STUDIES ON INFLAMMATION

V. The Mechanism of Fixation by the Inflammatory Reaction

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PLATE 7

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In previous communications it was shown (1) that trypan blue injected into the circulating blood rapidly enters the site of inflammation and is fixed there, so that the tissues are deeply stained. Furthermore trypan blue injected directly into the site of inflammation in the subcutaneous tissue or in the peritoneal cavity is fixed in the inflamed area and fails to reach the regional lymphatic nodes. Subsequent studies showed that the rapid accumulation of dye in an inflamed area is associated with increased capillary permeability (2). These studies were then extended and it was found that colloidal iron or ferric chloride injected directly into an inflamed area was fixed *in situ* by the inflammatory process, and that ferric chloride injected intravenously rapidly entered inflamed cutaneous areas, where its presence was identified by both qualitative and quantitative determinations (3). Further studies demonstrated that a foreign protein, as *e.g.* horse serum, injected into an inflamed peritoneal cavity penetrated into the blood stream less rapidly than when introduced into the normal cavity (4). When the foreign protein was injected into a cutaneous inflammatory area it was held *in situ* for a longer period than when injected into an inflamed peritoneal cavity. It was also found that foreign protein introduced into the circulating blood stream accumulated in an inflamed area, where it was present in greater concentration than in normal tissue.

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The earlier literature on the subject has been reviewed elsewhere (1, 2). With the demonstration that the dissemination of bacteria was retarded by an inflammatory reaction (5, 6, 7) and with the subsequent studies on the fixation of a dye, iron, and foreign protein at the site of inflammation, it became of interest to study the mechanism of fixation by the inflammatory reaction. It had been shown (2) that the accumulation of a dye in an inflamed area was doubtless connected with increased capillary permeability. In this paper an attempt is made to study the mechanism involved in the fixation of foreign substances by the inflammatory reaction.

The leucocytes are probably not a very significant factor in the mechanism of fixation for two reasons. Histologically no definite evidence could be obtained of phagocytosed particles in the leucocytes of the inflamed area at a time when fixation of foreign substances was already demonstrable by examining the tributary lymphatics. In the second place, fixation of trypan blue at the site of inflammation was shown to occur as early as 30 minutes after the injection of the inflammatory irritant (1). The occurrence of fixation at this early stage of the inflammatory reaction when there are as yet relatively few leucocytes present seems to point toward some other factor responsible for fixation.

Schade and Menschel (8) have found that in inflamed areas, especially those with suppuration, the accumulation of products of tissue disintegration may become so great that the osmotic pressure is raised to as high as eleven atmospheres, with a simultaneous marked increase in hydrogen ion concentration. They believe that inflammatory edema has primarily an osmotic origin. For this reason it seems reasonable enough to suppose that the increase in osmotic pressure at the site of inflammation might be a factor in influencing the flow of fluids and perhaps indirectly any contained substances.

A factor which might explain fixation is mechanical obstruction. It is conceivable that a network of fibrin and thrombosed lymphatics at the site of inflammation might arrest the passage of particulate material injected into such an inflamed area. The dissemination of fluids would probably also be retarded by mechanical obstruction of this kind, though probably not as effectively as solid particles which would be more readily caught in a fibrinous network.

In this connection it is interesting to note that some years ago Opie (9) showed that when cantharidin is administered intramuscularly thoracic duct lymph flow is at first diminished but later may be increased. The decrease of lymph flow was accompanied by acute edema of the liver and gall bladder. This edema was due to plugging by fibrin of the afferent lymphatics and the sinuses of lymph nodes which drain these organs. The observations of Adami (10) are also significant in this connection:

"Even when inflammation (as in pericarditis) affects the whole extent of a serous cavity, the layer of fibrin acts as a protective coat closing the lymphatic 'stomata' hindering the free absorption of the morbid material by the lymph and blood vessels, and filtering bacteria out of such fluid as does find its way through to the tissues beneath."

