STUDIES ON INFLAMMATION

XII. Mechanism of Increased Capillary Permeability. A Critique of the Histamine Hypothesis*†

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Inflammatory changes are initiated with disturbances in local fluid exchange. There is an early vasodilatation accompanied by an increase in capillary pressure (1). This seems to be referable to a stimulation of the axone reflex. Many years ago Cohnheim pointed out that capillary permeability increased as a result of direct endothelial injury (2). The augmentation in seepage through the capillary wall may be detected as early as 2 minutes after the introduction of an irritant (3). The studies of Ricker and Regendanz, of Florey, and of Landis indicate that it is primarily injury and not mere dilatation of the capillary wall which increases its permeability (4–6). The consequent outpouring of plasma proteins reduces the osmotic pressure of the plasma colloids in the blood and thus enhances the effectiveness of the hydrostatic pressure in promoting the passage of fluid into the extracapillary spaces. The studies of Hudack and McMaster (7, 8) indicate that in the initial phase of the inflammatory reaction there seems likewise to be an increase in the permeability of the afferent lymphatics with probably an accompanying increase in the lymph flow. As the inflammatory reaction develops and if it progresses in intensity the lymphatic drainage becomes impeded by the presence of

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thrombi in the lymphatic channels and coagulated plasma in the inflamed area (9–11).

In earlier studies we pointed out that the initial increase in capillary permeability is readily demonstrable by the accumulation from the circulating blood of various substances at the site of inflammation. These include a dye, graphite material, and bacteria. With the types of irritants employed quantitative measurements revealed that the capillary permeability was increased twofold in acute inflammation (12–14).

The precise mechanism involved in the initial augmentation in capillary permeability in injury is obviously of considerable importance. Several years ago Lewis postulated the development of a type reaction primarily referable to a chemical H substance liberated from injured tissue. The H substance is presumably histamine or a substance having similar properties and therefore resembling it closely (15). According to Lewis and Grant the type reaction as elicited by the cutaneous injection of histamine manifests itself in three ways: (*a*) a local vasodilatation of capillaries, venules, and arterioles by direct action; (*b*) a widespread dilatation of outlying arterioles resulting from a local reflex; and (*c*) an increase in the permeability of the minute vessels by direct action (16). This type reaction leads to local edema of the skin. They conclude that:

"The vascular reactions of the skin in urticaria facticia in response to single strokes, and incidentally of normal skins to repeated strokes, are closely compared. The reactions are in general and in detail identical with those of the type reaction. From this comparison, and from further evidence of a more direct kind, it seems clear that the reactions of the skin to stroking result from the liberation of a chemical substance in the skin, this substance having a histamine-like action on the blood vessels and nerves. Less extensive comparisons of the skin's response to scratches and pin pricks, and to excessive heat, indicate that these responses are of a similar or identical kind and that these are determined by a chemical stimulus."

The conclusions of Lewis and Grant appear to be based largely on an analogy of the type reaction obtained by histamine with that of a variety of other injurious agents. When more direct tests were performed on the effect of the skin wheal fluid on the contraction of the guinea pig uterus, they were unable to obtain any evidence that histamine was liberated in larger quantities from injured tissue than was found in normal plasma. Nevertheless Lewis (15) concludes later that:

"It is difficult to refrain from stating without reserve the simple conclusion that the vasodilator substance considered and the H-substance are one and the same, and that this substance is histamine, free or held in loose combination." Further on he continues: "Nevertheless there are instances of response to skin injury in which disintegration products cannot be neglected entirely as a possible factor. It is desirable that they should still be remembered and further considered, and for this reason I shall continue to refer to H-substance in preference to histamine."

