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A Note on the Mechanism of Fixation in an Area of Sterile Inflammation.*

VALY MENKIN.

From the Department of Pathology, Harvard University Medical School.

In explaining fixation of various foreign substances at the site of inflammation as the result of mechanical obstruction by thrombosed lymphatics and by a fibrin network in tissue spaces of the affected area, 3 types of evidence were presented: (1) the appearance of thrombosed lymphatics and of a fibrinous network; (2) the inability of substances to enter the site of inflammation when injected at its periphery; (3) finally, the inhibition of fixation upon the addition to the inflammatory irritant of a fibrin solvent, concentrated urea.[1, 2, 3] It has been pointed out that both factors, coagulated plasma in tissue spaces and fibrinous clots occluding lymphatics at the site of inflammation, can well account for fixation. Such clots in lymphatics have been seen *in vivo* as well as in fixed preparations.[4] At no time, however, has the writer maintained that either one of these 2 factors was more responsible for fixation than the other. In the types of inflammation studied previously with aleuronat or croton oil, both factors, *i. e.,* a fibrinous network in tissue spaces and lymphatics occluded by thrombi had been found. This, though, does not preclude in the least that with some types of inflammatory irritants, fixation may not be predominantly due to one of these 2 factors.

Recently Field, Drinker and White[5] on the basis of the large amounts and increased pressure of free flowing lymph obtained from the main lymphatic trunk of the hind ankle in the anesthetized dog some hours after immersion of the paw in boiling water, conclude that it is more reasonable to ascribe the fixation of trypan blue to its inability to diffuse through the "gelatinous"-appearing subcutaneous tissue which was observed in such inflamed areas rather than to generalized thrombosis of lymphatics.

The view as expressed by these investigators is difficult to accept

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1 Menkin, V., *Arch. Path.*, 1931, **12**, 802.

2 Menkin, V., *J. Exp. Med.*, 1931, **53**, 171.

3 Menkin, V., *J. Exp. Med.*, 1932, **56**, 157.

4 Menkin, V., *J. Exp. Med.*, 1931, **53**, 179.

5 Field, M. E., Drinker, C. K., and White, J. C., *J. Exp. Med.*, 1932, **56**, 363.

for several reasons. In the first place the measurements on lymph pressure obtained from a main lymphatic trunk at the ankle do not indicate the status of all the lymphatics throughout so extensive an area of injury as that produced by immersion of a paw in boiling water. The possible presence of occluded lymphatics in the superficial more intensely affected layers of tissue in which trypan blue was injected is not ruled out by the finding of an increase in the lymph flow and a rise in the pressure of a main lymphatic trunk. As has been recently pointed out by Hudack and McMaster,[6] it is to be noted that when the degree of damage is relatively mild, the drainage through the lymphatics is increased. Consequently the simultaneous occurrence of increased lymph pressure in a main lymphatic trunk draining a considerable area injured to an unequal degree and the fixation of trypan blue at its point of injection which is situated in a relatively small superficial area of presumably lethal damage does not preclude the possibility of local thrombosed lymphatics as the cause for the fixation of the dye. In brief, the measurements as recorded by these investigators do not indicate the status of all the lymphatics at the site of trypan blue inoculation. The writer has pointed out previously[2] that additional information is desired on the relation between exudation from blood vessels and changes in flow of lymph from the inflamed area. Such information is not provided in the study of Field, Drinker and White. An absolute increase in lymph flow in a main lymphatic trunk does not necessarily prove that there may not be a relative fall in the lymph flow if the increased amount of fluid passing out through the vascular capillary wall in an inflamed area is taken into consideration in evaluating changes of flow in the lymphatic vessels with inflammation.

