller amount of a dextran. The levan is produced in either liquid or solid media, resulting in the latter case in large mucoid colonies. When grown on a well-buffered nutrient agar containing 5 per cent sucrose, *Streptococcus salivarius* produces a large, clear, soft, mucoid colony of about the diameter of those produced by coliform bacteria and yeasts. No other known species of streptococcus was found to produce large mucoid colonies on sucrose agar with the exception of occasional strains of *Streptococcus bovis*, but the polysaccharide formed in this case was an insoluble dextran.

TABLE 1

Some basic negative characteristics of the throat cultures which preclude the presence of pneumococci, betacocci, staphylococci, enterococci, the so-called lactic group of streptococci, and certain viridans streptococci

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Bile solubility: Not soluble in bile.
Homofermentative nature: Carbon dioxide not produced from glucose.
Catalase: Catalase was not produced.
Proteolysis: No digestion of casein; gelatin not liquefied.
Bile tolerance: No growth in 30% bile in blood agar.
Salt tolerance: No growth in broth containing 6.5% NaCl.
Methylene blue tolerance: No growth in 0.1% solution in milk.
Reducing action: Litmus in milk not reduced before curdling.
Thermal resistance: Killed at 60°C. for 30 minutes in skimmed milk.
Minimum temperature: No growth at 10°C. in broth or milk.

CULTURES STUDIED

The 331 cultures employed in this work for detailed study were isolated from the throats of normal persons. Although a number of kinds of non-hemolytic streptococci may occur in smaller numbers in human saliva, if the throat is swabbed, and the isolations made in a semi-quantitative manner, the predominating flora is found to be a relatively simple one.

That the present collection did not contain heterofermentative streptococci (the genus *Betacoccus* or *Leuconostoc*), pneumococci, nor members of the so-called lactic and enterococcus groups of streptococci is shown by table 1. It may be added that the low thermal resistance and low bile tolerance of these organisms would also appear to preclude the presence of such members of the viridans group as *Streptococcus bovis*, *Streptococcus equinus* and *Streptococcus thermophilus*.

In any definitive study of the so-called viridans streptococci it is essential to apply a number of "qualifying tests" in order to exclude members of certain related genera as well as representatives of the other groups of non-hemolytic

streptococci. As compared with the other major groups of the genus, the taxonomy of the viridans streptococci is in a highly unsatisfactory condition; but the common impression that the viridans streptococci compose a hopelessly heterogeneous conglomeration is largely due to the general failure to determine the basic nature of the organisms dealt with. Among the many cultures sent to this laboratory under the label of *"Streptococcus viridans"* have been not only non-hemolytic enterococci, group B streptococci and others, but betacocci and even staphylococci.

STREPTOCOCCUS SALIVARIUS

When the strains of the throat streptococci are limited to those which synthesize a levan from sucrose, a most remarkably homogeneous group of organisms results. This group agrees entirely with what was previously designated

TABLE 2

Additional characteristics of Streptococcus salivarius showing the remarkable homogeneity of the group

Polysaccharide synthesis: Large mucoid colonies on 5% sucrose agar.

Temperature limits: Growth at 45°C; no growth at 10°C. (Tests were conducted in broth and in milk.)

Blood agar: "Indifferent" reaction by more than 95% of cultures; no strain produced strong greening.

Final acidity: In glucose broth all strains gave pH values between 4.4 and 4.0; milk was curdled by all strains which fermented lactose.

Esculin: Split by all strains in broth, without bile.

Arginine; Sodium Hippurate: Not hydrolyzed by any strain.

Inulin; Raffinose; Sucrose; Maltose; Salicin: Fermented.

Mannitol; Sorbitol; Glycerol; Arabinose; Xylose: Not fermented.