Krogh (17) accepts unreservedly the view first foreshadowed by the work of Ebbecke (18) and later chiefly sponsored by Lewis that under certain stimuli the tissue cells will liberate substances having a dilator effect on capillaries; but at the same time he finds it impossible to assume that in all cases the action is referable to a single chemical H substance. For this reason Krogh postulates the possibility of two effective substances liberated from injured cells: a diffusible factor closely related to histamine, if not histamine itself; and an H colloid substance which is probably less diffusible. Rous and Gilding (19) raise considerable doubt concerning the validity of a hypothesis which refers all local vasodilatation to the action of a single chemical substance liberated within tissues. They clearly demonstrated that the vascular contraction in Bier's spots prevailed over the local reddening induced by mechanical injury, whereas it was without effect upon the local vasodilatation induced by cutaneous injection of histamine. More recently Goldschmidt and McGlone (20) have studied the failure of a reactive hyperemia occurring with arrested circulation in an oxygen atmosphere. They have compared their findings with the histamine reaction in a similar oxygenated and ischemic environment. Their observations on the human forearm seem incompatible with the view that the vasodilatation responsible for reactive hyperemia is due to an H substance identical with histamine.

The present study was undertaken in order to determine whether one or more substances could be obtained from inflammatory exudates which, when introduced into normal cutaneous tissue, would induce local vasodilatation and an increase in the permeability of the capillary wall. Furthermore the properties of the active fractions which have been obtained from inflammatory exudates have been compared with histamine in an endeavor to test Lewis' hypothesis. In brief, the experiments about to be described indicate that a diffusible crystalline-like material capable of increasing capillary permeability is

present in inflammatory exudates. By appropriate tests this active principle has been shown to lack the properties characteristic of histamine, thus apparently ruling out the latter as of any primary significance in inflammation. Studies reported in a preliminary communication suggest that the twofold increase in potassium content found in exudates, as compared to blood serum, even as early as the first few hours of the inflammatory reaction may be connected in some way with the active factor (21).[1] In addition it was pointed out that organic compounds, other than histamine, including various products of proteolytic breakdown, such as amino acids, usually found increased in concentration in an exudate, likewise seem to have some effect in augmenting the permeability of capillaries during the course of the inflammatory process. The present report, however, will be confined to a description of the procedure adopted in isolating the active principle from an inflammatory exudate and to compare it in its properties to histamine. Studies on its chemical identification will form the subject of separate future communications.

EXPERIMENTAL

Inflammatory exudates, obtained by several different techniques, were studied. A convenient method of obtaining an abundance of exudative material is through the intrapleural injection of about 2 cc. of turpentine in dogs (22). Several experiments were also performed with exudates produced by the injection of minute amounts of croton oil in olive oil into the thoracic cavity of rabbits. In addition small quantities of exudate were obtained in the rabbit from cutaneous areas of inflammation induced by *Staphylococcus aureus*. Finally, in a few instances, to rule out the possibility of an admixture of an injected irritant with the exudate, sufficient edematous material was recovered by immersion, under ether anesthesia, of a rabbit's fore limb into almost boiling water for 1½ minutes.

The Presence in Inflammatory Exudates of a Substance Inducing Increased Capillary Permeability

As a rule the exudates were obtained from the thoracic cavity of dogs. The duration of inflammation ranged from 1 to several days. 0.2 cc. of the centrifugalized

[1] The average potassium content of 14 blood serum determinations on dogs was 18.2 mg. per 100 cc. in comparison with an average level of 36 mg. per 100 cc. in a similar number of determinations on exudates. Osterhout pointed out that with injury to a cell there is a fall in potential difference and a consequent establishment of a current with outward migration of potassium ions (25). The possible implications of these findings to problems of permeability in inflammation are obvious and are accordingly being studied further.

cell-free exudate was injected into the normal skin of a rabbit.[2] This was immediately followed by the injection of 10 or 15 cc. of 1 per cent trypan blue in saline into the ear vein of the same animal. In the great majority of cases the treated cutaneous areas stained intensely and practically homogeneously with the dye. The accumulation of trypan blue occurred as early as 1 to 3 minutes after the intracutaneous inoculation of the cell-free exudate. The intensity of the staining in the injected area increased with time, reaching a maximum in about 10 to 15 minutes (see areas 4 and 4 a, Fig. 1). The cell-free exudate, when diluted 1 part in 10 with serum or water, still manifested an effect on the permeability of normal capillaries. In this dilution, however, the staining was relegated to the periphery of the treated skin areas.

