THE RELATION BETWEEN HEMOLYTIC AND NON-HEMOLYTIC STREPTOCOCCI, AND ITS POSSIBLE SIGNIFICANCE.

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If streptococci are separated into those which produce hemolysis and those which do not, it will be found that each of these two groups follows definite and different lines of classification. Of the various means of effecting a classification, the complement fixation reaction has proven the most satisfactory.

Study (1) of the non-hemolytic group by this method has shown that these streptococci are not uniform in character. This variability is due to the fact that there are two opposite elements or features in this group, and the position in the group, or individuality of any one member depends upon whether it partakes more of one or the other of these features. This variation is orderly and simply for convenience one of these opposite elements is called the right side, and the other, the left side of the group. On the other hand, study (2) of the hemolytic group, using the same criterion, has shown that all the hemolytic streptococci studied were nearly identical, the variation being too slight to measure by the complement fixation reaction. If these two studies are correct and the same orderly miscibility of characters prevails among streptococci as among other species, then, first, the two varieties should be related, and, second, since a unique element cannot be related in the same way to two opposites, the hemolytic variety should show a relation to either the right side or the left side of the non-hemolytic group. It was the purpose of the present study to answer this question and at the same time to compare the two varieties of streptococci in other ways.

The power to hemolyze red blood cells has been regarded as the feature which divides streptococci into two groups. This division was made possible largely through the work of Schottmüller (3), who by noting the effect of streptococcus colonies on blood agar was able to separate those which produce clearing about the colony, from those which either produce no effect, or produce a greenish discoloration. The term *viridans*, which is descriptive only of this last group, is often incorrectly applied to the whole non-hemolytic group. No descriptive term has been given to the streptococci which produce no effect on red blood cells, other than the term indifferent. In the present study these indifferent streptococci and the so called *Streptococcus viridans* have been included in the same group, called non-hemolytic streptococci. This capacity to produce hemolysis is of fundamental importance because the streptococci which possess this feature stand in striking contrast to those which do not. These points of difference embrace certain features which seem inconstant and of doubtful importance, and others which are constant and probably very important. Of the former class, there are differences in morphology, staining properties, growth in plain broth, solubility, and carbohydrate reactions; of the latter class, differences in immunizing power, in frequency of occurrence in the human body, in power to invade and produce disease, and finally, in immunological classification. The evidence on most of these points is derived from previous studies (1, 2) of twenty-eight strains of each group of streptococcus as well as from the work of others, which will be referred to later.

Differences in Morphology.—One difference in morphology already noted by others is the tendency of hemolytic streptococci to maintain their coccoid shape while the non-hemolytic streptococcus is usually a diplococcus in chains. But a more striking difference is the quite constant appearance of hemolytic streptococci in clumps of small cocci, when growing on blood agar. Such organisms usually show well developed chain formation when transplanted to fluid medium but in cultures on blood agar it is possible to distinguish the chain-forming non-hemolytic diplococcus from the clumps of smaller cocci and diplococci which are hemolytic.

Differences in Staining Properties.—In attempting to raise the virulence of certain non-hemolytic streptococci by mouse passage, it was noticed that the strains showed a tendency to become partially decolorized when stained by Gram's method. Under ordinary circumstances non-hemolytic streptococci are strongly Gram-positive. Hemolytic streptococci much more often are only weakly Gram-positive and sometimes strains are encountered which are Gram-negative when growing on blood agar The explanation of this inconstant phenomenon was not determined.

Differences in Growth in Plain Broth.—It was found that many more strains of hemolytic streptococci grew in plain broth in the form of a flocculent sediment with clear supernatant fluid than of the non-hemolytic variety. Although this was not a constant phenomenon with any strain and while many strains when first isolated grew in broth with clear supernatant fluid and later with turbidity, still after many subcultures fourteen out of twenty-eight hemolytic strains grew as a flocculent sediment in plain broth, while only three out of twenty-eight non-hemolytic strains grew in broth without turbidity.

Differences in Solubility.—The observations on the relative solubility of the two groups were confined to the action of antiformin on these bacteria, in the process of making antigens, as described elsewhere (2). The ease and rapidity with which powdered hemolytic streptococci went into solution in 2 per cent antiformin were in striking contrast to the tenacity with which powdered non-hemolytic streptococci resisted solution under the same conditions. In this respect the hemolytic streptococci resemble pneumococci.