Lactose; Trehalose: Of the 184 strains, 16 failed to ferment lactose; 14 did not ferment trehalose.

as the "typical" *Streptococcus salivarius* but is further defined by so many other correlating and apparently constant characteristics that one would appear to be justified in considering it a "species." It will be noted from table 2 that practically no variations were experienced in the more basic characters studied, this homogeneity extending even through the fermentation tests, with the exceptions only of lactose and trehalose which were not fermented by a few strains.

A striking characteristic of *Streptococcus salivarius*, as opposed to the *Streptococcus mitis* group and most other streptococci, is the relatively large size of the individual cells produced in liquid media, especially milk. This is true in neutral broth cultures, but may not be noticeable in those which reach a low final pH.

It is idle to try to define a "species" of bacteria, or even say whether or not such an entity can be defined, but *Streptococcus salivarius* as limited by the characters here presented forms as distinct, as clearly defined, and as easily identified and differentiated a unit as any group in the genus *Streptococcus*. Among the other correlating characters, all cultures of *Streptococcus salivarius*

which synthesize levan from sucrose ferment inulin, and *vice versa*, but other inulin-fermenting species of streptococci do not synthesize levan; although it would scarcely seem likely that this correlation in *Streptococcus salivarius* will be found to hold inviolably, no exception has yet been encountered among several hundred isolations which have been made from the human throat.

The question may logically be asked as to whether or not *Streptococcus salivarius*, so defined and differentiated from the other non-hemolytic throat streptococci, actually constitutes a true species in the biological sense. Probably not. However, the classification of bacteria is of value for practical purposes, the "species" being units which can be identified and differentiated from other closely related groups with some degree of certainty and ease. *Streptococcus salivarius* meets these requirements admirably. The pneumococci probably represent as well established and universally accepted species as any group of bacteria. In general practice the identification of pneumococci depends upon their synthesis of type-specific polysaccharides, but it is well known that the non-mucoid variants of the pneumococci do not produce their specific capsular polysaccharides. (Although it is true that the pneumococci contain a species-specific "C" antigen, so far as we are aware this important discovery of Tillett and Francis (1930) is not made use of in the practical problem of identifying the species.) It would therefore not seem unduly radical to consider the production of a chemically-specific polysaccharide by *Streptococcus salivarius* as a species-specific characteristic.

In other work in this laboratory (Niven and Smiley, 1942) it has been shown that *Streptococcus salivarius* can be grown on a chemically defined medium containing only certain amino acids and vitamins in addition to glucose, a reducing substance and inorganic salts. This medium has been tested with representative cultures of all of the more important groups and species of hemolytic and non-hemolytic streptococci. None of these, with the exception of a few strains of *Streptococcus bovis*, will grow on this medium. *Streptococcus salivarius* will not grow on the synthetic medium which has been used for enterococci and group B streptococci, nor will these organisms grow in the *S. salivarius* medium. Of especial interest in the present connection is the fact that the other more heterogeneous non-hemolytic throat streptococci, the *Streptococcus mitis* group, do not grow in this synthetic medium. It therefore appears that among the other claims of *Streptococcus salivarius* to the status of a "species" may be added its specific nutritive requirements.

Although tedious and detailed studies are essential in order to define more definitely the limits of a species, the main value of such work as this is to arrive at some simple tests which can be applied in the practical problem of quickly identifying the organism. The obvious simple presumptive test for *Streptococcus salivarius* is for the ability to produce large mucoid colonies on 5 per cent sucrose agar. Based on experience thus far this appears to be a relatively safe criterion in dealing with cultures from *human sources*. However, in accurate work it is necessary to be constantly on guard to exclude the heterofermentative betacocci and the occasional strains of *Streptococcus bovis* which have the ability to syn-

thesize polysaccharides from sucrose. The betacocci can be easily detected by their ability to produce carbon dioxide from glucose; in addition, they grow at 10°C. but do not grow at 45°C.—the opposite of the temperature growth limits of *Streptococcus salivarius*. As compared with *Streptococcus salivarius*, *Streptococcus bovis* differs markedly in its greater bile tolerance, higher thermal death point, the production of marked greening in blood agar, and usually by the ability of the majority of strains to ferment arabinose and actively hydrolyze starch (Sherman, Stark and Safford, 1938).