In the second place the term "gelatinous" as used by Field, Drinker, and White in describing the inflamed subcutaneous tissue is not quite clear unless it refers to the gross physical appearance of coagulated serum in the injured area which on microscopic examination is seen to be composed of a fibrinous reticulum. If the term refers solely to the edematous fluid held in tissue spaces acting as a mechanical barrier, then it is difficult to explain all of the writer's previous findings when the peritoneal cavity was chosen as the site of inflammation and aleuronat as the irritant. This irritant as a rule yielded scarcely any edematous fluid in the peritoneal

[6] Hudack, S. S., and McMaster, P. D., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 944.

cavity. Yet foreign substances injected into the inflamed cavity were fixed and the retrosternal lymphatics were found to be thrombosed.

In the third place, Field, Drinker, and White reported no histological examination of tissue subjected to water at 100°C. Precise information as to what structural changes have occurred at the site of inflammation can be readily obtained by microscopic study.

For these 3 reasons similar studies were performed on the forelimb of rabbits. This animal was chosen since the writer's previous experiments had been performed on either the rabbit or the frog.

Under ether anesthesia the foreleg of a rabbit was dipped for about 2 minutes in water heated to 100°C. The animal was allowed to come out of the anesthetic. After a variable interval of time 3 cc. of 1% trypan blue in saline was injected subcutaneously into the inflamed foreleg and into the opposite foreleg to act as control. The axillary lymph node and lymph drawn from the efferent lymphatic vessel in each foreleg were studied for the presence of the dye. In one animal (Rabbit 10-50) both forelegs were dipped into water at 100°C. inasmuch as a sufficient number of controls had been reported in previous studies.[7] The degree of swelling was unusually extensive as compared with that obtained with other types of inflammatory irritants. Some areas of the skin were distinctly discolored with evident patches of necrotic tissue surrounded by a zone of erythema. The swelling extended well into the axilla and in some cases over the anterior chest wall. At the conclusion of an experiment the inflamed tissue was removed and placed in 10% formaldehyde for subsequent histological examination.

The results of these experiments appear in Table I. When trypan blue is injected only several hours after dipping the foreleg in water at about 100°C. no definite evidence of fixation is obtained in spite of a prominent gelatinous-like exudate in the injured area. Microscopic examination of such inflamed areas reveals considerable edema, dilatation, and congestion of small vessels and a very slight cellular infiltration. There is no fibrinous network and the lymphatic lumina are unoccluded and somewhat dilated. The microscopic picture is entirely in accord with the findings, *i. e.*, in regard to the prompt diffusibility of the dye to the tributary lymphatics. These results in the early stage of the inflammation are contrary to the statements of Field, Drinker, and White, claiming that the dye was fixed. This discrepancy may perhaps be ascribed to the different

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

type of animal used and possibly to the fact that, as indicated in the communication of these investigators, the injection of the dye and the readings concerning its drainage into the lymph channel were made while the animal was inactive and under general anesthesia. This was not the case in any of the writer's experiments; the anesthesia was discontinued immediately after exposing the limb of the