Differences in Fermentation Reactions.—A comparison of the fermentation reactions of the two groups showed that the hemolytic variety was eminently a salicin fermenter. Thus twenty-two out of twenty-eight strains fermented this substance, while fourteen out of twenty-eight non-hemolytic strains showed similar activity The non-hemolytic streptococci frequently fermented raffinose and inulin, while the hemolytic streptococci rarely fermented these substances. Lyall (4) studied 99 strains of hemolytic streptococci and found that 75 per cent fermented salicin alone and 8 per cent fermented salicin and mannite. Hopkins and Lang (5),

Kligler (6), and Holman (7) present similar results. In general it can be said that the hemolytic streptococcus is more restricted in its fermentative activity than the non-hemolytic variety, and shows an almost selective tendency to ferment salicin.

Differences in Immunizing Power.—A marked difference was noted in the powers of hemolytic and non-hemolytic streptococci to produce immune bodies in rabbits. The method of immunization was the intravenous injection of washed bacteria. After four to six injections with non-hemolytic streptococci, the serum of the rabbits contained large amounts of agglutinating and complement-fixing antibodies. But after several months of similar injections of six different strains of hemolytic streptococci, the serum of the rabbits did not contain antibodies. When, however, the supernatant broth of the culture fluid was injected with the organisms, the appearance of the antibodies in the serum could be detected after six to ten injections of hemolytic streptococci. Neufeld (8) succeeded in immunizing rabbits actively against hemolytic streptococci and the sera of these rabbits contained both agglutinating and protective antibodies. His method was to inject intravenously killed bacteria, and after 10 days small doses of living organisms subcutaneously. The latter injection resulted in inflammatory reactions.

Subcutaneous injection of rabbits with living non-hemolytic streptococci in this study has failed to elicit agglutinating or protective antibodies. The fact that the products of growth activity were necessary to bring about immune bodies in the serum suggests the presence, in the case of the hemolytic streptococcus, of a separable substance such as is described for pneumococci by Dochez (9). In this connection attention must be directed to the fact that Neufeld in making agglutination reactions with hemolytic streptococci used the supernatant fluid of ascitic broth cultures. Even though this fluid contained streptococci there is a possibility that the resultant reactions were largely precipitin reactions. In the present study it was not possible to effect a satisfactory agglutination using whole broth cultures.

Occurrence in the Human Body.—Hemolytic streptococci are rarely found in normal throats. This has been found true by Smillie (10) and by Holman. In studying the throat cultures of patients with

acute rheumatic fever and subacute streptococcic endocarditis, hemolytic streptococci were rarely found. Non-hemolytic streptococci, on the other hand, were present in the mouths of normal individuals and we have only observed their absence in cases where frank infection by other bacteria resulted in their temporary exclusion from the cultures. Non-hemolytic streptococci, moreover, were found by Andrewes and Horder (11) to be present even in dust. They apparently live under a wide variety of conditions.

Differences in Invasive Power.—This striking difference in distribution between hemolytic and non-hemolytic streptococci runs parallel with the difference in invasive quality of these two varieties. The hemolytic streptococcus has established its position not only as an active invader and producer of disease but also as a cause of epidemics. The numerous milk-borne epidemics studied by Davis (12), Smith and Brown (13), and others were shown to be caused by this type of streptococcus. Certain purulent infections, abscesses, and puerperal sepsis have been ascribed to the agency of this organism. Recently it has played a most important part in the etiology of the bronchopneumonia and empyema following measles. On the other hand, non-hemolytic streptococci have played no proven part as the constant causes of disease. They depend, for their parasitic growth on the previous preparation of a focus of lowered resistance. The term "facultative parasites" used by Andrewes (14) in describing their invasive power is suitable. Even in subacute bacterial endocarditis when this type of streptococcus is found constantly in the blood stream, the growth of this variety of streptococci on a previously injured heart valve seems purely saprophytic. The possibility that certain non-hemolytic strains may be primary invaders of sound tissue is obvious, but emphasis is laid on the fact that in comparison with the hemolytic type the non-hemolytic type is extremely inert.