A DOMINANT SEROLOGICAL TYPE OF STREPTOCOCCUS SALIVARIUS

As is well known, the so-called viridans streptococci have not been shown to contain *group* or species-specific antigens comparable to those of the Lancefield (1933) serological groups of hemolytic streptococci. Although there is perhaps no reason why the viridans streptococci should possess such antigens, this nevertheless seems a little strange inasmuch as group-specific "C" substances are contained not only by hemolytic streptococci, but by the pneumococcus, the non-hemolytic enterococci and non-hemolytic varieties of other serological groups, and the so-called lactic group (*Streptococcus lactis*) of non-hemolytic streptococci. (Tillett and Francis, 1930; Lancefield, 1934, 1941; Sherman, 1938; Sherman, Smiley and Niven, 1940.) However, the investigations of Lancefield (1925a, 1925b) and Hitchcock (1924b, 1928) gave no evidence of such group-specific antigens in the viridans streptococci, although the existence of an antigen of broader than type specificity in the hemolytic streptococci was at that time known through the work of Hitchcock (1924a). On the other hand, the existence of many serological *types* among the viridans streptococci has of course long been known and these organisms have been considered very heterogeneous from the serological point of view, some investigators finding almost as many serological types as cultures which they studied.

Hitchcock (1928), working with inulin-fermenting "indifferent streptococci," principally from the human throat, found that about 50 per cent of these organisms belonged to one serological type, as shown by precipitin and agglutination reactions, which he designated as Type I. The rest of his cultures were serologically heterogeneous and he referred to them loosely as "Group X." Birkhaug (1927) likewise reported that 75 per cent of the inulin-fermenting, non-methemoglobin-forming streptococci, isolated in connection with his studies of rheumatic fever, belonged to one agglutinative type; and Hitchcock found Birkhaug's type to be physiologically identical with his own indifferent streptococci and to belong to his type I.

Small (1927) described "*Streptococcus cardioarthritidis*" which he considered a new species having importance as a causative agent in arthritis and rheumatic fever. He gave a brief description of his organism, the most revealing characteristics from the standpoint of its identity being its ability to ferment raffinose and inulin, its inability to ferment mannitol, and its inability to produce any change on blood agar. Of the 21 strains described, all were homogeneous in their action on blood, raffinose, inulin, and mannitol, but 7 of them failed to

ferment lactose. It should be added that although some of Small's cultures were obtained from clinical specimens most of them were isolated from the throats of patients. Small did not claim that all cultures of his "new species" were strictly homogeneous serologically but stated that 31 strains had been obtained which belonged to one serological type.

Although our own efforts to produce group-specific sera for *Streptococcus salivarius* have been unsuccessful, we have obtained a number of type-specific sera which have enabled us to make a partial survey of the serological types within this species. Of particular interest is the fact that about 40 per cent (83 of 184 strains) belong to one serological type as determined by the precipitin technique. That the unit with which we are dealing is a serological *type* rather than a *group* is indicated by the facts that all of the strains appeared to belong to one agglutinative unit and that in the immunization of rabbits there did not appear to be any broadening of the specificity of the sera as the injections were continued over prolonged periods of immunization. As this appears to be the largest serological type of *Streptococcus salivarius* we have designated it as "Type I."