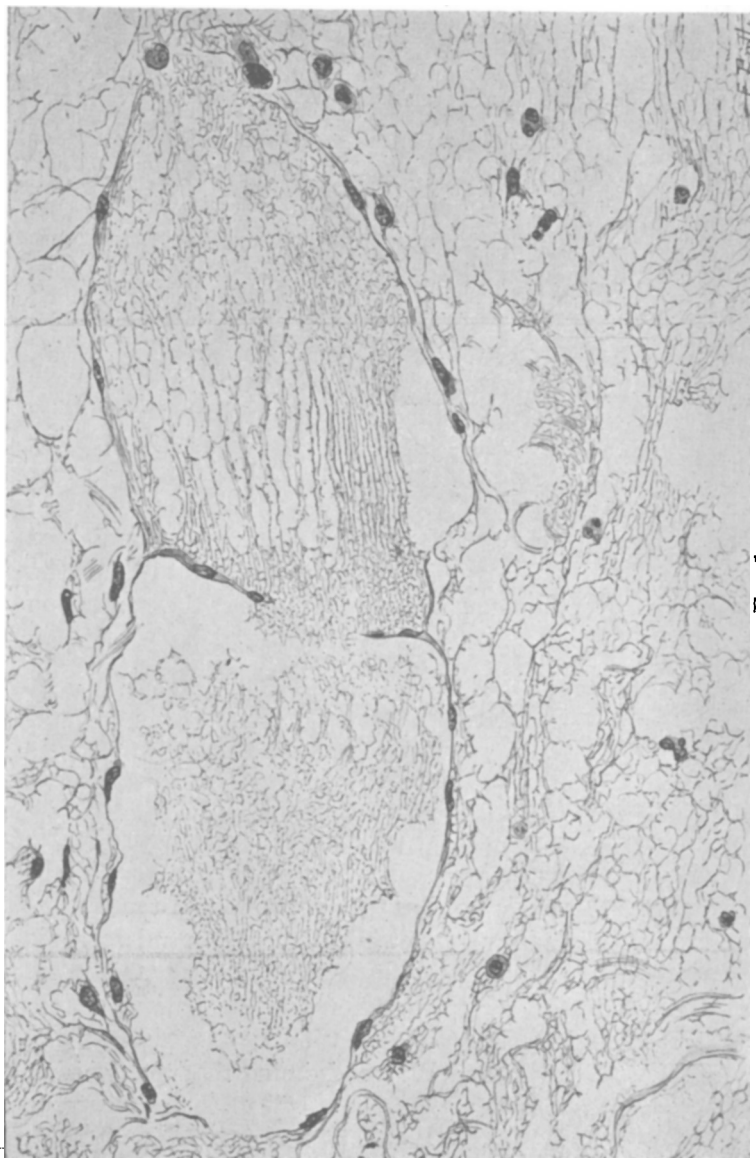

FIG. 1.

Drawing of a lymphatic vessel in an area of cutaneous inflammation of about 22 hours' duration. The inflammatory reaction was induced by water heated to 100°C. The lumen is occluded by a delicate fibrinous reticulum. (Rabbit No. 10-50.) Magnification about 500.

TABLE I.
Retention of Trypan Blue at the Site of Inflamed Areas Induced by Water Heated to 100°C.

Rabbit No.	Interval between exposure to irritant and injection of dye Hr. Min.	Total duration of inflammation Hr. Min.	Presence of dye on inflamed side Lymph of efferent lymphatic	Lymph node	Presence of dye on normal side Lymph of efferent lymphatic	Lymph node
7-10	4:55	7:10	+	+	++	+
7-03	5:35	8:25		+		+
10-50	19:35	22:15	0	0		
			0	0		
6-25	18:11	21:18	0	FT	++	++
7-11	47:05	50:15	T	0	+	++

T = trace. FT = faint trace.

animal for about 2 minutes in hot water. It is quite conceivable that a dye injected and studied while an animal is under general anesthesia may be delayed from reaching readily lymphatic channels.

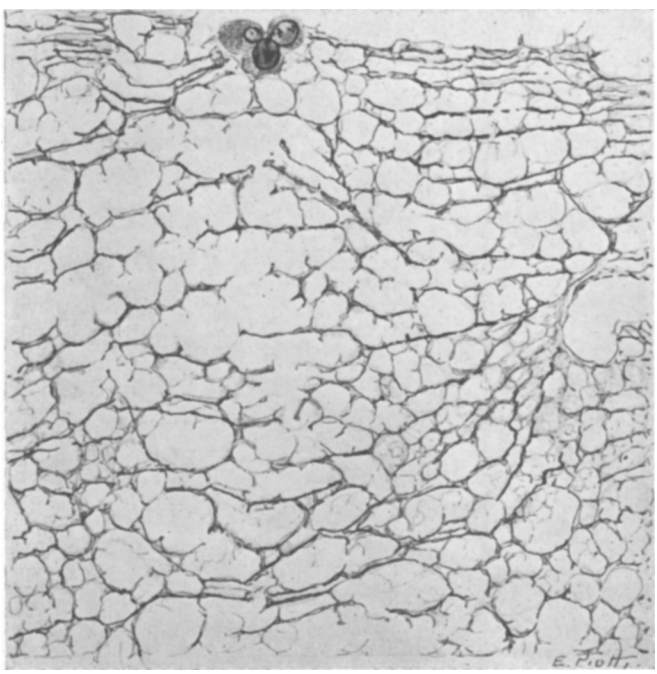

FIG. 2.