Careful comparative studies have shown that the presence of small amounts of the active factor in a diluted exudate or as found in undiluted blood serum will invariably produce staining only of the outlying portion of the treated skin area. This difference in staining pattern is a convenient gauge of the concentration of active substance affecting capillary permeability in a given amount of body fluid material.

To rule out the effect of turpentine *per se*, this substance was suspended in a volume of water corresponding to the amount of exudate likely to be recovered from an intrapleural injection of 2 cc. of this irritant. The intracutaneous injection of this turpentine emulsion failed to produce, in the time required for the usual experiment, an accumulation of dye from the circulating blood stream. Furthermore an exudate obtained from inflamed tissues induced by a burn responded as actively as did the exudates produced by turpentine. When the edematous fluid of a 1 day old inflammatory area caused by *Staphylococcus aureus* was inoculated into normal skin tissue and a dye was injected intravenously, the cutaneous area was diffusely stained within several minutes. The active factor was evidently not the microorganism *per se*, for in an equal interval of time a heavy broth

[2] The same experiment was repeated by injection of the cell-free exudate, obtained from a dog's pleural cavity, into the skin of a normal dog. In a few instances a pleural exudate removed from a rabbit was injected into its own normal skin; and this was followed by intravenous injection of the dye. These additional controlling experiments were done in order to rule out any question of protein specificity on capillaries by using body fluids of one animal and injecting this material into another animal.

suspension of *Staphylococcus aureus* failed to cause any accumulation of dye when injected intracutaneously into the same rabbit.

When the serum of a normal dog or rabbit is treated in the same manner as an exudate, the dye, in the interval of time studied, usually either fails to permeate from the circulating blood, or only a relatively small amount passes through, staining exclusively the periphery of the injected cutaneous area. For the reason stated above, this seems to indicate that the active factor inducing increased filtration through the endothelial wall exists in definitely lower concentrations in blood serum than in exudates. The foregoing observations suggest that a substance other than the inflammatory irritant which is capable of inducing almost immediate increased capillary filtration is present in an inflammatory exudate.

Studies on the Concentration and Isolation from Exudates of a Factor Effective in Increasing Capillary Permeability

Experiments were set up in an endeavor to determine the nature of the active factor liberated in injured tissue which is capable of inducing an increase in capillary permeability. Various obvious questions presented themselves from the start. Was one dealing with the protein fraction of the exudate, and if so, precisely which of the recognized plasma proteins were involved? On the other hand, was it not possible that one was concerned with a diffusible crystalline substance, such as histamine, or one of its derivatives, as implied in the studies of Lewis? Or perhaps an inorganic ion was the active factor. Then finally might not the increased capillary permeability be the resultant of several different but reinforcing substances some of which might have been introduced with the irritant, and others liberated as the degree of local injury progressed? The first of these questions was studied in the following manner.

Varying amounts of exudate, obtained as described above, were treated with an equal volume of saturated ammonium sulfate. A heavy precipitate formed. This readily redissolved in distilled water, saline (0.16 M NaCl), or in a phosphate buffer mixture at pH 7.35. As a rule the resulting solution produced a prompt increase in the filtration of the normal capillary as indicated by the immediate accumulation of trypan blue from the circulating blood into the treated cutaneous area of a rabbit (see area 2, Fig. 1). The reaction was not referable to distilled water which invariably induced vascular contraction, as manifested by conspicuous local blanching. In such an area the dye failed to accumulate for a consider-

