THE CLASSIFICATION OF HEMOLYTIC STREPTOCOCCI.

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In previous work (1) upon the classification of streptococci of the non-hemolytic variety, it was shown that no two of the twenty-eight strains investigated were exactly identical. The complement fixation reaction was the chief test by means of which the relation between the various strains was studied. This result was regarded as indicating a high degree of variability in this type of streptococcus.

The object of the present study was to determine whether the same variability prevailed among streptococci of the hemolytic type. Twenty-eight strains from various pathological sources were studied and their cultural characteristics, power to produce hemolysis of red cells, and behavior in the complement fixation reaction are reported in this paper.

As in the study of non-hemolytic streptococci the chief conclusions as to variability are drawn from a consideration of the complement fixation reactions between these hemolytic strains and their corresponding antisera. On account of the frequency with which these strains showed spontaneous agglutination when grown in plain broth, the agglutination test, which was likewise employed at first, had to be abandoned as a means of differentiation.

Methods.

1. For testing the effect of streptococci on red blood cells dilutions of a 24 hour broth culture were made in a row of small tubes, using plain broth as a diluent. Each tube contained 0.5 cc. of culture dilution, and the doses were graduated in the following manner: the first tube contained 0.5 cc. of culture, the second, 0.25 cc., the third, 0.12 cc., etc. To each tube 0.5 cc. of 5 per cent saline suspension of sheep red blood corpuscles was added. After incubation in

the water bath, at 37°C. for 1 hour, the mixtures were examined to determine whether hemolysis had taken place. Streptococci either (*a*) hemolyze the cells, (*b*) produce methemoglobin in the unhemolyzed cells, or (*c*) have no effect upon the cells. The method is not accurately quantitative but the results are more accurate than are those obtained with the blood agar plate method. Both methods were employed, however, for comparison.

2. Fermentation reactions were made in litmus milk and in media containing lactose, raffinose, inulin, salicin, and mannite as test substances. The media were prepared by adding 1 per cent of the carbohydrate test substance to Hiss serum water. In examining the effect of the streptococci on these carbohydrates a tube of each was inoculated with about 0.2 cc. of actively growing broth cultures and incubated for 10 days.

3. For the animal immunization a rabbit was used for each of the twenty-eight streptococci. Attempts were made at first to immunize these animals by intravenous injections of saline suspensions of killed streptococci at 4 day intervals. Although increasing doses equivalent finally to 50 cc. of broth culture were reached, the serum of these animals, except in a few instances, did not show complement-fixing antibodies, and much time was lost. Neither did a long series of small doses succeed in producing immune bodies in the serum. Finally, freshly killed broth cultures were resorted to. The first dose consisted of an injection of 1 cc. of a broth culture heated at 56°C. for 1 hour, the second, 2 cc., the third, 5 cc., the fourth, 10 cc., the fifth, 20 cc. When this dose was reached a second series of inoculations was begun, commencing again with 1 cc. of a broth culture and increasing the dose in the same manner as was done in the first series. All the injections were made at 4 day intervals. Animals treated by this method responded after six to ten injections by the appearance of complement-fixing bodies in the serum. As soon as a serum showed definite fixation with its own antigen, it was tested on the following day against the other twenty-eight antigens.

4. In the complement fixation reaction the various constituents were used in the following quantities: 0.05 cc. of streptococcus antigen, two units of complement; two units of anti-sheep ambo-

ceptor, and the following amounts of immune serum: 0.1, 0.05, 0.025 cc., etc. The complement-antigen-serum mixtures were made up to 1.5 cc. with saline solution and incubated in the water bath at 37°C. for 1 hour. Sensitized cells prepared by mixing 0.5 cc. of amboceptor dilution and 0.5 cc. of a 5 per cent suspension of cells were added and the tubes again placed in the water bath at 37°C. for 1 hour. At the end of this time the reactions were read.

