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Rôle of Coagulating Principle of Staphylococcus Aureus in Relation to Invasiveness of this Microorganism.*

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Previous studies by one of us had shown that the localizing property of *Staphylococcus aureus* was referable to its ability to induce a powerfully injurious reaction in tissues inoculated with it. The severe injury to the capillaries, by increasing their permeability, doubtless allowed plasma proteins, including fibrinogen, to pass out into the inflamed area and be deposited there as coagulated plasma. The intense damage on the part of the pathogenic organisms to the draining lymphatics induced their early occlusion by the formation of fibrinous thrombi. The infected area became thus "walled-off" as early as one hour following the inoculation of the staphylococcus organism.[1] Trypan blue injected into such an area failed at this stage to diffuse to the tributary lymphatic vessels. Similar results were obtained with the Berkefeld filtrate of this microorganism.[2, 3] These results were in marked contrast to the delayed fixation of the dye found in the case of hemolytic streptococcus infection. In skin areas of rabbits inoculated with the latter organism, the lymphatic channels maintained their patency for almost 2 days before the inflammatory reaction became sufficiently intense to induce lymphatic blockage.[1] The conclusion was drawn that the *Staphylococcus aureus* is primarily a non-invading organism owing to its intense local effects which cause it to be circumscribed promptly by the formation of a mechanical barrier in the form of a fibrinous meshwork as well as thrombosed lymphatics in tissues distended with edema. The generalized systemic effects caused by the hemolytic streptococcus was referred to the relatively mild local effects produced by this organism which thus allowed its relatively free dissemination into the regional lymphatic vessels.

A number of authors including Much, Gratia, Gross, and Gengou, have shown that the *Staphylococcus aureus* organism and the filtrate

*This study was aided by grants from the DeLamar Mobile Research Fund and from the William W. Wellington Memorial Research Fund.

1 Menkin, V., *J. Exp. Med.*, 1933, **57**, 977.

2 Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 162.

3 Menkin, V., *Am. J. Med. Sci.*, 1935. In press.

of its culture are capable of causing oxalated blood to clot in the test tube.[4-7] Much called the clotting principle of *Staphylococcus aureus* a specific ferment which he designated *staphylokinase*. The material was found to be thermostable by Gratia, who gave it the name of *staphylocoagulase*.

Does "Staphylocoagulase" play a significant rôle in circumscribing a tissue area infected with *Staphylococcus aureus* by favoring the formation of a local fibrinous barrier? In other words, is the early fixation of trypan blue in the skin of rabbits treated with *Staphylococcus aureus* referable to the production of this clotting principle by the organism or is it due rather to the injurious action *per se* of the microorganism and its toxin? Evidence here presented definitely shows that fixation or the early "walling-off" reaction induced by staphylococcus has apparently no connection with the *in vitro* coagulating principle elicited by this microorganism or its products.

Gross had shown that the clotting reaction is readily elicited from the filtrate of a culture of *Staphylococcus aureus*.[6] For this reason all observations, with the exception of one experiment, were made on Berkefeld filtrates of a single strain of *Staphylococcus aureus* (strain 40). Most of the filtrates were obtained from 7-day cultures of the organisms in broth containing half the usual amount of meat and Parke-Davis peptone in place of the usual Difco peptone. Filtration was performed through a Berkefeld V filter. The filtrate inoculated in rabbit 11-86 was from a 20-day broth culture filtered through a Berkefeld N filter.

These filtrates were titrated in test tubes for their clotting potency. 1.5 cc. of the filtrate was injected intracutaneously in the foreleg of a rabbit. On the following day 2.5 cc. of 1% trypan blue in saline were injected into the inflamed area and a similar amount of dye was injected into the opposite forelimb to serve as control. Two to 3 hours later the regional lymphatics were exposed and studied for the presence of the dye by a technique previously described.[1,8] It was found that by treating the filtrate of *Staphylococcus aureus* with glacial acetic acid the "staphylocoagulase" substance could be separated from the principle that induces fixation. The acetic acid treated fractions of the filtrates were obtained by

[4] Much, H., *Biochem. Z.*, 1908, **14**, 143.

[5] Gratia, A., *C. R. Soc. de Biol.*, 1920, **83**, 584.

[6] Gross, H., *Z. f. Immunit.*, 1931, **73**, 14.

[7] Gengou, O., *Ann. de l'Inst. Pasteur*, 1933, **51**, 14.

[8] Menkin, V., *J. Exp. Med.*, 1929, **50**, 171.

adding to the filtrate 1/100 of its volume of glacial acetic acid and allowing this to stand overnight in the icebox. A precipitate formed. The supernatant fluid was decanted off and the precipitate taken up in its original volume of sterile distilled water; it was then brought up to a pH varying from 7.4 to 8.2 by the addition of N/20 NaOH. In this range the precipitate readily dissolved. The clotting capacity of a filtrate was found to be unaffected by varying its pH within the range described. Sterility was assured throughout by the addition of 0.5% phenol to the substances to be tested.