It would be of particular interest to know what relation, if any, our type I *Streptococcus salivarius* has to the organisms which have been described by others. A culture of Small's "*Streptococcus cardioarthritidis*," obtained from the American Type Culture Collection, has been studied in detail and found to be not only an entirely typical strain of *Streptococcus salivarius* but also to belong to our serological type I. Through the kindness of Dr. Rebecca C. Lancefield we have obtained two cultures of Hitchcock's indifferent streptococci which belonged to his "Group X," his serologically heterogeneous group. These cultures have also been found to be typical polysaccharide-synthesizing strains of *Streptococcus salivarius*, but not to belong to our type I. It would therefore seem probable that Hitchcock's type I indifferent streptococcus and our type I *Streptococcus salivarius* are the same, since both have been found as the dominant serological type among the indifferent streptococci isolated from the human throat, but we have not as yet been able to locate any strains of Dr. Hitchcock's type I organism with which to test this point.

Our type I *Streptococcus salivarius* serum has given no cross reactions with representative cultures of the various serological groups of streptococci or other species of non-hemolytic streptococci, with the single exception that a very few strains of the so-called *Streptococcus mitis* group of viridans streptococci from the human throat gave reactions, usually weak, with the serum. Especial attention was given to the related species of viridans streptococci, *Streptococcus bovis* and *Streptococcus equinus*, but not a single cross reaction was experienced with these organisms although more than one hundred strains were tested. It should be recalled in connection with the few reactions which were obtained with other throat streptococci that Hitchcock also reported that a few strains of *Streptococcus viridans* reacted with his type I serum, which he was inclined to consider a cross reaction.

We have not done enough work with other serological types of *Streptococcus salivarius* to justify a report at this time. It may be stated, however, that there is at least one other type which encompasses a fairly large proportion of the strains of this species, and that an unknown number of other types exist. It is possible, however, that when these studies are extended, with the limitation of strains strictly to the physiologically homogeneous, "typical" *Streptococcus salivarius*, the group may not be found to be quite so hopelessly heterogeneous from the standpoint of numbers of serological types as has heretofore been thought.

The interesting work of Solowey (1942) appeared as this paper was being prepared for publication. She reports on the serological study of 205 strains of viridans streptococci obtained from subacute bacterial endocarditis, human throats and extracted teeth, a large proportion of which she identified as belonging to the *Streptococcus salivarius* group. Of the entire collection, 136 strains, or 66 per cent, could be classified as belonging within 14 serological units, 3 of these units (her groups I, II and IV) containing a significant number of strains. The dominant unit, "group I," contained 77 strains, about 38 per cent of the entire collection. Although referred to as "groups" it is not clear from her paper whether these serological units are *groups*, as this term is applied to the hemolytic streptococci, or serological *types*. It is hoped that through an exchange of cultures it may be learned whether or not our serological type I *Streptococcus salivarius* corresponds with one of Dr. Solowey's major units.

STREPTOCOCCUS SALIVARIUS AS AN INTESTINAL ORGANISM

As *Streptococcus salivarius* is usually the predominating organism in the human throat it is obvious that many millions of cells of this bacterium are swallowed each day by everyone. Because of some of its characteristics, such as its relative lack of tolerance for bile, there is considerable doubt as to whether or not it is able to grow in the human intestine, but it would seem probable that at least a small proportion of these organisms survive the acidity of the stomach and thus occur in the intestine. So far as we are aware, however, *Streptococcus salivarius* is not definitely known to occur in the human intestine, although in the older literature non-hemolytic streptococci from fecal samples have been recorded as *Streptococcus salivarius* on the inadequate bases that they did not ferment mannitol or did ferment raffinose.

Sodium azide has been used by a number of investigators as an inhibitory agent in studying respiratory enzymes and as a selective substance to facilitate the isolation of streptococci. Snyder and Lichstein (1940) have shown that the addition of sodium azide (0.01 per cent) to blood agar prevents the spreading of *Proteus* and largely inhibits coliform bacteria while permitting the growth of hemolytic and non-hemolytic streptococci.