Difference in Immunological Classification.—The difference in classification of these two groups has already been described. The classifications referred to are based on immunological reactions and should, therefore, be of fundamental value. To study the relation between the two groups the same immunological reaction was used. At the time this study was made the sera of fourteen rabbits immunized to hemolytic streptococci were available, while the sera of only

TABLE I.

Relations between Sera Immune to Hemolytic Strains and Antigens of Representative Non-Hemolytic Strains.

Sera immune to streptococcus.	Antigens.																			
	Left side.								Right side.											Homologous antigen.
	MB	A84	A140	38D	A102	B4	B23	A141	A135	A30	A179	R	A65	A49	B39	B29	B26	A4		
B31a	4	4	4	4	4	3	4	2	0	0	0	0	0	0	0	0	1	0		4
	4	3	4	4	4	4	4	1	0	0	0	0	0	0	0	0	0	0		4
	4	2	3	3	4	3	4	0	0	0	0	0	0	0	0	0	0	0		4
	3	0	0	0	4	1	3	0	0	0	0	0	0	0	0	0	0	0		4
MC	4	4	4	4	4	4	4	3	0	0	0	0	0	0	0	0	0	0		4
	4	4	4	4	4	4	4	3	0	0	0	0	0	0	0	0	1	0		4
	4	3	4	3	4	3	4	3	0	0	0	0	0	0	0	0	1	0		4
	3	1	3	2	4	1	4	1	0	0	0	0	0	0	0	0	0	0		3
D10	4	4	3	3	4	4	4	1	0	0	0	0	0	0	0	0	0	0		4
	4	4	4	3	4	3	4	1	0	0	0	0	0	0	0	0	1	0		4
	4	4	4	2	4	1	3	1	0	0	0	0	0	0	0	0	0	0		4
	4	3	1	2	3	1	3	1	0	0	0	0	0	0	0	0	0	0		4
A96	4	4	1	2	4	1	1	0	0	0	0	0	0	0	0	0	0	0		4
	4	4	3	1	4	1	4	0	0	0	0	0	0	0	0	0	0	0		4
	4	4	1	2	4	1	4	0	0	0	0	0	0	0	0	0	0	0		4
	4	4	1	2	4	3	3	0	0	0	0	0	0	0	0	0	0	0		4
196	4	4	1	1	4	0	1	1	0	0	0	0	0	0	0	0	0	0		4
	4	3	4	2	4	2	4	1	0	0	0	0	0	0	0	0	0	0		4
	4	2	4	1	4	2	4	1	0	0	0	0	0	0	0	0	0	0		4
	3	0	3	0	3	0	3	1	0	0	0	0	0	0	0	0	0	0		4
B30	4	3	3	3	4	4	4	4	0	0	0	0	0	0	0	0	0	0		4
	4	3	3	3	3	4	4	3	0	0	0	0	0	0	0	0	0	0		4
	3	3	3	1	3	4	4	2	0	0	0	0	0	0	0	0	0	0		4
	3	1	2	0	3	3	3	0	0	0	0	0	0	0	0	0	0	0		4
β40	4	2	3	2	4	3	4	2	0	0	0	0	0	0	0	0	0	0		4
	4	2	2	2	3	3	3	1	0	0	0	0	0	0	0	0	0	0		4
	3	1	2	1	1	1	2	0	0	0	0	0	0	0	0	0	0	0		3
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0

