A STUDY OF STREPTOCOCCI FROM POST-GONORRHEAL PROSTATITIS BY A QUANTITATIVE METHOD OF AGGLUTINATION AND ABSORPTION

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This study includes 28 strains of streptococci from a series of chronic postgonorrheal infections of the prostate, 4 strains of streptococci from the normal urethra and 16 from various sources outside the genito-urinary tract (feces, nasopharynx, sputum of chronic bronchitis, empyema, tonsillitis, pleural fluid, scarlet fever throat, puerperal sepsis, and mastoid infection).

The general grouping of these streptococcal strains according to their action on blood agar is indicated in table 1.

Rabbits were immunized with 6 selected strains (table 1), all of which were found present in 2 successive cultures of prostatic exudate. Three of these were typical streptococci of the viridans type, causing the alpha type of hemolysis of Smith and Brown; one caused a narrow zone of hemolysis corresponding (alpha prime); the other two were typical hemolytic streptococci causing the wide hemolytic zones (beta). The rabbits were injected once a week with a 16-hour growth on ascites phosphate agar. Suspensions were made in salt solution and heated for 30 minutes at 53-56 C. The first injection was ¼ of a slant intravenously and with each succeeding injection the dose was increased by that amount until a maximum of one slant was given. The animals were bled and tested on the seventh day after the fourth injection. In the case of those animals whose serums did not contain sufficient agglutinins, the injections were continued at weekly intervals until a satisfactory titer was obtained, serum being taken for tests preceding each later inoculation.

The green streptococci generally seemed to produce agglutinins more readily than the hemolytic.

A standard technic for agglutination was used which gave consistent results on repeated trials.

A solid medium was used because homogenous suspensions then could be obtained regularly. The basis of this medium was nutrient agar in which dibasic sodium phosphate was substituted for sodium

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TABLE 1

AGGLUTINATION TITERS OF ANTISTREPTOCOCCIC SERUMS BEFORE AND AFTER ABSORPTION

Source of Streptococci	Classification of Streptococci According to Growth on Blood Agar	No. of Strain	Agglutination Titers of Serums for Prostatic Streptococci						Agglutination Titers of Serums for Homologous Prostatic Streptococci After Absorption						Nos. of Streptococcus Strains With Which Serums Were Treated
			Serum Against Strain 1	Serum Against Strain 12	Serum Against Strain 13	Serum Against Strain 14	Serum Against Strain 20	Serum Against Strain 22	Serum 1	Serum 12	Serum 13	Serum 14	Serum 20	Serum 22	
Prostate	Viridans-alpha	1	3200	400	100	10	0	0	0	4000	1200	800	400	600	1
Prostate	Viridans-alpha	2	3200	400	10	10	0	0							
Prostate	Viridans-alpha	3	3200	400	100	10	0	10	0	4000	800	800	400	800	3
Prostate	Viridans-alpha	4	3200	400	100	10	0	0							
Prostate	Viridans-alpha	5	3200	400	10	10	0	0	0	4000	800	800	400	800	5
Prostate	Viridans-alpha	6	3200	200	100	10	0	0							
Prostate	Viridans-alpha	7	3200	200	100	10	0	10							
Prostate	Viridans-alpha	8	3200	400	100	10	0	0	0	6000	800	800	400	800	8
Prostate	Viridans-alpha[1]	9	2800	200	10	10	0	10	0	4000	800	600	400	800	9
Prostate	Viridans-alpha	10	3200	400	100	10	0	0	0	6000	800	800	400	600	10
Prostate	Viridans-alpha[1]	11	3200	100	100	10	0	0							
Prostate	Viridans-alpha	12	1600	8000	800	800	0	10	2800	0	0	0	200	800	12
Prostate	Viridans-alpha	13	1600	8000	1200	800	0	10	2800	0	0	0	400	800	13
Prostate	Viridans-alpha[1]	14	1200	4000	1200	800	0	10	2400	0	0	0	400	600	14
Prostate	Viridans-alpha	15	1600	8000	800	800	0	10							
Prostate	Viridans-alpha	16	1600	8000	1200	800	0	0	2800	0	0	0	200	800	16
Prostate	Viridans alpha	17	1600	8000	1200	800	0	10							
Prostate	Viridans-alpha	18	1200	8000	1200	800	0	10							
Prostate	Viridans-alpha[1]	19	1600	8000	1200	800	0	10							
Prostate	Hemolytic-beta	20	10	10	10	10	400	10	2400	4000	1200	800	0	800	20
Prostate	Hemolytic-beta	21	0	0	0	10	400	0	2800	4000	800	800	0	600	21
Prostate	Hemolytic-beta	22	0	0	0	0	0	800	2800	6000	800	800	400	0	22
Prostate	Hemolytic-beta	23	0	0	0	0	0	800	2800	4000	800	800	400	0	23
Prostate	Viridans-alpha[1]	24	0	0	100	10	0	0	2400	6000	800	600	200	800	24
Prostate	Viridans-alpha	25	0	0	0	10	0	10	2800	4000	800	800	400	800	25
Prostate	Hemolytic-beta	26	10	10	10	10	0	0							
Prostate	Hemolytic-beta	27	10	0	0	10	0	0							
Prostate	Viridans-alpha	28	0	0	0	10	0	0							
Normal urethra	Hemolytic-beta	29	0	0	0	0	0	0	2800	6000	800	800	400	800	29
Normal urethra	Hemolytic-beta	30	0	0	0	10	0	0	2800	4000	800	600	400	800	30
Normal urethra	Viridans-alpha	31	0	0	0	0	0	0	2800	4000	800	800	400	600	31
Normal urethra	Viridans-alpha	32	0	0	0	0	0	0							
Feces	Viridans-alpha	33	2800	400	100	10	0	0	0	4000	800	800	400	800	33
Feces	Hemolytic-beta	34	0	0	0	0	0	0							
Nasopharynx	Hemolytic-beta	35	10	10	10	10	10	10							
Nasopharynx	Hemolytic-beta	36	10	10	10	10	0	100	2800	4000	800	800	400	600	36
Nasopharynx	Hemolytic-beta	37	10	10	10	100	0	10							
Nasopharynx	Viridans-alpha	38	100	10	0	10	0	10							
Sputum	Viridans alpha	39	10	100	0	10	0	0	2800	4000	800	600	400	800	39
Sputum	Viridans-alpha[1]	40	0	0	0	0	0	10							
Sputum	Hemolytic-beta	41	0	0	0	10	0	0							
Empyema	Hemolytic-beta	42	0	0	10	10	0	0							
Tonsil	Hemolytic-beta	43	0	0	0	10	0	10	2800	6000	800	800	400	800	43
Tonsil	Viridans-alpha	44	0	10	0	0	0	0	2400	4000	800	800	400	600	44
Pleural fluid	Hemolytic-beta	45	10	10	0	10	0	10							
Scarlet fever throat	Hemolytic-beta	46	10	10	0	0	0	0	2800	6000	1200	800	400	600	46
Puerperal sepsis	Hemolytic-beta	47	10	10	10	10	10	10							
Mastoid	Hemolytic-beta	48	10	10	10	10	0	10	2800	4000	800	800	400	800	48