The antigens were prepared as follows: The washed sediment of a 24 hour broth culture was desiccated *in vacuo*. The addition of absolute alcohol to the suspension of washed culture for the purpose of producing precipitation, which was the method employed in the study of non-hemolytic streptococci, was omitted, since this procedure was found to be unnecessary. After desiccation the sediment was ground into a fine soft powder and weighed. 10 mg. were dissolved in 5 cc. of a 2 per cent antiformin solution in the water bath at 56°C. and the solution was neutralized by using litmus paper as an indicator with 0.1 N sulfuric acid. The free chlorine was liberated by adding one or two drops of 5 per cent sodium thiosulfate. The absence of free chlorine was determined by testing with potassium iodide starch paper. The solution was then made up to 10 cc. with carbolized normal salt solution and centrifugalized. If a sediment appeared it was discarded. 1 cc. of the antigen then represented 1 mg. of dried ground bacterial sediment. This method was strictly adhered to, and thus the antigens used in these experiments represented equivalent amounts of the streptococci from which they were prepared.

RESULTS.

Table I contains a list of the organisms, their sources, and the chief cultural characteristics as outlined above. The sources of these streptococci include most of the pathological processes in which hemolytic streptococci are known frequently to operate. All are true streptococci as can be seen from their insolubility in bile. The observation of others that hemolytic streptococci are more often round individual cocci in chains, than diplococci in chains such as the non-hemolytic streptococci, was confirmed in the study of these strains.

TABLE I.

Source and Chief Cultural Characteristics of the Strains Used.

Streptococcus.	Source.	Gross appearance in plain broth.	Length of chains in plain broth.	Solubility in bile.	Appearance of colony on blood agar plate.	Effect of colony on blood agar plate.	Effect on suspension of red blood cells.
4	Abscess.	Turbid.	10–30 cocci in a chain; many short chains.	Insoluble.	Small round colony.	Clear zone of hemolysis.	Hemolysis; no methemoglobin formed.
B30	Preagonal septicemia; chronic endocarditis.	Nearly clear; soft sediment.	Short chains; 6–10 cocci.	"	" " "	" " "	" " "
B31	Blood culture. Sore throat.	Turbid; pasty sediment.	Many short chains; 6–10 cocci.	"	" " "	" " "	" " "
B31a	Metastatic abscess in B31.	" " "	Many short chains.	"	" " "	" " "	" " "
200	Pus. *Tenosynovitis.*	Nearly clear; soft sediment.	Moderately long chains; 6–20 cocci.	"	" " "	" " "	" " "
201	Preagonal septicemia; cirrhosis.	Clear; *flocculent* sediment.	*Long chains;* 10–20 cocci.	"	Moist, spreading.	" " "	" " "
202	Pus. Tenosynovitis.	Turbid; soft *sediment.*	Short chains; 4–12 cocci.	"	Small round colony.	" " "	" " "
203	Pus. Cellulitis.	Nearly clear; soft sediment.	Mixed lengths; 4–20 cocci.	"	" " "	" " "	" " "
204	Spinal fluid. Meningitis.	Clear; flocculent sediment.	Long chains; 10–20 cocci.	"	" " "	" " "	" " "
205	Throat culture. Sore throat.	Turbid; soft sediment.	*Many short chains.*	"	" " "	" " "	" " "

174	Blood culture. Sore throat.	Turbid; soft sediment.	Mostly short chains.	Insoluble.	Small round colony.	Clear zone of hemolysis.	Hemolysis; no methemoglobin formed.
175	Spinal fluid. Meningitis.	" " "	" " "	"	" " "	" " "	" " "
182	" " "	" " "	" " "	"	Moist, spreading.	" " "	" " "
184	Blood culture. Puerperal sepsis.	" " "	" " "	"	Checker colony.	" " "	" " "
186	Pus. Peritonitis.	" " "	" " "	"	Small round colony.	" " "	" " "
189	Pus. Knee-joint.	" " "	" " "	"	" " "	" " "	" " "
190	Pus. Mastoiditis.	" " "	" " "	"	" " "	" " "	" " "
196	Blood culture. Scarlet fever.	Clear; flocculent sediment.	Long chains; 30–40 cocci.	"	" " "	" " "	" " "
A96	Postmortem blood culture. Erysipelas.	" " "	Long chains.	"	" " "	" " "	" " "
C6	Pus. Pelvic abscess.	" " "	" "	"	" " "	" " "	" " "
C7	Blood culture. Septicemia.	Turbid; flocculent sediment.	Short chains and clumps.	"	" " "	" " "	" " "
CK	Pus. Pharyngeal abscess.	Clear; flocculent sediment.	" " "	"	" " "	" " "	" " "