TABLE I—*Concluded.*

Sera immune to streptococcus.	Antigens.																		Homologous antigen.
	Left side.								Right side.										
	MB	A84	A140	38D	A102	B4	B23	A141	A135	A30	A179	R	A65	A49	B39	B29	B26	A4	
Ch	4	3	1	3	4	0	4	0	0	0	0	0	0	0	0	0	0	0	4
	4	4	3	3	4	1	4	0	0	0	0	0	0	0	0	0	0	0	4
	4	3	1	1	4	1	4	0	0	0	0	0	0	0	0	0	0	0	4
	4	4	1	1	4	0	3	0	0	0	0	0	0	0	0	0	0	0	4
B31	4	4	1	3	4	1	4	0	0	0	0	0	0	0	0	0	1	0	4
	4	4	4	3	4	3	4	1	0	0	0	0	0	0	0	0	0	0	4
	4	3	2	1	4	3	4	0	0	0	0	0	0	0	0	0	0	0	4
	4	0	3	2	4	2	4	0	0	0	0	0	0	0	0	0	0	0	4
200	4	4	1	1	4	3	2	1	0	0	0	0	0	0	0	0	0	0	3
	4	4	2	2	4	4	3	0	0	0	0	0	0	0	0	0	0	0	4
	4	4	1	3	4	3	2	0	0	0	0	0	0	0	0	0	0	0	4
	4	4	2	3	3	1	3	0	0	0	0	0	0	0	0	0	0	0	4
201	4	4	2	2	3	1	3	0	0	0	0	0	0	0	0	0	0	0	4
	4	4	3	1	3	3	4	0	0	0	0	0	0	0	0	0	0	0	4
	4	2	1	1	2	1	2	0	0	0	0	0	0	0	0	0	0	0	4
	3	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	3
C6	4	3	0	1	4	0	1	1	0	0	0	0	0	0	0	0	0	0	4
	4	4	1	1	4	1	2	0	0	0	0	0	0	0	0	0	0	0	4
	4	4	1	2	4	1	2	0	0	0	0	0	0	0	0	0	0	0	4
	4	3	2	3	3	3	3	0	0	0	0	0	0	0	0	0	0	0	4
4	4	3	2	4	4	2	2	2	0	0	0	0	0	0	0	0	0	0	4
	4	0	1	3	3	1	1	1	0	0	0	0	0	0	0	0	0	0	3
	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
βad	4	1	0	0	4	0	4	1	0	0	0	0	0	0	0	0	0	0	4
	4	1	0	0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	4
	4	0	0	0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	4
	2	0	0	0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0

The numbers indicate plus signs, and the reading from above downward indicates the following dilutions: the top figure in each group, a dilution of 0.1 cc.; the second figure, 0.05 cc., the third, 0.025 cc.; the fourth, 0.012 cc.

In the tables 4 indicates no hemolysis; 3, 25 per cent hemolysis; 2, 50 per cent hemolysis; 1, 75 per cent hemolysis; 0, negative reaction or complete hemolysis.

two rabbits immunized to non-hemolytic streptococci of the left side and sera of three rabbits immunized to non-hemolytic streptococci of the right side were available. Limited time was responsible for the use of a limited number of sera. The plan was to test the sera immune to hemolytic streptococci against a number of antigens made

TABLE II.

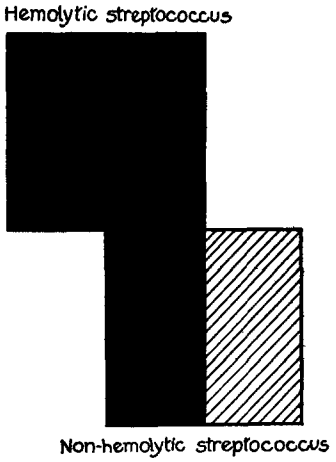
Relations between Sera Immune to Non-Hemolytic Strains and Antigens of Hemolytic Strains.

Sera immune to non-hemolytic strains.	Antigens.																											
	4	B30	B31	B31a	200	201	202	203	204	205	174	175	182	184	186	189	190	196	A96	C6	C7	CK	G	MC	D10	D14	βad	β40
Of the left side. MB	1	4	1	4	3	1	4	1	4	3	1	1	4	1	4	3	4	4	1	1	3	4	1	4	1	4	1	4
	3	3	1	3	1	1	3	0	3	2	4	1	3	0	3	3	4	3	2	1	1	3	0	3	0	1	0	3
	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A102	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4
	4	4	3	4	3	4	4	2	3	4	4	1	4	3	4	4	2	4	4	3	3	4	3	4	1	3	4	4
	2	2	0	1	2	1	2	0	1	0	3	0	3	0	2	3	0	2	2	1	1	3	1	1	1	1	2	1
	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Of the right side. B29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

from non-hemolytic streptococci. Eighteen antigens were used and they are arranged in Table I in the order that they maintained in their previous classification (1). In that classification the first eight antigens were on the left side of the chart, while the remaining ten came in the order printed, on the right side. The methods of im-

munizing animals, of preparing the reagents for the complement fixation reactions, and of performing the tests were the same as described elsewhere (2). Negative and positive controls were used in each test.

