THE PARTIAL PURIFICATION OF STAPHYLOCOAGULASE AND THE EFFECT OF CERTAIN PRESUMPTIVE INHIBITORS UPON ITS PLASMA-COAGULATING ACTION[1]

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Staphylocoagulase (Gratia, 1919) appears to require a cofactor present in plasma (Smith and Hale, 1944). Gerheim, Ferguson, and Travis (1948) propose "prostaphylocoagulase" as the name of the staphylococcal product, which would tacitly leave staphylocoagulase as the term indicating the active adduct of bacterial product and plasma cofactor. The plasma cofactor has been reported to be associated with plasma albumins (Gerheim, Ferguson, and Travis, 1947) and with plasma globulins (Tager, 1948).

The coagulation of plasma by living cultures of staphylococci is not inhibited by citrate, hirudin, fluoride (Much, 1908), oxalate (von Gonzenbach and Uemura, 1916), heparin (Rigdon, 1942), cobra venom, chlorazol fast pink, hydroquinone, or low oxygen tension (Walston, 1935). Gengou (1933) reported no inhibition following storage of broth cultures of staphylococci in the presence of 0.15 per cent phenol, 0.1 per cent permanganate, or 0.1 per cent trypaflavine, but complete inactivation by 0.3 per cent formaldehyde in 48 hours at 37 C. Neter (1942) reported that zephiran chloride 1:10,000 prevented, and 1:100,000 delayed, coagulation of oxalated human plasma by a broth culture of living staphylococci. Adequate controls ruled out any effect of zephiran at these concentrations on the coagulability of the plasma itself; the experiments were not decisive as to whether the action was on the organism or on the staphylocoagulase. Spink and Vivino (1942) found that sulfathiazole (in concentrations of 100 to 1,000 mg per 100 ml) accelerated the clotting action of sterile broth filtrates of coagulase-positive strains of *Staphylococcus aureus* on citrated human plasma. A similar effect of slightly less magnitude was shown by sulfanilamide; on the other hand, sulfapyridine was slightly, and sulfadiazine strongly, inhibitory to coagulation. All the sulfonamides studied showed an accelerating action in the presence of *p*-aminobenzoic acid, which by itself also had an accelerating action. Lominski and Roberts (1946) found, in 212 out of 348 human sera, a substance that neutralized coagulase. The inhibitory substance is precipitated with the serum globulins by ammonium sulfate and resists heating at 63 C for 30 minutes. Agnew, Kaplan, and Spink (1947) tested coagulating action and viability of staphylococci in the presence of penicillin and streptomycin. Penicillin inhibited growth in concentrations of 3,125 units (1.87 mg crystalline penicillin G sodium) per ml or higher; the inhibition of coagulase was minimal at a concen-

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tration of 49 units per ml, and almost complete at 25,000 units (15 mg) per ml. Streptomycin inhibited growth in concentrations of 4 mg per ml and higher; coagulation was inhibited in all concentrations studied, the lowest being 0.25 mg per ml. Farkas (1947), using Berkefeld filtrates of *S. aureus* cultures, found that bromine in a concentration of 0.3 per cent or more would inhibit plasma coagulation, and that similar inhibition occurred with 0.8 per cent iodine. The same author reports an inhibitory effect with tetrathionate, but does not specify the amounts used.

PREPARATION OF CONCENTRATED STAPHYLOCOAGULASE

The procedure which follows yields a concentrate of the bacterial product commonly called staphylocoagulase, possibly better called prostaphylocoagulase. A strongly coagulase-positive strain of *S. aureus* (which strain we designate arbitrarily as "L" and which was originally isolated from a chronically infected mastoid wound) is grown for 1 week in 100-ml portions of beef heart tryptic digest medium (pH 7.6). The cultures are then autoclaved 20 minutes at 120 C. This drastic step is justified by the observation of Gengou (1933) that the coagulating substance is highly resistant to thermal inactivation; it is easily inactivated by proteolytic enzymes (Walker, Schaffer, and Derow, 1947). Autoclaving has the advantages of removing certain interfering proteins by coagulation, rendering the cocci more readily separable in the centrifuge, and removing the hazard of contaminating the subsequent preparations with living cocci. The supernatant of the autoclaved cultures is adjusted to pH 4 with hydrochloric acid at room temperature, cooled to zero C, and centrifuged at that temperature. The precipitate is washed three times with one-tenth the original volume of filtrate: first with cold sodium acetate buffer, pH 4, ionic strength 0.1 (Boyd, 1945); next with cold acetate buffer of the same pH, ionic strength 0.01; and finally with cold distilled water. It is then suspended in a convenient volume (about 50 ml) of distilled water at room temperature and brought to pH 7.5 with sodium hydroxide, spun at room temperature, and the small insoluble residue discarded. The supernatant is adjusted to pH 4, centrifuged, and washed as before. It is finally resuspended in a minimal volume of distilled water, redissolved at pH 7.5, and dried in a vacuum from the frozen state. The product is promptly water soluble; it is not consistently stable either when dry or in solution. Depending upon the potency of the original culture supernatant, final yields vary between 25 and 60 mg per 100 ml of original supernatant, containing from 4 to 10 mg of nitrogen.

Coagulase activity of solutions of this product is determined by the same serial dilution procedure previously described (Walker, Schaffer, and Derow, 1947). The extent of purification is indicated by the determination of the ratio $\frac{\text{coagulase titer}}{\text{mg of nitrogen}}$ which in crude supernatants has a value of about seven and in our best preparations has reached values over 9,000.

TESTING PROCEDURE FOR INHIBITORS

Each substance under test was dissolved in a known concentration in human plasma. The coagulase titer of a staphylocoagulase solution was then determined simultaneously using the same plasma as a control substrate, and the plasma plus inhibitor as the test substrate. The results of tests performed are shown in table 1.

TABLE 1

Results of inhibition tests on staphylocoagulase

SUBSTANCE TESTED	FINAL CONCENTRATION PER ML	COAGULASE TITER	
		Test	Control
Tyrothricin in propylene glycol	0.05 mg 0.055 ml	16	256
Propylene glycol	0.055 ml	32	256
Propylene glycol	0.25 ml	8	256
Gramicidin in propylene glycol	0.1 mg 0.1 ml	8	256
Tyrocidin in propylene glycol	0.1 mg 0.1 ml	8	256
Propylene glycol	0.1 ml	8	256
Gramicidin in propylene glycol	0.01 mg 0.01 ml	32	64
Tyrocidin in propylene glycol	0.01 mg 0.01 ml	32	64
Propylene glycol	0.01 ml	32	64
Penicillin G	12,500 units	512	256
Streptomycin	62.5 mg	2	512
Bacitracin	250 units	256	256
Bacitracin	50 units	128	256
Hydrazine sulfate	5.2 mg	256	128
Sulfadiazine	2.5 mg	256	256
Sulfathiazole	2.5 mg	512	256
Sodium azide	1 mg	8	32
Sodium azide	0.33 mg	16	32
Sodium azide	0.1 mg	32	32
Zephiran	0.25 mg	128	128

SUMMARY

Concentrates of coagulase (prostaphylocoagulase) have been made from culture filtrates of *Staphylococcus aureus* in which the coagulase titer per milligram of nitrogen has been increased over a thousandfold. The coagulant activity of such partially purified staphylocoagulase has been found to be inhibited by streptomycin, propylene glycol, and sodium azide. It is not inhibited by penicillin, zephiran, bacitracin, tyrothricin, gramicidin, tyrocidin, hydrazine, sulfathiazole, or sulfadiazine.

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