In the endeavor to throw some light upon the mechanism involved in fixation, a series of experiments was undertaken to determine whether the inflammatory exudate in itself possessed some property which might facilitate the fixing of foreign substances in the inflamed area.

The Effect of Adding Ferric Chloride, Horse Serum, or Trypan Blue to the Inflammatory Exudate and to Blood Serum

The inflammatory exudate was obtained by injecting 0.5 cc. of 10 per cent croton oil in olive oil into the peritoneal cavity of rabbits. 24 to 72 hours later the exudate was removed and centrifugalized. A sample of blood was also removed from the heart and likewise centrifugalized. The tests were made by the addition of 0.2 cc. of varying dilutions of a 0.25 per cent ferric chloride solution to 0.3 cc. of either exudate or blood serum. The final volume was brought up to 1 cc. with saline (0.9 per cent).

Addition of ferric chloride to the inflammatory exudate gave rise in each case to a heavy precipitate. Addition of the undiluted iron salt to blood serum produced a precipitate which on slight shaking immediately redissolved. At higher dilutions of ferric chloride solution no precipitation occurred when it was added to blood serum. That the precipitate caused by undiluted 0.25 per cent ferric chloride solution and blood serum is transient and redissolves on slight shaking is of some interest in explaining the possibility of injecting the ferric salt intravenously (3, 11) without fatal embolic effect on the animal.

In view of the work of numerous investigators it is probable that the precipitate formed by the addition of ferric chloride to the inflammatory exudate is a ferric proteinate (12).

Having shown the direct effect of exudate on an iron salt, experiments were then undertaken to determine the influence of the inflammatory exudate and of the blood serum on foreign protein such as horse serum.

The inflammatory exudate was obtained as described above. After centrifugalizing the exudate a piece of tissue, either kidney or lymph node, was added to the supernatant fluid to bring about prompt and firm coagulation. The coagulated material was again centrifugalized and the supernatant fluid used for the experiments. 0.5 cc. of varying dilutions of horse serum was added to 0.5 cc. of exudate or to 0.5 cc. of blood serum. For each experiment two control test tubes were set up, one containing 0.5 cc. of the exudate and the other 0.5 cc. of blood serum. Both were brought up to 1 cc. with saline. The tubes were then placed in a water bath at 37°C. for from 15 to 25 minutes.

The control tubes and those containing blood serum showed no trace of coagulation on the addition of horse serum, whereas when the latter was mixed with inflammatory exudate marked coagulation resulted in all cases.

Experiments similar to the above were performed with trypan blue, but in no instance could it be shown that either exudate or blood serum had any precipitating or coagulating effect on this dye. Yet trypan blue is fixed *in situ* by the inflammatory process. For this reason it is believed that fixation *per se* involves primarily a different mechanism than precipitation. However, since it has been found that iron is more effectively fixed than trypan blue in an inflamed peritoneal cavity (1, 3) it is possible that precipitation of the ferric salt plays some part in accentuating the effect of the fixation mechanism. Precipitation or coagulation of a foreign substance when injected in an inflamed area may be a secondary factor in preventing its rapid dissemination from the site of inflammation.

Histological Studies of Area of Inflammation

Sections were made of the inflamed tissue of rabbits in experiments in which either trypan blue or ferric chloride had been shown to be fixed *in situ* by the inflammatory process. There is as a rule a central area of dense leucocytic infiltration. The intensity of the inflammatory reaction in the immediate neighborhood of veins and arteries is noteworthy. It is to be recalled (1) that when the dye was injected

intravenously it would not always penetrate into the central zone of the inflamed area. This is evidently due to thrombosis of the small vessels for sections of such areas reveal some thrombosed vessels with acute inflammatory changes in the surrounding tissue.

Histologically there is little evidence of phagocytosed particles of trypan blue or of iron within the leucocytes at a time when retention of these substances at the site of inflammation is clearly demonstrable.