The results of these tests are shown in Table I. The lack of reaction with antigens on the right side was very noticeable, while definite reactions only with antigens on the left side was equally striking. The variation in the action of the different sera suggested either variations in the hemolytic streptococci used in producing these sera, or in the relative strength of the sera, or variations in the reactions themselves. The explanation of these variations was not established.



TEXT-FIG. 1. Scheme to represent the relation between hemolytic and non-hemolytic varieties of streptococcus.

Having shown that sera immune to hemolytic streptococci were related in a definitely limited way to non-hemolytic antigens of the left side, we undertook to inquire whether sera immune to non-hemolytic streptococci maintained a similar relation to hemolytic antigens. Table II illustrates the results of these tests. The two sera immune to non-hemolytic streptococci of the left side, gave definite, though at times weak reactions with the entire series of hemolytic antigens. The three sera immune to non-hemolytic streptococci of the right side gave no positive reactions with any of the hemolytic antigens.

In attempting to represent this relation graphically, the non-hemolytic variety can be pictured as a square consisting of two elements as in Text-fig. 1 and the hemolytic variety as a solid homogeneous square. Their relation is indicated by apposing the left side of the non-hemolytic streptococcus to the hemolytic streptococcus.

DISCUSSION.

The relation between these two varieties of streptococcus as established by these results seems to be orderly and definite. Any classification, to be considered valuable, should be fundamental. This will depend on the criterion used. With streptococci, the older classifications based on morphology and various cultural characteristics have proven unsatisfactory because the criteria do not bear any relation to the more fundamental questions of distribution in man and pathogenicity. These two factors determine the importance of bacteria in medicine. An immunological basis should be the test for classifying pathogenic bacteria.

If analogy with other species is instituted, this classification of the two varieties of streptococci as well as the relation between the two varieties is compatible with certain facts regarding the relative pathogenicity of the two groups. It may be stated that the non-hemolytic streptococcus is a heterogeneous variety, has wide distribution in man, and invades and causes disease only under exceptional conditions of preexisting infection or lowered resistance. The hemolytic streptococci encountered in this study form a unique variety, have very limited occurrence in man, and highly developed invasive and disease-producing qualities.

An analogous situation prevails with pneumococci. Group I pneumococcus is a unique variety (15), is not found in normal throats according to Stillman (16), and has marked pathogenicity. Group IV in the same classification of Dochez is a heterogeneous variety according to Olmstead (17), has moderately widespread distribution in normal throats, but is considered the least pathogenic of the pneumococci. Stillman (16) concludes:

"Among the pneumococci found in the mouths of healthy individuals, Type IV predominates, Type III is fairly frequent, and atypical organisms of Type

II are occasionally encountered. Organisms of these types give rise to a minority of cases of lobar pneumonia."

In the colon-typhoid group it is not possible to institute a perfect comparison because complete immunological studies of the colon group are not available. But the typhoid bacillus has been shown by Hooker (18) to be a nearly homogeneous variety. Its absence from normal individuals and its high invasive and disease-producing powers are well established. By fermentation reactions, at least, the colon bacillus has been shown to be a definitely heterogeneous variety. The other features of occurrence in normal individuals and low pathogenicity are well known.

These considerations indicate the value of the classification of streptococci using the complement fixation reaction as the criterion. They also argue for a fundamental correctness of the classification so established. If it can be proven by further investigation of these points that the variable groups of bacteria are the least invasive while the non-variable groups are the most invasive, a valuable principle in epidemiology would be established.

CONCLUSIONS.

1. The relation between hemolytic and non-hemolytic streptococci is orderly and arises from the fact that the former variety is unique while the latter is heterogeneous.

2. Analogous considerations of the classification, distribution, and pathogenicity of the streptococcus group and the pneumococcus and colon-typhoid groups show a definite parallelism.

3. These considerations suggest that unique varieties of bacteria associated with man are the more highly invasive, while the heterogeneous varieties are more saprophytic.

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