Fibrinous meshwork in an area of inflammation of about 22 hours' duration. The inflammatory reaction was induced by water heated to 100°C. Trypan blue injected into this area failed to reach the tributary lymphatic nodes. (Rabbit No. 10-50.) Magnification about 1200.

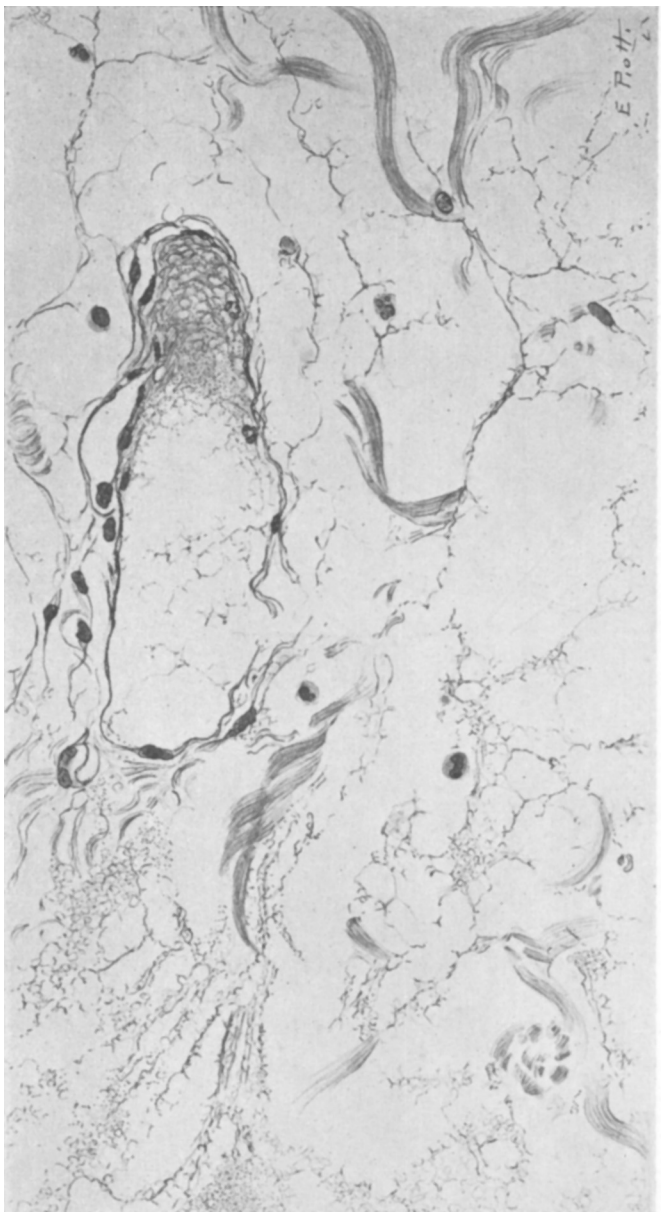

FIG. 3.

Drawing of a lymphatic lumen from an area of inflammation of about 22 hours' duration. The reaction was induced by water heated to 100°C. The lumen is only partially occluded by a delicate fibrinous thrombus. (Rabbit No. 10-50.) Magnification about 500.

owing to muscular atonicity. Mere handling of the leg might under such circumstances favor the passage of the dye into the lymph. However, a retardation of a dye at the site of inflammation in such an abnormal condition as inactivity under general anesthesia can-

not certainly be regarded as fixation in the way that the author's previous studies have demonstrated.