It is of interest to note the meshwork of fibrin which is found usually at the periphery of the zone of dense infiltration (Fig. 1). In the same region careful study reveals many lymphatic vessels which are thrombosed. Fig. 2 shows very clearly an occluded lymphatic vessel. The thrombus is characterized by numerous leucocytes within a delicate fibrinous reticulum. The fact that there are many occluded lymphatics and a dense network of fibrinous strands within tissues that are distended with edema at the site of inflammation supports the view that foreign substances, especially solid particles, such as precipitated iron salts, can disseminate only with difficulty from the inflamed area through the regional lymphatic vessels.

The Failure of Trypan Blue to Penetrate into an Inflamed Area When the Dye Is Injected at Its Periphery

If, as described above, the thrombosed lymphatics and the network of fibrin in an acutely inflamed area are instrumental in preventing mechanically the free passage of substances from the site of inflammation, it follows that for the same reason similar substances injected at the periphery of the inflamed area should fail to enter it. To test this hypothesis, the following experiments were conducted at the suggestion of Professor Eugene L. Opie.

An inflammatory reaction was induced by the injection of 0.4 cc. of a saline suspension of *Staphylococcus aureus* into either the skin of the abdomen or into the subcutaneous tissue of the foreleg of a rabbit 2 or 3 cm. from the shoulder joint. After a variable interval of time about 1 cc. of 1 per cent trypan blue was injected into four to six areas of the skin immediately adjacent to the site of inflammation. Thus the inflamed area became circumscribed by a colored band of blue. In a normal skin area of the abdomen of the same size as the inflamed area or in the normal foreleg similar injections of dye were made to serve as controls. In two experiments 0.5 to 0.6 cc. of concentrated broth was used as the inflammatory irritant. Several hours later both inflamed and normal areas were examined for the presence of dye.

The results are shown in Table I. It is seen that the dye failed to penetrate into the inflamed area when injected at its periphery, whereas it disseminated readily into the control area. The area of inflammation usually appeared as a definite round spot free from blue coloration. At the conclusion of two of the experiments (1 and 2) 10 cc. of 1 per cent trypan blue were injected into the circulating blood stream of each animal. It is interesting to note that within a short interval of time the inflamed area into which no trypan blue had penetrated when injected at its periphery was now distinctly stained by the dye.

TABLE I

The Penetration of Trypan Blue into an Inflamed Cutaneous Area When the Dye Is Injected at Its Periphery

Experiment	Interval between injection of irritant and that of dye	Total duration of inflammation	Penetration of dye into inflamed area	Penetration of dye into normal area
	hrs.:min.	*hrs.:min.*		
1*	0:40	4:00	0	+
2*	1:00	4:30	0	++
3**	18:00	19:00	0	++
4	19:45	21:20	0	++
5**	21:45	25:30	0	+
6	22:05	25:40	0	++

* The inflammatory reaction was caused by concentrated broth.

** The site of inflammation was located in the foreleg.

Similar results obtained on the frog will be reported in a separate paper.

DISCUSSION AND CONCLUSIONS

Microscopic studies show the presence of a network of fibrin within the tissues and numerous thrombosed lymphatics at the site of inflammation. Precipitated iron compounds, possibly coagulated horse serum, or particulate matter caught in this fibrinous reticulum will disseminate less readily than trypan blue from the site of inflammation.

Trypan blue injected at the periphery of an inflamed area fails to enter the site of inflammation. This failure of penetration is caused

by the occlusion of lymphatic vessels and by the presence of a fine network of fibrin in the tissue spaces of the inflamed area.

Fixation of foreign substances by the inflammatory reaction is therefore primarily due to mechanical obstruction caused by a network of fibrin and by thrombosed lymphatics at the site of inflammation.

There is another phase of the problem which still requires more accurate information. This concerns the relation between exudation from blood vessels and changes in flow of lymph from the inflamed area. Further experiments are being conducted to investigate this question.

The reaction of fixation which occurs extremely early in the inflammatory process circumscribes the irritating substance and allows a definite period of time for the leucocytes to assemble for the purpose of phagocytosis.

It is through a delicate regulating mechanism of this kind that, to use the expression of Opie (7), "the vital organs are protected at the expense of local injury."