TABLE I—*Concluded*

Strepto-coccus.	Source.	Gross appearance in plain broth.	Length of chains in plain broth.	Solubility in bile.	Appearance of colony on blood agar plate.	Effect of colony on blood agar plate.	Effect on suspension of red blood cells.
G	Pus. Purulent pericarditis.	Clear; flocculent sediment.	Short chains and clumps.	Insoluble.	Small round colony.	Clear zone of hemolysis.	Hemolysis; no methemoglobin formed.
MC	Blood culture. Septicemia.	" " "	" " "	"	" " "	" " "	" " "
D10	Blood culture. Mastoiditis.	" " "	" " "	"	" " "	" " "	" " "
D14	Blood culture. Septicemia.	Turbid; flocculent sediment.	" " "	"	Both small round and moist, spreading colony.	" " "	" " "
βad	β-hemolytic types of Smith and Brown.	Nearly clear; soft sediment.	" " "	"	Small round colony.	" " "	" " "
β40		" " "	" " "	"	Flat colonies. Checker " Ring "	" " "	" " "

All the strains caused hemolysis of red cells and produced a perfectly clear zone around their colonies on blood agar plates. Colony formation varied, apparently due to the condition of the medium, and both round and so called "checker" colonies were seen to grow from the transplant of a single round colony. Colonies also varied in their capacity to produce hemolysis on blood agar. Often a strain would produce more hemolysis when growing in the depths of the medium than when growing on the surface. A single strain might produce a zone of marked clearing around the colony at one time and a zone which showed scarcely any clearing at another.

Another feature of hemolytic streptococci is their occasional lack of Gram positiveness. This tendency to become Gram-negative was noticed in non-hemolytic strains, after many mouse passages, in an attempt to increase virulence. But the condition is frequently met with in hemolytic strains. The cause of this could not be determined.

Spontaneous agglutination in broth cultures was frequent in this series. Some of the strains which were transplanted many times gradually lost this characteristic and grew with greater turbidity, resembling non-hemolytic strains in this respect.

Only a few of the strains were tested as to their virulence and it was found that this could be raised by repeated passages through animals so that 0.0001 cc. of an 18 hour broth culture killed a white mouse in 24 hours. The experience of many workers has shown that the virulence of hemolytic streptococci can be raised to a high degree. The large number of mice required to determine the virulence of so many strains made it inadvisable to pay further attention to this point which has already been well established.

The fermentation reactions of these strains are strikingly limited and quite uniform (Table II). Although all the strains do not ferment the same test substances there is a noticeable uniformity in their action. In general it can be said that as a class they are weak fermenters compared with the non-hemolytic streptococci. The time necessary to produce acid and a clot in milk, for instance, is seldom less than 48 hours, and it usually requires longer, whereas most of the non-hemolytic strains produce this effect in 24 hours. None of the strains fermented raffinose or inulin. Only six failed to ferment salicin. Four fermented mannite; six fermented only milk

TABLE II.

Fermentation Reactions.

