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RELATIONSHIP BETWEEN AGGLUTINABILITY AND CERTAIN IN VITRO TESTS OF STAPHYLOCOCCI, STREPTOCOCCI, AND COLON BACILLI ISOLATED FROM PERSONS SUSPECTED OF HAVING CHRONIC INFECTION*

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AGGLUTINATION reactions have been used in attempts to appraise the significance of bacteria isolated from suspected foci of infection. In efforts to determine the reliability of these reactions it was reasoned that pathogenicity of the organisms should play a part in their relationship to the disease process. However, it would have been impractical to make animal inoculation tests on the twelve thousand cultures of staphylococci, streptococci, and colon bacilli used in these experiments. Therefore, the following tests were used because they had previously been shown to have given results parallel with certain pathogenic properties of the cultures. Pigment, hemolysis, and coagulase tests[1] were used for staphylococci; resistance to the bactericidal action of fresh, diluted, defibrinated guinea pig blood[2-4] was used for streptococci; and the electrophoretic migration velocity[5, 6] was used for the colon group. These will be referred to as in vitro tests.

For the agglutination reactions, pure cultures of streptococci were grown in brain heart infusion overnight, tested for purity, washed once with 1.0 per cent phenol in 0.85 per cent salt solution, and resuspended in it. The final concentrations were about 10 billions per c.c. Other bacteria were grown on solid media and suspended in the phenol saline without washing. Unless the suspensions were decanted before use, the control tubes often contained a deposit of bacteria and extraneous matter which was difficult to distinguish from specifically agglutinated particles. The methods described by Spicer[7, 8] and Mueller and Klise[9] were found useful for preparing suspensions of autoagglutinative cultures.

A series of tubes containing 1.0 c.c. of progressive dilutions of the patient's serum, ranging from 1:40 to 1:5,120, together with a saline control, were placed in a rack. To each tube was added 1.0 c.c. of the bacterial suspension. After being shaken for two minutes, the tubes were placed in a water bath at 50° to 55° C. for one hour. The tubes were inspected periodically, and the results read whenever the control tube began to show excessive sedimentation. Otherwise, the rack was left on the laboratory table until the following morning, and the results read at that time.

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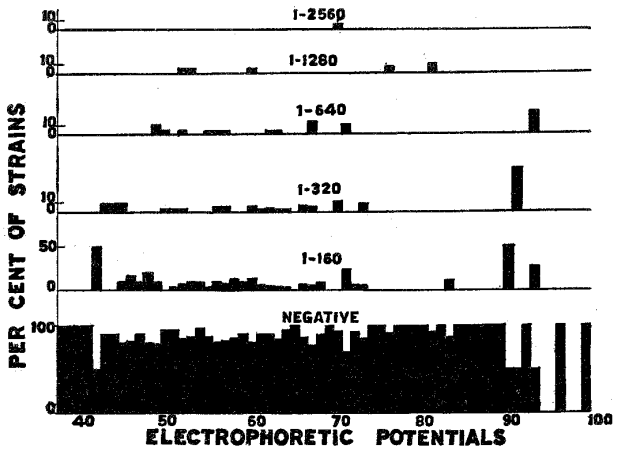

Fig. 1.—Comparison of electrophoretic migration velocity and agglutinability of 893 *B. coli-aerogenes* cultures.

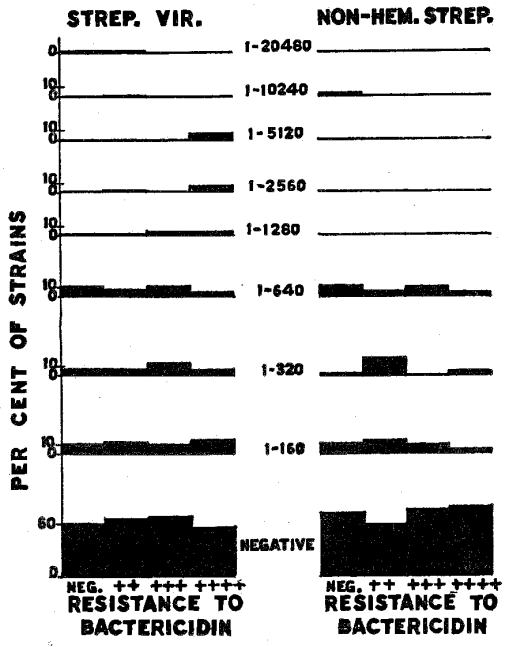

Fig. 2.—Comparison of resistance to the bactericidal action of fresh, diluted, defibrinated guinea pig blood and agglutinability of 627 cultures of *Streptococcus viridans* and 107 cultures of gamma type streptococci.

Some of the tests gave an increased titer when re-examined. This was found to have been caused by jarring the tubes when they were dropped back into the racks. In subsequent tests the tubes were lifted about 1 cm. and dropped back into the racks, and this was repeated a short while later. The results were read soon afterward. Because many of the sera agglutinated most of the organisms in dilutions up to 1:80, only reactions of 1:160 and over were considered positive.

Certain sera agglutinated a wide variety of bacteria, some of them in high dilution. Other sera gave only weak reactions. This suggested that agglutination was as much a function of the serum as of the bacteria.