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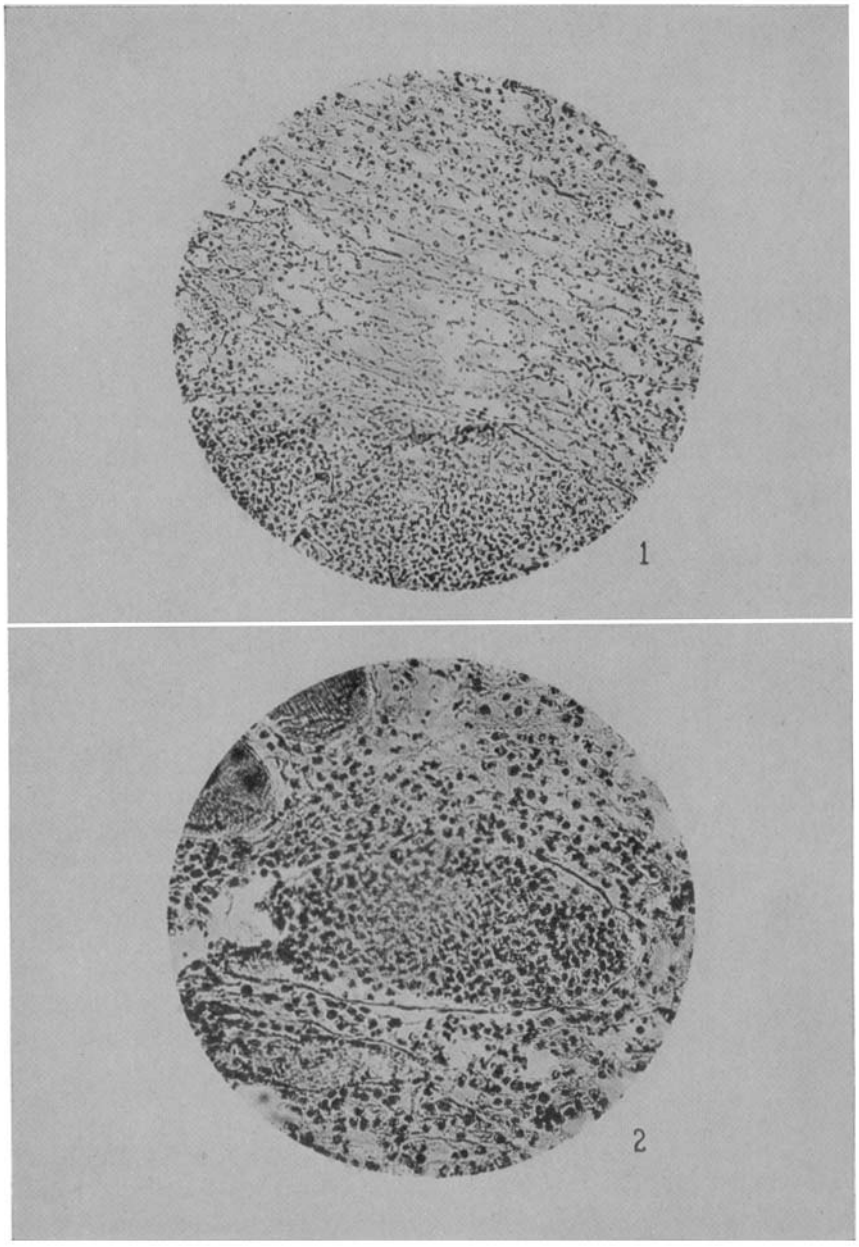
EXPLANATION OF PLATE 7

FIG. 1. Site of inflammation showing network of fibrin in the subcutaneous tissue of rabbit. Trypan blue was injected directly into this area and was shown to be fixed *in situ*. Low power magnification.

FIG. 2. Site of inflammation. Same section as in Fig. 1 but in a different field. A lymphatic vessel occluded by a large thrombus. High dry magnification.



(Menkin: Studies on inflammation. V)