Streptococcus.	Effect on.					
	Milk.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.
4	±	+	−	−	−	−
205	+	+	−	−	−	−
174	+	+	−	−	−	−
175	⁄	+	−	−	−	−
189	+	+	−	−	−	−
MC	+	+	−	−	−	−
B30	+	±	−	−	±	−
B31	+	+	−	−	+	−
B31a	+	+	−	−	+	−
201	+	+	−	−	+	−
202	+	+	−	−	+	−
203	+	+	−	−	+	−
204	+	+	−	−	+	−
182	+	+	−	−	+	−
184	⁄	+	−	−	+	−
190	+	+	−	−	+	−
C6	+	+	−	−	+	−
CK	+	+	−	−	+	−
G	+	+	−	−	∓	−
D10	+	+	−	−	+	−
D14	+	+	−	−	+	−
A96	+	+	−	−	⁄	−
βad	+	+	−	−	+	−
β40	+	+	−	−	+	−
200	+	+	−	−	+	+
186	+	+	−	−	+	+
196	+	+	−	−	+	+
C7	−	+	−	−	+	+

− indicates no change; +, acid and clot; ∓, acid, partial clot; ±, acid, no clot; ⁄, slight acid, slight clot.

and lactose. These results agree, in the main, with those of Lyall (2) who studied 99 strains.

From Table III, which shows the results of the complement fixation reactions, it can be seen that there exists a striking uniformity in the action of this variety of streptococci. The antiserum of each strain gives fixation with the antigens of all the other strains, or, in other words, each strain causes fixation of complement with all the antisera.

TABLE III.

Results of Complement Fixation Reactions.

Rabbit sera immune to streptococcus.	Streptococcus antigens.																										
	C6	200	202	203	204	βad	B30	B31a	174	C7	D14	B31	201	CK	MC	G	186	175	196	189	β40	D10	A96	190	182	4	205	184
C6	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
200	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
200	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
203	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
204	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
βad	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	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	4	4	4	4	4	4
B30	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	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	4	4	4	4	4	4	4
B31a	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	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	4	4	4	4	4	4	4	4	4
174	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	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	3	4	4	4	4	3	4	4	4	3	4	4	4	4	4	4	4	3	4	3	4	4	4	4	4	4	3

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. Only three readings are given on account of lack of space.

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.

TABLE III—*Continued.*

Rabbit sera immune to streptococcus.	Streptococcus antigens.																											
	C6	200	202	203	204	βad	B30	B31a	174	C7	D14	B31	201	CK	MC	G	186	175	196	189	β40	D10	A96	190	182	4	205	184
C7	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	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	4	3	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4
D14	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	1	4	4	4	4	4	4	4
B31	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
201	4	3	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	3	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
CK	4	4	4	4	4	4	4	4	4	4	3	4	4	4	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	3	4	4	4	3	4	4	4	4	3	4	4	4	4	4	4	4
	3	4	4	3	4	3	4	4	4	3	1	3	4	3	4	2	4	4	3	4	0	4	4	4	4	4	4	4
MC	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	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	4	4	4	4	4
	3	3	4	1	4	4	4	4	4	4	4	3	4	4	4	3	4	3	4	4	4	4	4	4	4	4	4	4
G	4	4	4	4	4	4	4	4	4	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	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	3	4	4	3	4	2	4	4	4	4	4	4	4	4	4	2	3	3	4	4	3	3	4	4	4	4	4	4
186	4	4	4	4	4	4	4	4	4	4	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	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3
	2	3	3	3	4	4	3	4	4	4	3	4	3	4	4	4	4	4	4	4	3	3	4	4	4	4	4	2
175	4	4	4	4	4	4	4	4	4	4	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	3	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	2	3	3	3	4	4	2	4	1	3	1	3	4	3	3	4	3	3	3	4	1	1	2	4	3	3	3	2
196	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	2	4	4	4	4	4	2	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	3	3	4	2	3	4	4	4	4	2	1	1	3	4	4	4	4	4	4	4	3	3	4	4	3	4	3	4
189	4	4	4	4	4	4	4	4	4	4	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	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	3	3	1	1	3	4	0	4	0	3	3	1	4	3	3	3	4	3	3	3	3	1	3	4	1	3	3	1