The proportion of agglutinable cultures in different groups was as follows: *Staphylococcus aureus,* 47 per cent; hemolytic streptococci, 42 per cent; *Streptococcus viridans,* 36 per cent; *Staphylococcus albus,* 32 per cent; gamma type

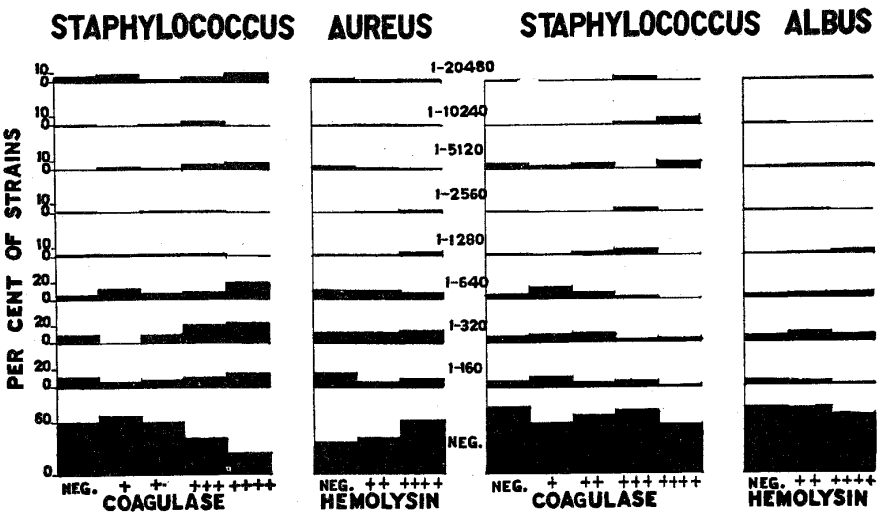

Fig. 3.—Comparison of hemolytic and coagulating properties with the agglutination titer of 368 cultures of *Staphylococcus aureus* and 885 cultures of *Staphylococcus albus.*

streptococci, 28 per cent; gamma type enterococci, 25 per cent; colon bacilli, 14 per cent; and alpha type enterococci, 10 per cent. In a series of 110 normal adults, Steinberg and Wiltsie[10] obtained with colon bacilli a titer of 1:100 in 3, 1:300 in 4, and 1:1,000 in 1 person.

TABLE I

RELATIONSHIP BETWEEN AGGLUTINABILITY AND IN VITRO TESTS OF BACTERIA ISOLATED FROM PERSONS SUSPECTED OF HAVING FOCAL INFECTION

TYPE OF ORGANISM	CULTURES EXAMINED	IN VITRO POSITIVE		IN VITRO NEGATIVE		PER CENT AGREEMENT (COLUMNS 1 + 4)
		AGGL. + (1)	AGGL. 0 (2)	AGGL. + (3)	AGGL. 0 (4)	
Staphylococcus albus	2975	7.2	11.3	16.3	65.3	72.5
Staphylococcus aureus	1122	52.3	40.6	3.5	3.7	56.0
Hemolytic streptococci	309	26.4	32.3	21.3	19.7	46.1
Nonhemolytic streptococci	861	13.8	29.8	16.7	39.7	53.5
Streptococcus viridans	4980	19.5	39.9	18.2	22.8	42.3
Colon bacilli	2148	1.9	20.4	12.2	65.5	67.4

Reactions considered positive:

Staphylococci—any strain which coagulated human and rabbit plasma.

Streptococci —cultures giving ++++ and +++ resistance to fresh, diluted defibrinated guinea pig blood (see references 2 and 3).

Colon bacilli —electrophoretic migration velocities of less than 52 microns/100 volts/sec./3.45 cm. (see references 4 and 5).

Agglutination—titers of 1:160 and over.

There did not seem to be a relationship between the agglutination titer and the strength of the in vitro reaction (Figs. 1, 2, and 3). Therefore, it will simplify comparison to consider only qualitative relationships between the in vitro tests and agglutinability (titers of 1:160 and over). These showed agreement in 72.5 per cent of *Staphylococcus albus* and in 67.4 per cent of colon bacilli, but other groups showed agreement in only about one-half of the cultures (Table I).

Agglutinable cultures did not give stronger in vitro reactions than inagglutinable cultures. Certain cultures which gave strongly positive in vitro reactions were inagglutinable. Rawls and Chapman[2] found that, when a streptococcus which was resistant to fresh guinea pig blood was not agglutinated by the patient's serum, it had greater power to produce arthritis in rabbits than did similar but agglutinable strains.

CONCLUSIONS

In vitro tests which had given results parallel with certain pathogenic properties were applied to cultures of staphylococci, streptococci, and colon bacilli isolated from persons suspected of having chronic infection. The cultures were also tested for agglutinability by the serum of the person from whom they had been isolated.

There was no close relationship between the in vitro reaction and the agglutination titer.

There was agreement in 67.4 per cent of colon bacilli and in 72.5 per cent of *Staphylococcus albus*, but there was agreement in only about one-half of the cultures of streptococci and *Staphylococcus aureus*.

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