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Further Studies on Mechanism of Invasiveness by Pyogenic Bacteria.

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The writer has recently demonstrated that the dissemination of a foreign substance from its site of inoculation is at least in part a function of its irritating capacity.[1, 2] *Staphylococcus aureus* induces a lesion sufficiently intense to occlude draining lymphatics and to cause the formation of a fibrinous network as early as one hour after cutaneous inoculation of the organism. The degree of "walling-off" was determined by studying the dissemination of trypan blue from the site of injury to the regional lymphatics. In the case of Type I *pneumococcus* the area is circumscribed at a somewhat later stage (about 6 hours subsequent to the inoculation of the organism). When *Streptococcus hemolyticus* is inoculated into the skin the lymphatics maintain their functional patency for about 2 days, and throughout that time these vessels are virtually unoccluded by thrombi. This histological observation adequately accounts for the delayed fixation and consequently the relative ease with which the dye penetrates to the regional lymphatics in a hemolytic streptococcal inflammation.

These results, while offering an explanation for the well-known localizing tendencies of the staphylococcus as against the disseminating properties of streptococcus, present an interesting paradox. Staphylococci tend to remain localized and produce relatively slight systemic effects because of their pronounced local injurious action which serve to fix them *in situ*. Hemolytic streptococci, on the contrary, produce far greater systemic sequelae owing to the invasiveness resulting from their relatively mild local effects.

The experiments of the writer have recently been confirmed by Dennis and Berberian.[3] Subsequent to the writer's observations, Tillett and Garner[4] have demonstrated that broth cultures of hemolytic streptococci are capable of liquefying the normal human fibrin clot. In contrast to this they pointed out that the normal rabbit fibrin clot is totally resistant to dissolution by such means. This

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

[2] Menkin, V., *Arch. Path.*, 1931, **12**, 802.

[3] Dennis, E. W., and Berberian, D. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 976.

[4] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

finding renders it difficult at present to accept the opinion of some writers[3] that the *in vitro* lytic element observed by Tillett and Garner is an essential factor in accounting for the delayed fixation of a dye demonstrated by the author in the streptococcal type of inflammation in rabbits. It is possible that the fibrinolytic effect of hemolytic streptococci on the human plasma clot plays a definite rôle in human infections; but this is rather in the nature of a reinforcing or accessory factor added presumably to a more fundamental property of the hemolytic streptococcus which holds true for both the human and rodent types of infection.

In an attempt to analyze the problem further, experiments were performed with Berkefeld filtrates of the various types of pyogenic organisms previously studied. The details of this investigation will form the subject of a future communication, but the essential findings are summarized as follows:

1. The sterile filtrate of a several day old culture of *Staphylococcus aureus* induces an intense inflammatory reaction in the dermis of rabbits indistinguishable from the reaction obtained when the viable organisms are inoculated. Trypan blue is fixed as early as one hour after injection of the filtrate. Microscopic sections through the inflamed areas reveal many lymphatics occluded by a fibrinous reticulum. The tissue spaces are in many regions distended by coagulated plasma.

2. The staphylococcal filtrate is inactivated when heated for about one hour and a half at 58°C., for its cutaneous injection causes no fixation of the dye. Histologically, the lymphatic vessels are found to be entirely patent.

3. *Staphylococcus aureus* filtrate in contact with leucocytes obtained from an exudate causes these cells to become swollen, vacuolated, and, in many instances, degenerated. There is also some evidence that the total leucocyte count is lowered when this bacterial filtrate is maintained for some time in contact with an exudate. No such effect on leucocytes is produced with the heated and therefore inactivated staphylococcal filtrate. These observations strongly suggest that the active principle in the filtrate of *Staphylococcus aureus* which causes early blockage is somewhat similar, if perhaps not identical, to leucocidin.

4. On the other hand, the filtrates of both *Streptococcus hemolyticus* and Type I *pneumococcus* fail to induce retention of trypan blue at the site of cutaneous inoculation. There is no evidence of fixation even as late as 50 hours after skin injection of the streptococcal filtrate.

5. The strain of *Streptococcus hemolyticus* (S-23) used in all

these studies fails to inhibit the coagulation of rabbit blood; this holds true for the filtrate as well.

In conclusion, the foregoing preliminary data indicate that the localizing property of *Staphylococcus aureus* is probably referable not merely to the severe irritating property of the organism *per se,* but also to its additional ability to release a powerful soluble exotoxin-like product, identical in many respects with leucocidin, and capable in itself of inducing a sufficiently intense injury to cause obstruction of normal lymphatic drainage. *Pneumococcus* Type I and *Streptococcus hemolyticus,* on the other hand, fail to form any such detectable accessory substance able to cause damage to the lymphatic or capillary endothelium. Briefly, then, evidences obtained thus far are in accord with the writer's original view, that the delayed fixation of dye and hence the invasive capacity of hemolytic streptococcus is referable to the mild local effects of this organism in contrast to the pronounced injurious action of *Staphylococcus aureus.* These studies are being continued in an attempt to obtain more precise information concerning the chemistry and the rôle of leucocidin-like substances.

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Anticoagulants of the Blood.

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Any agent which inactivates or removes from blood, calcium, prothrombin, or thrombin is an anticoagulant. Theoretically, antifibrinogen and antithromboplastin are also possible but have never been convincingly demonstrated. The removal of calcium by oxalates, citrates, or fluorides is well known. As a type of antiprothrombin, aluminum hydroxide is an excellent example. On mixing and incubating oxalated plasma (0.5 cc.) with aluminum hydroxide cream (0.05 cc.) a plasma is obtained which after the removal of the aluminium hydroxide, will not clot on recalcification, whereas the untreated plasma clots in 2 minutes when calcium is added. The loss of clotting power is not due to removal of thromboplastin, for on adding an active preparation made from rabbit's brain, the normal clotting time is not restored. Furthermore, fibrinogen is not removed, for thrombin (fresh serum) will cause clot-