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**Streptococcal Inhibition of Non-Specific Inflammatory Fixation.**

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Dennis and Berberian<sup>1</sup> recently described the ability of certain strains of *Streptococcus hemolyticus* and *Streptococcus viridans* to inhibit (delay) the process of inflammatory fixation in the skin of the rabbit when *Staphylococcus aureus* whole-culture was used as the irritant. Berkefeld filtrates, as well as the whole-cultures of streptococci exhibited the property of delaying inflammatory fixation as long as 4-6 hours. The experiments reported below were carried out with the hope that the inhibitory effect might be even more sharply defined if a substance such as aleuronat were used as the irritant, thus avoiding the multiplicity of factors which must be active in the antagonistic association of streptococci and staphylococci as in our earlier experiments. Menkin<sup>2</sup> has previously reported that the injection of a suspension of aleuronat into the skin of the rabbit induced the inflammatory fixation of trypan blue within a period as short as 30 minutes.

Can *Streptococcus hemolyticus* whole-culture inhibit or delay the inflammatory fixation normally elicited by aleuronat in the skin of the rabbit?

Aleuronat (5%) was suspended in a 3% solution of soluble starch in plain broth. The suspension was autoclaved at 10 lbs. pressure for 10 minutes, and thoroughly agitated every few minutes while cooling. After standing for several hours the upper portion consisted of a uniform suspension of finely divided particles suitable for injection through a 27G needle.

Two series of rabbits, Nos. 41-45, and 46-50 inclusive were used in the experiment. Nos. 41-45 each received 0.5 cc. of 18 hr. whole-culture of *Strep. hemolyticus* (strain "Gay") grown in dextrose phosphate broth, plus 0.5 cc. of aleuronat suspension intradermally into the right foreleg; the culture and aleuronat suspension were mixed in the syringe. 0.5 cc. of aleuronat suspension plus 0.5 cc. of dextrose phosphate broth adjusted to pH 6 (pH of 18 hr. whole culture used) were mixed in the syringe and injected into the skin of the left foreleg of each rabbit as the control. Rabbits 46-50

<sup>1</sup> Dennis, E. W., and Berberian, D., *J. Exp. Med.*, 1934, **60**, 581.

<sup>2</sup> Menkin, V., *Ibid.*, 1929, **50**, 171.

were treated in an identical manner except that strain S 23M of *Strep. hemolyticus* was employed for the injections into the right foreleg; the left foreleg was used for the control injection. The degree of inflammatory fixation was determined at intervals of 2, 4, 6, 8, and 24 hours after the injection of the aleuronat and aleuronat streptococcus mixture by injecting 0.8 cc. of 1% solution trypan blue in saline into the inflamed skin of both forelegs of the rabbits to be examined at that period. Exactly one hour after the injection of the dye into a rabbit, the animal was placed under ether anesthesia and the regional lymph nodes in the axilla examined for the presence of the dye. The inflamed areas of skin were removed and fixed in formalin for subsequent histological examination.

The results are presented in Table I. The degree of discoloration of the lymph nodes, indicative of the degree of patency of the lymphatics and absence of the inflammatory fibrinous barrier (controlled by microscopic examination of stained sections) in the area of local inflammation is indicated by an appropriate number of plus signs. Conversely, inflammatory fixation is indicated by the failure of the dye to reach the lymph nodes in significant quantity. As shown in Table I, aleuronat suspension alone promptly produced

TABLE I.  
Inhibition of Nonspecific Inflammatory Fixation by Streptococci.

Rabbit No.	Duration of inflammation hrs.	Passage of dye to lymph nodes			
		Right*		Left†	
		S (a)	D (b)	S (a)	D (b)
4-1	2	+++	+++	+	—
4-2	4	++	++	+	±
4-3	6	+++	+++	±	—
4-4	8	+++	++	—	—
4-5	24	++	+	—	—
4-6	2	++++	+++	±	—
4-7	4	++	+++	++	—
4-8	6	++++	+++	+	±
4-9	8	+++	++	+	—
5-0	24	++	+	±	±

\*Aleuronat plus streptococcus whole culture injected into right foreleg. Nos. 4-1 to 4-5 received strain "G"; Nos. 4-6 to 5-0 received strain S 23 M.

†Aleuronat plus plain broth injected into left foreleg.

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 aleuronat and aleuronat-streptococcus mixtures. The presence of the dye in the nodes indicates lack of inflammatory fixation and inadequacy of the inflammatory barrier; absence of the dye indicates the establishment of an efficient barrier.

(a) S = superficial lymphnodes of the injected region.

(b) D = Deep lymphnodes of the injected region.

fixation of the dye (within 2 hours). In the presence of *Streptococcus hemolyticus* whole-culture, inflammatory fixation, *i. e.*, barrier formation was delayed for at least 24 hours.

*Conclusion.* *Streptococcus hemolyticus* whole-culture is capable of inhibiting the inflammatory fixation which is normally elicited by aleuronat in the skin of the rabbit.

It has been clearly demonstrated that the dissemination of streptococci, staphylococci, and *Pseudomonas aeruginosus* from an inflammatory focus in the skin to the regional lymphnodes and thence to the blood stream is largely governed by the efficiency of the fibrinous barrier which is responsible for what Menkin has termed "inflammatory fixation".<sup>1, 2, 3</sup> It appears to be quite clear that the necrotizing property of *Staphylococcus aureus* is responsible for the characteristic localized nature of staphylococcal infections, by eliciting a prompt and intense inflammatory response.<sup>3, 4</sup> Menkin<sup>3, 5</sup> has suggested and maintained that *Streptococcus hemolyticus* paradoxically owes its characteristic invasiveness to a lack of irritating properties and consequent delay in eliciting an inflammatory response. The experimental data given by Dennis and Berberian<sup>1</sup> and the clearly defined results given above indicate that one need not resort to a paradoxical interpretation of the observed phenomena. If *Streptococcus hemolyticus* is capable of inhibiting the formation of an inflammatory barrier in the presence of an irritating substance such as aleuronat, it seems not unreasonable to suppose that this ability to interfere with the normal inflammatory response may play an important rôle in the dissemination of streptococci from a portal of entry under natural conditions.

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<sup>3</sup> Menkin, V., *Ibid.*, 1933, **57**, 977.

<sup>4</sup> Menkin, V., and Walston, H. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1259.

<sup>5</sup> Menkin, V., *Am. J. Med. Sci.*, **190**, 583.