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# Influence of Specific Antiserum on the Inflammatory Fixation of *Streptococcus hemolyticus.*

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It has been demonstrated[1, 2] that the formation of the inflammatory barrier and consequent "inflammatory fixation" is greatly delayed when invasive strains of hemolytic streptococci are inoculated into the skin of rabbits. The delayed fixation, in the case of *Streptococcus hemolyticus,* is due to the ability of this organism to actively suppress the formation of the inflammatory barrier to a considerable degree, as demonstrated by the ability to delay fixation even in the presence of such inflammatory irritants as *Staphylococcus aureus,*[2] and aleuronat.[3] Further experiments have been carried out to determine the influence of streptococcal antiserum on the fixation time in local streptococcal inflammation.

Will streptococcus antiserum induce inflammatory fixation of *Streptococcus hemolyticus?*

Five cc. of "Streptoserin" (Bayer) were added to 5 cc. of a fresh 16-hour dextrose phosphate infusion broth culture of *Streptococcus hemolyticus,* and 5 cc. of fresh normal horse serum were added to 5 cc. of culture for use as a control. The culture-serum mixtures were allowed to stand at room temperature for 30 minutes before use. One cc. of the antiserum-streptococcus mixture was injected into the skin of the right foreleg of each of eight rabbits; 1 cc. of the normal horse serum-streptococcus mixture was similarly injected into the skin of the left foreleg of each rabbit. The degree of inflammatory fixation was subsequently determined at regular intervals by injecting 0.8 cc. of 1% trypan blue solution into the center of each inflamed area and examining the regional lymph nodes for the presence of the dye 1 hour after its introduction into the area of inflammation. The inflamed areas of skin were fixed in Zenker-formol and subsequently studied microscopically.

The results of the experiment are shown in Table I. Complete fixation occurred in the presence of specific antiserum in less than 2 hours. On the control side the dye passed freely to the regional

1 Menkin, V., *J. Exp. Med.,* 1933, **50**, 977.

2 Dennis, E. W., and Berberian, D., *Ibid.,* 1934, **60**, 581.

3 Dennis, E. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 100.

lymph nodes, indicating lack of fixation and inadequacy of the inflammatory barrier, for at least 28 hours. The presence of a fibrinous barrier in the area which retained the dye was confirmed by histological study.

TABLE I.
Influence of Streptococcus Antiserum on Inflammatory Fixation in Acute Local Streptococcal Inflammation.

| Rabbit No. | Duration of inflammation, hr. | Passage of dye to lymph nodes | | | |
|---|---|---|---|---|---|
| | | Right* | | Left† | |
| | | S (a) | D (b) | S (a) | D (b) |
| 7-0 | 1 | ++++ | — | +++ | — |
| 7-1 | 2 | — | — | ++++ | +++ |
| 7-2 | 4 | — | — | ++++ | +++ |
| 7-3 | 6 | — | — | ++++ | ++++ |
| 7-4 | 8½ | — | — | ++++ | +++ |
| 7-5 | 10 | — | — | +++ | +++ |
| 7-6 | 22 | ± | — | +++ | ++++ |
| 7-7 | 28 | ± | — | +++ | ++ |

The number of plus signs indicates the relative amount of trypan blue which passed from the site of inflammation to the regional (axillary) lymph nodes at given periods of time after the injection of the streptococcus-serum mixtures. The presence of the dye in the nodes indicates lack of fixation and inadequacy of the inflammatory barrier; absence of the dye indicates the establishment of an efficient barrier.

*Received *Strep. hemolyticus* plus streptococcus antiserum.

†Received *Strep. hemolyticus* plus normal horse serum (control).

(a) S = superficial lymph nodes of the region of injection.

(b) D = deep lymph nodes of the region of injection.

*Conclusion.* Inflammatory fixation is promptly induced in acute local streptococcus inflammation *in the presence of streptococcus antiserum*, in contrast with the marked delay in fixation in the presence of normal serum.

The experiment presented above has been repeated on several occasions. Five different strains of *Streptococcus hemolyticus*, and 4 different commercial brands of polyvalent so-called antitoxic-antibacterial streptococcus antisera have been used with essentially identical results.

The data given above suggest that the factor produced by *Streptococcus hemolyticus* which is responsible for the active suppression of the formation of the local inflammatory barrier is antigenic and is readily neutralized by streptococcus antiserum. A possible alternative explanation is that the inflammatory fixation is part of an Arthus phenomenon due to the introduction of a mixture of antigen and antibody into the skin. However, in view of the ability of the "inhibitory factor" to suppress fixation in other types of inflammatory response, this explanation would seem to be improbable,

and attempts to test the hypothesis experimentally have given negative results.

Ward and Lyons[4, 5] have shown that the virulence of hemolytic streptococci is ultimately dependent upon the resistance of the organism to phagocytosis. However, invasiveness (*i. e.*, the ability of the organism to disseminate from the portal of entry) is a vitally important corollary of virulence. We have found that avirulent strains of streptococci may rapidly reach the blood stream following injection into the skin, but are quickly disposed of by the cellular defenses of the body; on the other hand the speed with which organisms which are resistant to phagocytosis kill the host may be determined by the ease with which they reach the blood stream. Menkin[6] has recently demonstrated that the early establishment of a nonspecific inflammatory barrier at the site of injection into the skin may protect rabbits against otherwise lethal doses of pneumococci known to be highly resistant to phagocytosis.

Thus it would seem that, despite the general lack of specific opsonins in commercial streptococcus antisera, the local introduction of adequate amounts of such antisera directly into the involved area of an acute local streptococcal inflammation, would offer a means of facilitating or maintaining the localization of the infectious process by the enhancement of the fibrinous barrier and "inflammatory fixation".

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## Sorbitol as a Diuretic.

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The hexahydric alcohol, sorbitol, has recently become available in quantities at a low price. It is non-toxic, has enormous water binding capacity, is rapidly excreted by the kidneys after intravenous injection and has 1.88 times the osmotic pressure of the same percentage sucrose solution. Its solutions are less viscous and more easily injected than those of sucrose and are entirely stable to

[4] Ward, H. K., and Lyons, C., *J. Exp. Med.*, 1935, **61**, 515.

[5] Lyons, C., and Ward, H. K., *Ibid.*, 1935, **61**, 531.

[6] Menkin, V., *J. Infect. Dis.*, 1936, **58**, 81.