Titrations of the clotting properties of the substance to be tested were performed as follows: Blood was obtained from a normal rabbit by heart puncture, the sterile syringe already containing a small amount of sterile 4% sodium citrate in saline. The blood was centrifuged after sufficient citrate had been added to bring the total concentration to 2%; the supernatant citrated plasma was pipetted off with sterile precaution.

Dilutions of the "staphylocoagulase"-containing substance were made in small aseptic test tubes with sterile broth in the case of plain filtrates, and with sterile distilled water in the case of the acetic acid precipitates, the total volume being 0.5 cc.; to each dilution was added 0.5 cc. of the citrated plasma. The mixtures were then shaken and placed in a 37°C. incubator, and read at varying intervals, the final reading being taken after 48 hours, this having been found to give the most consistent measure of "staphylocoagulase". The end-point was taken as being that dilution where a definite clot, as opposed to fibrin threads, was formed.

The results of all the experiments are recorded in Table I. It is quite evident that the ability of trypan blue to be retained at the site of skin inoculated with either the filtrate of *Staphylococcus aureus* or its acetic acid precipitate bears no relationship to the *in vitro* clotting reaction. The filtrate of one strain of the microorganism produced fixation of the dye in the animal, although it failed to coagulate the plasma *in vitro* (Rabbit 11-86, Table I). The reverse state of affairs was brought about in the case of rabbit 11-46. The 2 phenomena of fixation and staphylocoagulase production are evidently entirely independent of each other. The intensity of one of these reactions does not in any way predict the trend of the other (*cf.* rabbits 11-86 and 11-46). Furthermore the acetic acid fractions indicate that although the fixation principle may be destroyed by chemical treatment, yet the *in vitro* clotting reaction persists strongly. The supernatant fraction of the acetic acid treated filtrate or the acetic acid precipitate of sterile broth produced neither

TABLE I.

In Vitro Clotting Principle of *Staphylococcus aureus* in Relation to Fixation of Trypan Blue at Site of Inflammation.

Rabbit No.	*Staphylococcus aureus* or its acetic acid treated fractions	Time between injection of filtrate (or fraction) and dye	Total duration of inflammation	Fixation of dye at site of inflammation	*In vitro* clotting reaction in 48 hr.
		hr.:min.	hr.:min.		
11-86*	1.5 cc. filtrate	21:05	24:00	+	0
11-46	1.5 " "	20:45	22:55	0	+++ (titer 1:620)
11-59	1.5 " "	21:10	22:50	+	+ (" 1:80)
11-52	1.5 " acetic acid precipitate	21:23	23:30	0	Fair (" 1:20)
11-53	1.5 " " " "	17:25	19:30	0	+
11-54	1.5 " " " "	18:50	21:00	0	+++ (" 1:320)
11-44	1.5 " " " "	21:00	23:20	0	++ (" 1:300)
11-57	1.5 " supernatant fraction of acetic acid treated filtrate	18:40	20:45	0	0
11-30	1.5 " acetic acid precipitate of sterile broth	21:00	23:20	0	0

*Filtrate of a 20-day culture of a strain of *Staphylococcus aureus* different from that used in remaining experiments.

fixation of the dye when inoculated into animals nor did it induce coagulation of citrated plasma in the test tube.

The "staphylocoagulase" precipitated from the filtrate of a culture of the microorganism when injected intracutaneously in a rabbit is absorbed from the skin by the tributary lymphatics, as indicated by the mild cellular reaction in the regional lymph nodes. Yet it fails to exert a sufficiently powerful local reaction to obstruct lymphatic drainage by the formation of a fibrinous barrier. Trypan blue diffuses readily from the site of its cutaneous inoculation and the tributary lymphatics are found unoccluded. These observations indicate that "staphylocoagulase" evidently plays no rôle in inducing prompt mechanical obstruction to lymph flow. The latter is therefore primarily referable to the powerfully necrotizing action *per se* of the *Staphylococcus aureus* microorganism and of its soluble toxin.

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Oxidation of Carbohydrates and Polyhydric Alcohols by Luminous Bacteria.

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A comparative study of the oxidation of single substrates by the "resting" cells of the marine *(Achromobacter fischeri)* and the fresh water *(Vibrio phosphorescens)* luminous bacteria has revealed considerable difference in the ability of the 2 species to oxidize various carbohydrates and polyhydric alcohols. The rates of aerobic oxidation of 28 substrates by "resting" cells, prepared by washing thoroughly in the centrifuge and resuspending in phosphate buffer, were followed over a period of 3 hours in Warburg respirometers. The suspensions were adjusted to approximately the same density by means of a photronic cell turbiditimeter. The concentration of all substrates was M/60.

Although the per cent increase in oxygen uptake of the suspensions with substrate as compared with similar ones without the addition of substrate varied in some cases to a fairly wide degree, the ratio of the oxygen consumption with a given substrate to that with dextrose as a standard generally agreed to within less than 10% in repeated experiments. The controls, without added substrate, agreed with vessels containing substrates that were not oxidized to