In our efforts to isolate *Streptococcus salivarius* from human feces, we have successfully combined sodium azide and sucrose in order to get a medium which

has both selective and differential values for the isolation of this organism. The medium used has the following composition:

	per cent
Sucrose	5.0
Tryptone	1.0
Yeast extract	0.5
K_2HPO_4	0.3
Agar	1.5
Sodium azide	0.02
pH—7.0	

On this medium *Streptococcus salivarius* produces large mucoid colonies, as opposed to the characteristic small colonies of other streptococci, whereas coliform bacteria and other miscellaneous types which produce large colonies are

TABLE 3

Streptococcus salivarius and total streptococci in human feces as determined with sucrose-sodium azide agar

SAMPLE NUMBER	TOTAL STREPTOCOCCI (PER GRAM)	*Streptococcus salivarius*	
		(per gram)	(per cent of total)
1	9,100,000	9,000,000	99
2	540,000	530,000	98
3	8,000,000	6,800,000	85
4	1,000,000	840,000	84
5	159,000,000	129,000,000	81
6	1,780,000	1,320,000	74
7	23,000,000	15,400,000	67
8	33,000,000	7,200,000	22
9	14,200,000	2,000,000	14
10	4,700,000	500,000	11
11	81,000	5,000	6
12	67,000	2,400	4
13	1,600,000	13,000	0.8
14	4,300,000	2,000	0.05
15	1,640,000	1,200	0.07
16	192,000,000	<1,000	
17	3,800,000	<1,000	
18	3,500,000	<1,000	

largely or completely inhibited. It thus becomes possible to recognize *Streptococcus salivarius* colonies on sight with a high degree of accuracy. As was previously noted, occasional strains of *Streptococcus bovis* can produce mucoid colonies on sucrose agar and this organism is known to occur sometimes in small numbers in human feces, but mucoid strains of this species were not encountered in the samples of human feces examined.

Of 18 persons examined, *Streptococcus salivarius* was isolated from the fecal samples of 15. In these 15 samples the numbers of *Streptococcus salivarius* ranged from 1,200 to 129,000,000 per gram, 7 of the samples containing more than 1,000,000 per gram. As may be seen from table 3, in some samples *Strep-*

tococcus salivarius comprised a surprisingly large proportion of the total streptococci which grew on this medium.

Mucoid colonies which were counted as *Streptococcus salivarius* were verified as streptococci microscopically, and several cultures from each sample (80 strains in all) were subjected to detailed study. All of these proved to be typical strains of *Streptococcus salivarius* and to agree perfectly with strains obtained from the human throat. It is perhaps of interest to note that the fecal strains show no greater tolerance to bile than do those from the throat, all being inhibited by 30 per cent bile in blood agar while most of them fail to grow in the presence of 20 per cent bile. Of the 80 fecal strains tested, 36 (45 per cent) belonged to serological type I.

These results show the general occurrence of *Streptococcus salivarius* in the human intestine in appreciable numbers; however, they do not prove that this organism is able to grow in the intestine, though the large numbers found in some fecal samples would appear to indicate that growth probably does occur in some cases.

THE STREPTOCOCCUS MITIS GROUP

When *Streptococcus salivarius* is segregated, the remaining non-hemolytic streptococci of the human throat fall into a rather ill-defined and heterogeneous group which may be most appropriately designated as the *Streptococcus mitis* of Andrewes and Horder (1906). Although clearly differentiable from *Streptococcus salivarius* and several of the other species of viridans streptococci, the *Streptococcus mitis* group appears to be decidedly heterogeneous on the basis of the methods now available for the study of streptococci. It may well be a complex group which awaits more incisive methods for the segregation of its constituent units.

Some of the reactions which differentiate *Streptococcus salivarius* from *Streptococcus mitis*, and also show the relative heterogeneity of the mitis group, are given in table 4.