chloride. The buffer effect of the phosphate is well known. Ascites fluid was added to the melted agar in the proportion of 1 part of fluid to 3 parts of agar. The ascites fluid was previously heated to 56 C. for one hour. Plates were used because the surface available is several times that of a slant with an equal quantity of medium. Approximately the 12-hour growth on one plate of a stock strain was suspended in 1 c c of sterile distilled water. The resulting heavy suspension was taken up with a capillary pipet and 2 or 3 drops placed on the surface of each 10 plates. The pipet was then sealed at the end in the flame and bent at an angle of 90 degrees 4 or 5 cm. from the end; now the drops could be quickly and uniformly spread with the bent pipet in a similar manner to that used in making blood smears, and without tearing a less solid medium than could be inoculated with wire loops. The plates were incubated 12-18 hours, and suspensions of the growth in normal salt solution made in amounts of 1 c c per plate. The surface growth was scraped off with a bent capillary pipet and the suspension transferred to graduated tubes which were centrifugated for 20 minutes at high speed. A 50% suspension was made of the bacterial sediment and placed in the icebox as a stock emulsion for later agglutination and absorption tests. It was found that such concentrated suspensions in salt solution would keep several weeks in the refrigerator without deterioration so far as agglutination and absorption are concerned.

The streptococci and especially the hemolytic, which tend to clump spontaneously when grown in broth or on ordinary blood agar, lost this property after a few successive transfers of young cultures on ascites phosphate agar.

Agglutination tests were made with equal mixtures of serum dilutions and a 0.5% streptococcus suspension which were incubated at 52-56 C. for 2 hours when preliminary readings were made. The tubes were then reincubated over night and final readings made the next morning. However, there were only few variations in the two readings in a large number of tests.

A serum which agglutinated in a maximum dilution of 1 : 1,200 gave the same titers macroscopically with suspensions of 0.25, 0.5 and 1% of streptococci.

The tabulated agglutination results show that antistreptococcus serum 1 contains major agglutinations for 11 prostatic streptococci and minor agglutinins for 8 of similar origin. Only one strain of those tested from other sources gave a like reaction. This strain was isolated

from the feces of a patient with pyelonephritis. Three other anti-streptococcus serums—12, 13 and 14—contained specific agglutinins for 8 prostatic streptococci and group agglutinins for 11 other strains. The group agglutinins of serum 1 were present in rather higher dilution than usual but later absorption tests established the specificity. It may be recalled here that Barnes,[1] found group precipitins for streptococcus extracts in relatively high dilutions. Antistreptococcus (hemolytic) serums 20 and 22 each agglutinated its homologous strain and one other. The remaining strains, including 2 hemolytic, 2 viridans, and 1 alpha prime viridans could not be classified with any of the serums. Four streptococcal strains from the normal urethra were not agglutinated, and with the exception of one feces strain, the other 15 strains from sources outside the genito-urinary tract were not agglutinated except in low dilution. There was no cross agglutination between the hemolytic and viridans streptococci except in very low dilutions. Since two thirds of the streptococci or prostatic origin fall into two related groups and as streptococcus viridans is regarded as a heterologous group, the results seem to indicate some degree of specificity in the types which occur in chronic postgonorrheal prostatitis.