able interval of time. The buffer mixture or saline *per se* likewise induced no local staining reaction. Ammonium sulfate, as well as sodium sulfate, produced an entirely different picture when injected into the dermis. The appearance in the case of these two salts was identical, suggesting that the type reaction was referable to the $SO_4^=$ in both cases, rather than to the cations. This response was characterized by an inner colorless zone surrounded by an area of pronounced congestion peripheral to which a narrow zone of blue stood out conspicuously (see areas 7 and 8, Fig. 1). This pattern obviously differed entirely from the local reaction observed either when the untreated cell-free exudate or the saturated ammonium sulfate precipitated fraction were compared (areas 4, 4 a, and 2, Fig. 1). In a number of instances, though not invariably, the local effect on the capillary obtained with the ammonium sulfate fraction appeared to be more intense than when the untreated cell-free exudate was employed. Results of the same type were obtained when a precipitate was produced by the interaction of 20 per cent sodium sulfate with the cell-free exudate (see area 1, Fig. 1).

When the globulin fraction of the exudate was precipitated out by treatment with one-half or one-third saturated ammonium sulfate and the precipitate was redissolved in a buffer mixture, the solution, as a rule, produced no effect on the normal capillary wall. The suspended precipitate obtained either by treating the exudate with 95 per cent alcohol or 5 to 20 per cent trichloracetic acid likewise proved to be inactive. As compared to exudate, blood serum showed either no effect or at most a diminished activity on the endothelial wall. However when blood serum was treated with saturated ammonium sulfate a precipitate formed which, dissolved in a buffer mixture, often enhanced the filtration of dye into the treated skin area. This would suggest that the saturated ammonium sulfate precipitated fraction represents perhaps in a concentrated form a factor found in abundance in inflammatory exudates and in smaller quantity in blood serum.

For the convenience of the reader a type protocol of the experiment illustrated on Fig. 1 is presented (Table I). The data are self explanatory. The number of plus signs refers to the intensity of staining by the dye in the various local skin areas, and the time intervals are stated indicating the rapidity of passage of dye into the extracapillary spaces.

At this stage of the investigation the observations suggested that the active factor concerned might possibly be the albumin fraction of the exudate or a substance carried down with this protein upon treatment with certain precipitants. For this reason the analysis was pursued by adopting the scheme shown in Table II.

The precipitate obtained by treating the cell-free exudate with saturated ammonium sulfate was dialyzed for several hours in a cellophane bag against distilled water. The dialyzed protein material remaining in the cellophane bag was found

to be inactive when injected into the normal skin of a rabbit. Trypan blue failed to permeate into the site of skin inoculation which assumed an appearance of local blanching (area 3, Fig. 2). This dialyzed fraction was acid to phenol red, but when it was rendered alkaline by dissolving the protein in a buffer mixture of pH

TABLE I

Protocol of Dog 7-0. Experiment. Exudate and its fractions. Nov. 15, 1935.

Cutaneous area No., inoculated with	Time of inoculation	10 cc. 1 per cent Trypan blue in saline intravenously at 5:32:5		
		Presence of dye in inoculated area at		
		5:35	5:40	5:45
	hrs.:min.:sec.			
(1) Na_2SO_4 (20%) treated fraction dissolved in buffer pH 7.35	5 : 27	0	+	++
(2) $(NH_4)_2SO_4$ (saturated) fraction dissolved in buffer pH 7.35	5 : 27 : 5	(?) Slight trace	+	++
(3) Trichloracetic fraction suspended in buffer pH 7.35	5 : 28	0 (blanching)	0 (blanching)	0 (blanching)
(4) Untreated cell-free exudate	5 : 28 : 5	+	+++	++++
(4 a) Same as (4)	5 : 28 : 7	Slight trace	++	++++
(5) Evaporated $(NH_4)_2SO_4$ fraction and redissolved in buffer pH 7.35	5 : 29 : 0	+	++	+++
(6) Buffer pH 7.35	5 : 29 : 5	0	0	0
(7) 20% Na_2SO_4	5 : 30	Center 0. Peripheral zone of redness	+ Peripheral to inner zone of redness	Same as at 5 : 40
(8) Saturated $(NH_4)_2SO_4$	5 : 32	Same as (7)	Same as (7)	Same as (7)

7.43, it likewise failed to enhance seepage of dye from the circulating blood (area 3 a, Fig. 2).