The results in Table I show that fixation of trypan blue was obtained only 18 to 19 hours after exposure of the foreleg to hot water. Microscopic examination shows at this stage a very extensive fibrinous network in tissue distended by considerable edema (Fig. 2, Rabbit 10-50). Many lymphatic vessels are found. Some lumina are practically entirely occluded by a fine fibrinous reticulum (Fig. 1, Rabbit, 10-50); others, however, show only partial occlusion by a small fibrinous thrombi (Fig. 3, Rabbit 10-50) and finally, some lymphatic vessels are perfectly patent. These observations are in accord with the explanation that fixation of the dye is due, as in other types of inflammation, to the presence of mechanical obstruction in the form of a fibrinous network in tissues distended with edema and to the occlusion of some of the lymphatics by fibrinous thrombi. It is possible that in these types of inflammation the fibrinous network is of greater consequence than the thrombosis of lymphatics in permitting fixation to take place. However, this fact does not change the basic explanation for the mechanism of fixation. The two factors play the dominant part in mechanically arresting the free diffusion of foreign substances from the site of inflammation. That in some varieties of inflammatory reactions one factor may play a more prominent rôle than the other is quite obvious. The intensity of fixation is probably a function of the number of occluded lymphatics at a given time as well as of the presence of the fibrinous meshwork. As long, however, as some lymphatics vessels are patent, an absolute increase in lymph flow and pressure is to be expected in a main channel draining a large area of edematous swelling. As the inflammatory reaction progresses and more lymphatics become occluded, the lymph flow is likely to diminish. This seems to be precisely the case found by Field, Drinker, and White, as seen from their statement: "One observation was made on the inflamed leg 24 hours after the onset of inflammation. In this case the lymph was still flowing though not as abundantly as it had been 12 hours previously".[5]

Concentrated urea was shown[3] to dissolve fibrin and in this manner to prevent fixation. The foreleg of each of 2 rabbits was exposed to boiling water for about 2 to 3 minutes, followed immediately by subcutaneous injection in that limb of 7 cc. of 50% urea in distilled water. About 18 hours later the injured leg revealed a tremendous amount of edema and congestion. Trypan

blue was injected into the injured and normal forelimbs as described above. Two to 3 hours later the dye was seen to have penetrated to the regional lymphatics on the inflamed side as readily as under normal circumstances. The results reported in this communication are in agreement with previous observations and indicate that fixation at the site of a sterile inflammation induced by hot water is due to mechanical obstruction in the form of a fibrinous network, *i. e.*, coagulated plasma, and in part to the presence of a number of lymphatics that are occluded by fibrinous thrombi.

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Configuration and Anesthetic Activity of Aromatic Alcohols.*

P. K. KNOEFEL† AND G. A. ALLES. (Introduced by C. D. Leake.)

From the Pharmacological Laboratory, University of California Medical School, San Francisco, and the Laboratory of George Piness, M.D., Los Angeles.

In general, local anesthetics are deficient in duration of action rather than potency. A striking demonstration of prolongation of effect as a result of a small change in structure of a drug-compound has recently been afforded. It is definitely established in the sympathomimetic amines that the transformation of the side chain so that the carbon bearing the nitrogen becomes a secondary carbon atom results in prolongation of effect.[1] To test the validity of this principle in another field, we are now studying for local anesthetic activity some derivatives of ephedrine in which the same peculiarity of structure exists. Of other series, procaine type compounds do not readily lend themselves for this study because of the difficulty of preparing the desired amino-alcohols. However, the aromatic alcohols with pertinent structure are easily obtained. Some of these have been studied before; in fact it has been stated,[2] on rather invalid evidence, that the change from primary to secondary to tertiary alcohol reduces the activity.

Anesthesia of the rabbit cornea and the frog sciatic nerve has been determined for a series of primary, secondary, and tertiary alcohols,

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† Fellow of the National Research Council.

[1] Alles, G. A., *J. Pharmacol. Exp. Therap.*, 1933, **47**, 339.

[2] Hirschfelder, A. D., and Bieter, R. N., *Physiol. Rev.*, 1932, **12**, 190.