In addition to the inability of any culture of the *Streptococcus mitis* group to ferment inulin or to synthesize polysaccharide from sucrose, most of them differ from *Streptococcus salivarius* in a number of other characteristics. Among the 147 strains of the mitis group included in this work, not one differed from *Streptococcus salivarius* on less than five characters and the vast majority of them were different on six or more reactions. As opposed to *Streptococcus salivarius*, in addition to their inability to produce levan and to attack a number of the test substances, about 90 per cent of the strains of the *Streptococcus mitis* group produce marked greening of blood agar, very rarely a completely "indifferent" reaction; on the average, also, the mitis group does not reach so low a pH in glucose broth and many cultures fail to curdle milk.

That the *Streptococcus mitis* group may contain more than one distinct unit is futher indicated by the fact that an appreciable proportion of this group hydrolyzed arginine with the production of ammonia, a very rare property among the viridans streptococci (Niven, Smiley and Sherman, 1942). Cor-

related with the ability to hydrolyze arginine were the ability of a larger pro-portion of the strains to grow at 45°C., to produce a lower pH in glucose broth, to curdle milk, and to attack salicin and esulin. Although these statistical correlations are of no present practical value, they do add to the suspicion that *"Streptococcus mitis"* may indeed be a composite. But an attempt to divide the mitis group at this time would be a mere work of tessellation, without the emergence of a clearly-lined mosaic.

Inasmuch·as the heterogeneity of the *Streptococcus mitis* group has been emphasized, it should be pointed out, on the other hand, that it has some degree of homogeneity. Not one of the strains of this group hydrolyzed sodium hip-

TABLE 4

Relatively heterogeneous nature of the Streptococcus mitis group as opposed to the homogeneity of Streptococcus salivarius, and the differentiation of the two groups

	PER CENT GIVING REACTION	
	S. salivarius (184 strains)	*S. mitis* (147 strains)
Polysaccharide synthesized from sucrose.................	100	0
Inulin fermented..	100	0
Indifferent on blood (never strong greening)............	100	10
Esculin split...	100	21
Raffinose fermented.....................................	100	32
Growth at 45°C...	100	36
Arginine *not* hydrolyzed................................	100	57
Milk curdled (by lactose-fermenting strains)............	100	66
Salicin fermented.......................................	100	74
Trehalose fermented.....................................	93	15
Growth in 10% bile-blood agar..........................	91	13

Note. In addition to the characters given in table 1, all strains of the *Streptococcus mitis* group, as well as *Streptococcus salivarius*, were homogeneous in the following respects: sodium hippurate, mannitol, sorbitol, glycerol, arabinose, and xylose were not attacked; sucrose and maltose were fermented by all cultures. Final pH values in the *Streptococcus mitis* group ranged from 5.8 to 4.2, averaging about pH 4.5.

purate nor did a single strain ferment any of the following pentose sugars and higher alcohols: arabinose, xylose, mannitol, sorbitol and glycerol. These, however, are not very helpful as they are characteristics common to most species of the viridans streptococci. It should nevertheless be noted that, in addition to *Streptococcus salivarius*, a number of species of the viridans streptococci are clearly and easily differentiable from the *Streptococcus mitis* group: *Streptococcus bovis* by its greater bile tolerance and higher thermal death point, its ability to split esculin, and by its usual ability to ferment arabinose; *Streptococcus equinus* by its greater bile tolerance, its ability to split esculin, and its inability to ferment lactose; *Streptococcus thermophilus* by its much higher thermal death point and maximum temperature of growth, and its inability to ferment mal-tose. The little-known *Streptococcus acidominimus* of Ayers and Mudge (1922),

which occurs in the bovine vagina and occasionally in the udder and in milk, also differs markedly from the mitis group in that it hydrolyzes sodium hippurate and has extremely feeble acid tolerance, giving final pH values of from 6.5 to 5.6 in glucose broth and producing little or no visible change in litmus milk (Smith and Sherman, 1939).

Although the *Streptococcus mitis* group can be differentiated from the better defined species of viridans streptococci, it has not yet revealed clearly marked characteristics, uniquely its own, which allow its rapid and positive identification by the use of only a few simple tests.