These observations indicated that the albumin fraction of the inflammatory exudate was evidently not the factor responsible for increased capillary permeability in inflammation. Further substantia-

tion of this point was obtained when crystallized serum albumin or crystalline egg albumin[3] was introduced into the dermis of a normal rabbit. Trypan blue failed to accumulate in cutaneous areas treated with these pure proteins. The analysis was consequently directed towards testing for the possible presence of diffusible active substances in the dialysate.

The dialysate, whether untreated, or freed of its $SO_4^=$ ions by repeated treatment with 10 per cent barium chloride, was evaporated to dryness either on an ordinary water bath or, as in the preliminary experiments, in a vacuum oven under reduced temperature. The material obtained by this procedure appeared as a mass of crystals, heterogeneous in respect to size and shape. Its reaction was, as a rule, alkaline to phenol red. The Fehling test was positive, indicating the presence of reducing substances in the dialysate. The biuret and nitric acid tests for proteins were negative.

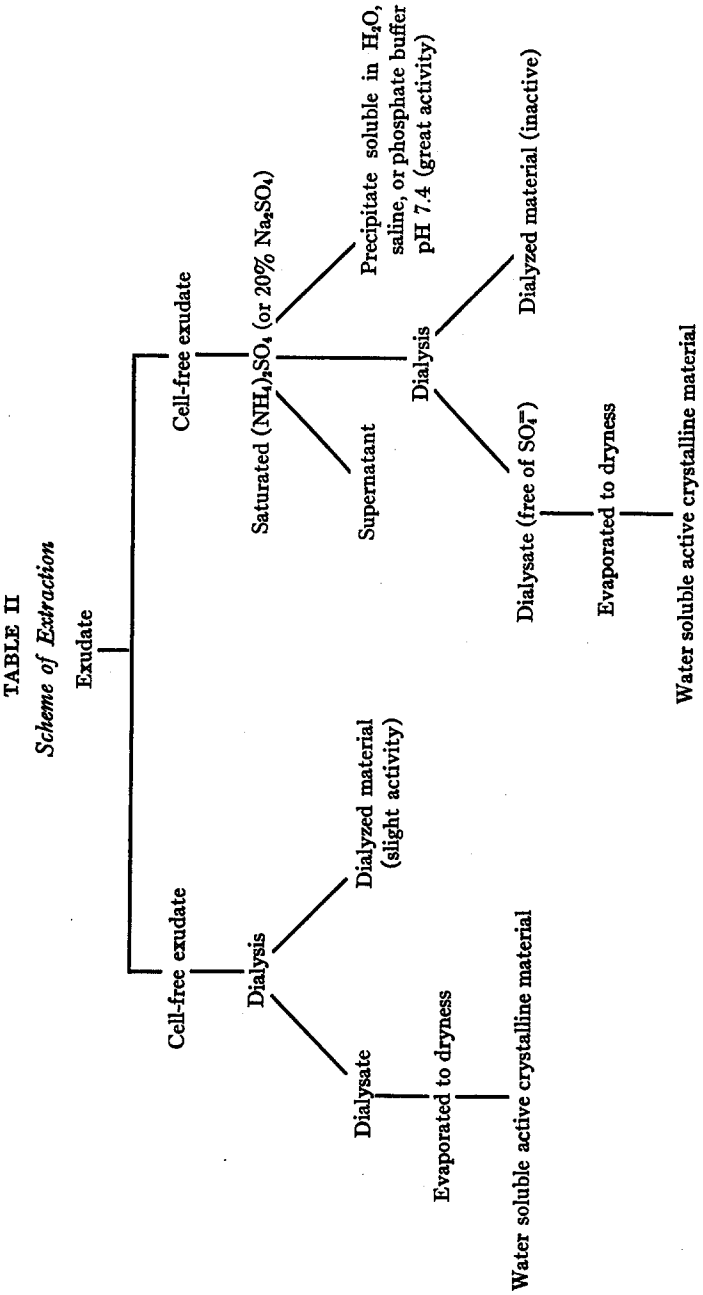
It is quite obvious that this crystalline material was in no sense purified and therefore doubtless represented a mixture of several diffusible substances. The crystalline water soluble fraction obtained from the evaporated dialysate showed definite activity in regard to the augmentation of capillary filtration (Table II). Trypan blue from the circulation accumulated throughout the local skin area, staining it relatively homogeneously. This occurred irrespective of whether the crystalline material was dissolved in water or whether the dialysate was simply concentrated to a very small volume by evaporation (see Fig. 2, areas 1 and 2 a).