Absorption tests were made with selected strains of each group and also with several other strains which did not agglutinate or only in very low dilutions.

It was found that in order to obtain complete absorption in low dilution, it is necessary to use concentrated suspensions, the serums of higher titer requiring proportionately heavier suspensions.

The following method was used: Titration was first made to determine the amount of streptococci necessary to completely exhaust the homologous serum. Varying dilutions of the stock 50% suspensions were made (25, 12.5, 6.25, and 3.125%) in amounts of 0.1 cc each in small precipitin tubes 4-5 mm. in diameter and to these were added 0.1 cc of serum diluted 1:5. The mixtures were incubated for 2 hours at 53-56 C. with occasional agitation. Control serum tubes without bacterial suspension were also incubated. After incubation 0.1 c c of the fluid was withdrawn from each tube and placed in another set of precipitin tubes and an equal amount of 0.5% streptococcus suspension added to each treated serum, the untreated serum, normal serum, and normal salt solution. If the supernatant fluid was not sufficiently clear it was centrifugated; when a large number of strans were being tested about 20 tubes were centrifugated at a time by plugging the tips of the centrifuge tube containers with cotton and placing 4 or 5 small tubes in each container. One and one-fourth times the smallest percentage of streptococcus suspension necessary to remove the agglutinins were used for absorption by heterologous strains in cross absorption tests. Complete absorption could be obtained in 2 hours by this method.

[1] Jour. Infect. Dis., 1918, 22, p. 230.

No advantage was noted by heating the emulsion to 65 C. before use, because these mixtures were incubated at higher temperature than usually the case.

The results of the absorption tests indicate clearly that the two main groups are distinctly specific even though the antiserum for one of them was rather strong in common agglutinins.

DISCUSSION

There is a great deal of interest at the present time in the immunologic classification of streptococci and the relation of streptococci to various diseases. Havens [2] classified 93% of 292 strains of hemolytic streptococci from various sources into 3 groups by agglutination; Tunnicliff,[3] Bliss,[4] and Gordon [5] have established a definite immunologic group of certain hemolytic streptococci, isolated from scarlet fever. Any definite grouping of nonhemolytic streptococci so far has not been established. Krumweide and Valentine,[6] made agglutination tests with antistreptococcus serums, produced with endocarditis and tonsil strains, and noted cross agglutination with 3 endocarditis strains while several other strains of the same origin were not agglutinated. One prostatic streptococcus, which they included was not agglutinated by any of their serums.

Holman,[7] and Kinsella and Swift [8] believe that streptococcus viridans constitute a heterogeneous group members of which cause disease only in states of lowered resistance from preexisting infection or other causes. Howell [9] states that a classification of streptococcus could not be made from the results of complement-fixation tests. Barnes,[1] however, found the precipitins relatively specific in high dilution. Clawson,[10] concludes that the nonhemolytic group is widely heterogeneous from agglutination and complement-fixation tests. Williams, Unneberg, Goldberg and Hussey [11] state that in a series of influenza cases the "alpha streptococci" which were dominant "consist of multiple strains from the results of carbohydrate reactions and the action on standard blood agar medium." Bumpus and Meisser [12]

[2] Jour. Infect. Dis., 1919, 25, p. 315
[3] Jour. Am. Med. Assn., 1920, 74, p. 1387.
[4] Bull. Johns Hopkins Hosp., 1920, 31, p. 174.
[5] Brit. Med. Jour., 1921, 1, p. 632.
[6] Jour. Infect. Dis., 1916, 19, p. 760.
[7] Jour. Med. Research, 1916, 34, p. 377.
[8] Jour. Exper. Med., 1918, 28, p. 169.
[9] Jour. Infect. Dis., 1919, 25, p. 46.
[10] Jour. Infect. Dis., 1920, 26, p. 93.
[11] Jour. Immunol., 1921, 6, p. 53.
[12] Arch. Int. Med., 1921, 27, p. 326.

find that their results from animal experiments indicate that certain green producing streptococci from focal infection of the mouth produce a specific pyelonephritis. However, they do not state whether their strains were all of the same immunologic type. It seems that the streptococci in postgonorrheal prostatitis possess sufficiently specific features to warrant efforts to trace them back to the sources of infection.

SUMMARY

A homogenous emulsion of streptococci can be obtained uniformly from young growths on ascites phosphate agar plates.

The quantitative method of making suspensions of centrifugated packed bacteria is more satisfactory than other methods of computation such as counting or comparison with standard barium sulphate suspensions.

Two thirds of the streptococci isolated from chronic prostatic infections can be classified by agglutination into two related groups. This specificity seems to be limited to the viridans (alpha and alpha prime) types of streptococci.