THE LACTOSE-NON-FERMENTING, NON-HEMOLYTIC STREPTOCOCCI FROM THE THROAT AND OTHER HUMAN SOURCES

Since Andrewes and Horder (1906) described *Streptococcus equinus*, the lactose-non-fermenting streptococcus which predominates in the intestine of the horse, it has been the custom to designate as *Streptococcus equinus* the non-hemolytic, lactose-non-fermenting streptococci which are isolated from human sources. Floyd and Wolbach (1914), Broadhurst (1915), Holman (1916), Blake (1917), Arnold (1920), Porch (1941), and many others, have reported as *Streptococcus equinus* such streptococci from human sources, most frequently from the throat but occasionally from urine and from infections. Large collections of non-hemolytic streptococci isolated from the human throat usually contain a few strains which are unable to ferment lactose. The propriety of classifying such human throat strains as *Streptococcus equinus* was questioned by Safford, Sherman and Hodge (1937), on the basis of only one culture, because their strain appeared to resemble *Streptococcus salivarius* and to differ in a number of respects from the typical *Streptococcus equinus* from the intestine of the horse (Hodge and Sherman, 1937).

In our investigations of the non-hemolytic streptococci of the human throat an occasional culture is isolated which fails to ferment lactose, and 21 such strains were studied in detail in the present work and identified as *Streptococcus salivarius* and *Streptococcus mitis*. That the 16 strains so classified are unequivocally *Streptococcus salivarius* is shown in table 5 which presents some selected characteristics of the organisms in comparison with those of 59 cultures of *Streptococcus equinus*. Of the 16 lactose-negative strains of *Streptococcus salivarius*, 8 belong to serological type I.

Although the *Streptococcus mitis* group does not have many satisfactory distinguishing characteristics, it is fairly clearly different from *Streptococcus equinus* on the bases of the great disparity in the bile tolerance of the two organisms and the inability of the mitis type to split esculin. Table 6 shows rather definitely that the remaining 5 lactose-negative throat strains belong to the *Streptococcus mitis* group.

The results reported would appear to establish the identities of these lactose-non-fermenting streptococci; and it is probable that most such lactose-negative, non-hemolytic streptococci from the human throat belong to the salivarius-mitis group, rather than to that of *Streptococcus equinus*. It does not follow

that this will prove true in the case of cultures isolated from other human sources. For example, Porch (1941) has reported, as *Streptococcus equinus*, lactose-non-fermenting streptococci from the human genito-urinary tract which reacted with group B serum. Through the kindness of Miss Porch (now Mrs. Frechtling) and Dr. Justina H. Hill of the Johns Hopkins Hospital, we were

TABLE 5

Identity of lactose-non-fermenting organisms from the human throat as Streptococcus salivarius, and not as Streptoccus equinus

ORGANISM	NUMBER OF STRAINS	GROWTH ON 30% BILE-BLOOD AGAR	MARKED GREENING OF BLOOD AGAR	LEVAN FROM SUCROSE	RAFFINOSE FERMENTED	INULIN FERMENTED	REACTION WITH TYPE I SERUM
							per cent
Human throat (lactose-negative)...............	16	−	−	+	+	+	50
S. salivarius...............	170	−	−	+	+	+	40
S. equinus.................	59	+	+	−	−(90%)	−(73%)	0

TABLE 6

Apparent identification of certain lactose-non-fermenting organisms from the human throat as members of the Streptococcus mitis group, and not as Streptococcus equinus

ORGANISM	NO. OF STRAINS	GROWTH IN BLOOD AGAR WITH			ESCULIN ATTACKED
		30% bile	20% bile	10% bile	
Human throat (lactose-negative).........	5	−	−	−	−
S. mitis..................................	142	−	−	−(85%)	−(76%)
S. equinus...............................	59	+	+	+	+

TABLE 7

Identity of lactose-non-fermenting organisms from the human genito-urinary tract as group B streptococci, and not as Streptococcus equinus