TABLE III—*Concluded.*

Rabbit sera immune to streptococcus.	Streptococcus antigens.																											
	C6	200	202	203	204	βad	B30	B31a	174	C7	D14	B31	201	CK	MC	G	186	175	196	189	β40	D10	A96	190	182	4	205	184
β40	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	1	4	4	4	4	4	4	4	4	4	4	4	4
D10	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	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	3	4	4	4	4	3	4	4	4	4	3	4	4	4	4	4	3	4	4	4	4	3	4
A96	4	3	3	4	4	4	4	4	4	4	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4
	4	4	1	3	4	4	4	4	4	4	3	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4
190	3	4	4	4	4	4	4	4	4	3	2	3	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	2
	2	4	4	4	4	4	4	4	4	3	2	3	4	4	4	4	4	4	4	3	3	3	4	4	4	4	4	2
	1	3	4	3	4	4	4	4	4	3	1	3	4	3	4	3	4	4	3	3	1	2	3	4	4	4	4	2
182	4	4	4	3	4	4	4	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	4	4	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	1	0	1	0	4	4	4	4	4	2	0	0	3	1	4	1	4	4	4	4	1	3	4	4	4	3	4	2
4	4	2	4	3	3	4	4	4	4	3	3	2	4	4	4	2	4	3	4	4	4	4	4	4	4	4	4	4
	4	2	4	2	2	4	4	3	4	3	3	2	4	4	4	2	4	3	4	4	4	3	4	4	4	4	4	3
	3	1	3	0	1	3	3	2	3	3	1	1	3	3	3	0	3	0	3	3	3	1	3	3	3	3	0	2
205	4	3	1	1	4	4	3	4	1	4	1	1	4	4	4	4	4	4	4	4	1	2	4	4	1	4	4	1
	4	3	3	4	4	4	4	4	4	4	2	2	4	4	4	4	4	4	4	4	3	4	4	4	3	4	4	4
	4	4	3	4	4	4	4	4	4	4	0	1	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4
184	3	1	4	1	1	2	4	3	2	1	0	0	1	4	4	0	3	3	4	0	4	1	4	4	4	4	0	4
	4	1	4	4	4	4	4	4	4	3	1	0	4	4	4	3	4	4	4	4	4	4	4	4	4	4	3	4
	3	3	4	1	3	4	4	3	4	4	4	0	3	4	4	2	4	4	4	4	4	4	4	4	4	4	3	4

There are slight variations. Thus Serum 184 does not give fixation with Strain B31, and in other instances, the reactions were very weak. Whether this irregularity in results was due to deteriorated reagents could not be determined on account of the limited time available for completing the work. The antigens often lost their fixing power after they had been kept for some time and it was therefore frequently necessary to make fresh antigens. The sera of fourteen of the rabbits used were tested before inoculation, and in no case was there evidence of the non-specific fixation sometimes ascribed to normal rabbit serum.

DISCUSSION.

The strains of streptococci which form the basis of this study, while limited in number to twenty-eight, can be said to represent fairly the hemolytic variety. The strains were derived from the pathological processes usually associated with infection by this variety of streptococcus. The several variations in fermentative activity noted by others were present in this series. Variations in colony formation were observed which do not seem to be fundamental but apparently depend upon such factors as changing conditions of the culture medium. Variations in the clear zone produced about a colony occurred but seem to represent transitory differences in the degree of hemolytic activity, rather than a constant characteristic of the particular strain of streptococcus. The differences in growth in plain broth were not constant. There was a tendency on the part of the strains which showed spontaneous agglutination in their early transplants after isolation to grow with more turbidity after repeated transplantation. This feature was previously noticed in the case of non-hemolytic strains. The fermentation reactions of this group of streptococci, while displaying differences, revealed the fact that the members of this variety of streptococcus were weak fermenters, because two of the test substances, inulin and raffinose, were not fermented by any of the strains.

The differences noted above were of minor significance. On the other hand, the similarity between all the strains studied was strikingly emphasized by the complement fixation reactions. Although the strains came from different pathological sources and displayed many superficial variations in cultural activity, judged by the complement fixation test all the strains were nearly identical.

CONCLUSIONS.

1. The hemolytic variety of streptococcus is homogeneous, consisting of members that are nearly identical.

2. This homogeneity is most strikingly displayed in the behavior of these streptococci in the complement fixation reaction, all the strains studied reacting in a nearly identical way with all the antisera.

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