The active permeability factor, as found in the untreated cell-free exudate, or as obtained in its dialysate, retains its potency when the material is kept on ice. In the types of acute inflammation studied (23) the activity is not altered by changes in the pH of the exudate, ranging from 7.4 to 6.5. The active substance is essentially thermostable. It may still display definite activity after being brought to the boiling point. Freezing it down to $-20°$ does not seem to alter its potent effect on the capillary wall. Dialysis of the untreated cell-free exudate without preliminary treatment with saturated ammonium sulfate produced practically the same type of result, except that here the material that remains in the cellophane bag sometimes retained a slight degree of activity in affecting the capillaries. It induced con-

[3] Obtained through the courtesy of Dr. E. J. Cohn.

TABLE II

Scheme of Extraction



centration of the dye, but only at the periphery of the treated skin area (Table II).

To conclude, the results obtained from various types of inflammatory exudates suggest the presence of some agent which almost immediately augments filtration of a dye through the normal capillary wall. Previous work (13, 14) indicated that this increased filtration was primarily referable to changes in the permeability of the capillary endothelium. Furthermore the active factor seems in large part to be of crystalloid dimensions and therefore diffuses fairly readily through a cellophane membrane. Finally the active diffusible factor appears to exist also in blood, although biological tests indicate that it is present there in definitely smaller quantities than in an inflammatory exudate.

Is Histamine the Active Factor Found in Inflammatory Exudates?

In the introductory section of this paper mention was made of Lewis' hypothesis to the effect that the mechanism of increased capillary permeability in injury was referable to the liberation of a single substance, presumably histamine, or at least a closely related compound, the so called H substance. In an endeavor to confirm this view the properties of histamine were compared with those of the active factor found in inflammatory exudates.

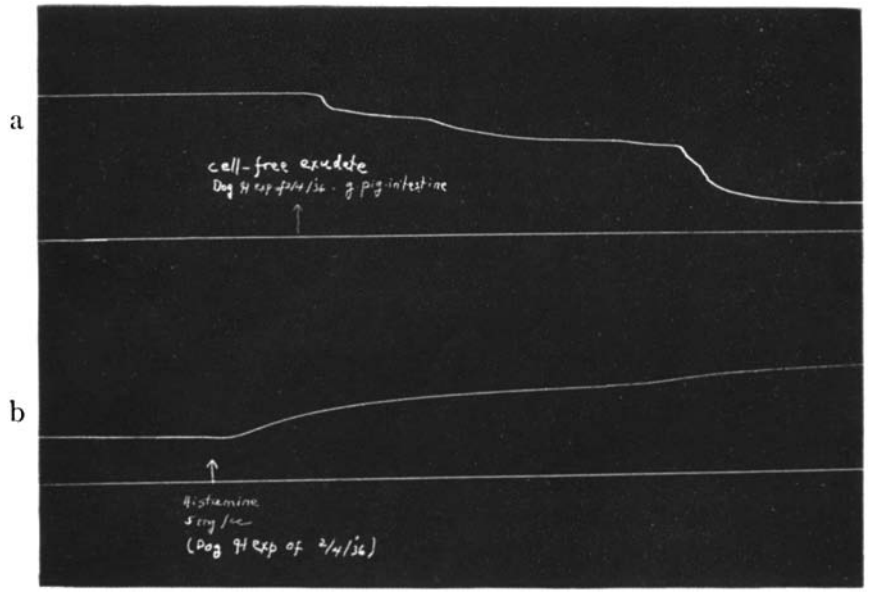
Histamine (ergamine acid phosphate) was dissolved in either water, serum, or saline in varying amounts ranging from 0.1 to 10 mg. In each case the given quantity of histamine was taken up in 0.2 cc. of fluid and injected intracutaneously into the abdomen of rabbits. Trypan blue was then introduced into the ear vein. The dye either failed to enter the histamine-treated area or else, in some cases, it diffused in a widespread flare-like formation at the periphery of the site of inocution, with a conspicuous area of local blanching appearing in the center (area 7, Fig. 2). This outlying zone of staining was more likely to occur with the high concentrations of histamine.