ORGANISM	REACTION WITH GROUP B SERUM	HYDROLYSIS OF			GROWTH AT 45°C.
		Hippurate	Arginine	Esculin	
Genito-urinary (lactose-negative).............	+	+	+	−	−
S. mastitidis.................................	+	+	+	−	−
S. equinus...................................	−	−	−	+	+

able to study 8 of those cultures. They not only gave good precipitin tests with group B serum, but hydrolyzed sodium hippurate and arginine, did not split esculin, and failed to grow at 45°C.—all characteristic reactions of the *Streptococcus mastitidis* group. *Streptococcus equinus* does not hydrolyze sodium hippurate nor arginine, does split esculin, and grows at 45°C. From time to time we have tested a good many representative cultures of *Streptococcus equinus*, isolated from the intestine of the horse, with group B sera without

noting a reaction. The identity of the lactose-negative cultures from the human genito-urinary tract is shown in table 7.

The lactose-negative cultures from the genito-urinary tract were subjected to detailed study and found to be entirely typical of group B streptococci in other respects. They did not grow at 10°C., had weak reducing action, fermented trehalose, sucrose and maltose, but did not ferment raffinose, inulin, mannitol nor sorbitol. Hemolytic group B streptococci are well known to occur commonly in the human urinary and reproductive tracts and it is probable that non-hemolytic strains are not rare in these habitats. The inability of some group B streptococci to ferment lactose is also well known and Brown (1939) has shown the frequency of such strains among hemolytic cultures from human sources. It is also known that some bovine strains of group B streptococci, non-hemolytic as well as hemolytic, fail to ferment lactose (Sherman, Greisen and Niven, 1941).

Although it would appear that the general labeling of lactose-negative streptococci of human origin as *Streptococcus equinus* is an error, the possibility of its occurrence in human sources, especially the intestine, is by no means ruled out. In fact, Winslow and Palmer (1910), with the best methods of identification then available, reported *Streptococcus equinus* from human feces more than thirty years ago.

SUMMARY

A collection of 331 cultures representing the predominating non-hemolytic streptococci of the human throat was studied in detail; 184 strains were identified as *Streptococcus salivarius*, and the remaining 147 as members of the *Streptococcus mitis* group.

Streptococcus salivarius appears to be a remarkably homogeneous and clearly defined species, especially marked by its ability to synthesize large amounts of polysaccharide from sucrose, to ferment both raffinose and inulin, its inability to produce marked greening in blood agar, and many other correlating and apparently constant characteristics.

About 40 per cent of the strains of *Streptococcus salivarius* belong to one serological type (Type I). There is at least one other serological type which includes a fairly large proportion of the strains of this species, and an unknown number of other types.

The so-called "*Streptococcus cardioarthritidis*" was physiologically and serologically identified as *Streptococcus salivarius*.

By the use of a nutrient agar containing sucrose (5 per cent) and sodium azide (0.02 per cent), which has both selective and differential values for this purpose, it was shown that *Streptococcus salivarius* commonly occurs in large numbers in the human intestine. *Streptococcus salivarius* was isolated from the fecal samples of 15 of the 18 persons examined, the numbers of this organism ranging from 1,200 to 129,000,000 per gram.

The organisms identified as *Streptococcus mitis* form a rather heterogeneous and perhaps complex group, giving diverse tests in many of the reactions studied.

The group has, however, a considerable degree of homogeneity and can be readily differentiated, not only from *Streptococcus salivarius*, but from several other species of viridans streptococci.

Lactose-non-fermenting, non-hemolytic streptococci from the human throat are generally considered to be *Streptococcus equinus*. Of 21 such strains studied, 16 proved to be *Streptococcus salivarius*, whereas the other 5 were identified as members of the *Streptococcus mitis* group. Eight strains of lactose-non-fermenting organisms from the human genito-urinary tract, which had been reported as *Streptococcus equinus*, were found to be group B streptococci.

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