The obdurate reaction to histamine on the part of the capillaries in the rabbit had been pointed out recently by Morgan (24). In the dog, however, trypan blue accumulated readily in a histamine-treated area, but here again the dye displayed the characteristic flare-like pattern at the peripheral portion of the local area of inoculation, the center of which remained blanched. This occurred even when 2.75

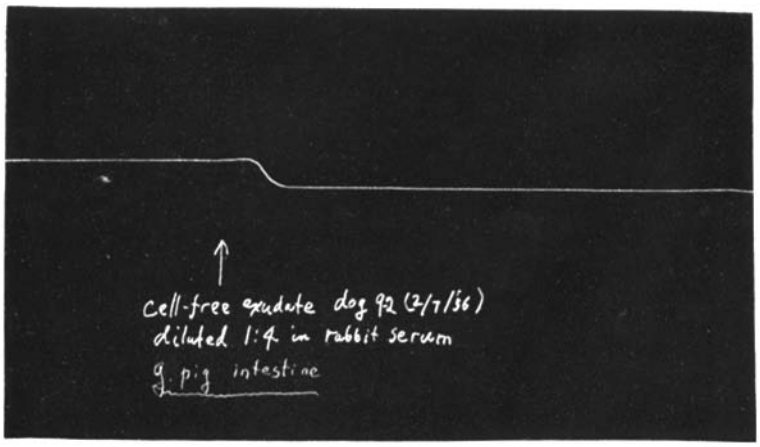
mg. of dissolved histamine had been introduced into the skin of the dog. In contrast to the staining pattern obtained with histamine, it has already been pointed out that the active factor found in an exudate or recovered from its dialysate produces a homogeneous or practically uniform effect on the capillaries of the injected skin area. This results in a relatively even staining by the dye as it concentrates from the blood stream into the extracapillary spaces of the inoculated area (see areas 4 and 4 a, Fig. 1; areas 1 and 2 a, and 6, Fig. 2). As previously stated, diluting the active factor found in an exudate results merely in a peripheral localization of the dye when the samples are tested on the normal skin. This is likewise true of undiluted blood serum. In view of the dilution experiments with the cell-free exudate, it has already been suggested that the active capillary factor is probably found in definitely smaller quantities in blood than in inflammatory exudates. Nevertheless, both with diluted exudate or undiluted serum, the local staining of the site of cutaneous inoculation never spreads in the outlying fashion characteristic of histamine. Furthermore the staining pattern of histamine occurs when as much as 10 mg. of this substance is introduced into a local skin area. It is doubtful whether such a high concentration of this substance would be exceeded in 0.2 cc. of a cell-free exudate. It seems therefore unlikely that the distinction between the tissue stained patterns of undiluted exudates and of histamine is in any way referable to a superabundance of histamine in inflammatory fluids.[4]

[4] An additional corroborative evidence of the antagonistic action of histamine to the active principle was obtained by the addition of 1 mg. of histamine to 0.2 cc. of an otherwise potent exudate. Areas on the dermis of the abdomen of rabbits treated with such preparations were found to be refractory in regard to seepage of dye from the circulation.

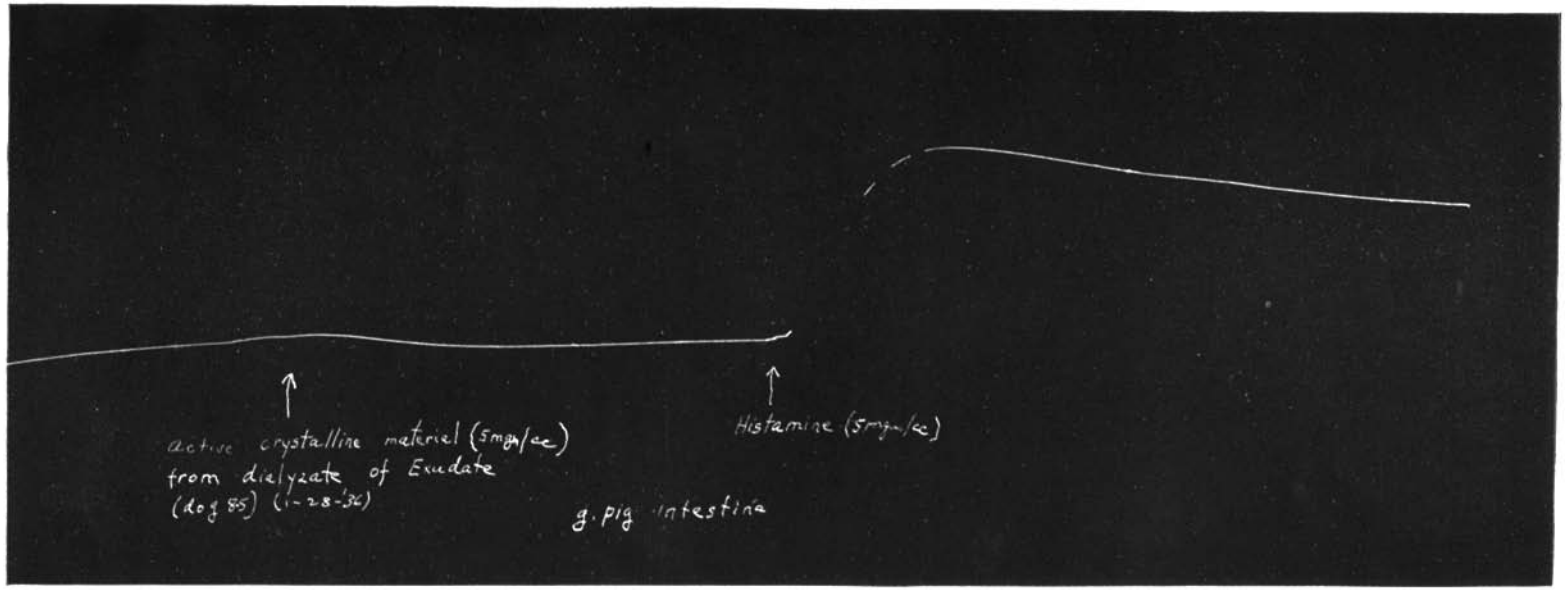
The studies of G. S. Barsoum and J. H. Gaddum recently pointed out that if the addition of histamine to an unknown extract induces the disappearance of the original contractile effect of the extract on an isolated intestinal segment this may serve as indication that the effect of the extract is due to histamine (*J. Physiol.*, 1935, **85**, 1). For this reason an experiment was set up in which, after treating the intestinal guinea pig segment with the active dialysate of an exudate, a solution of histamine was added to the same bath. The usual depressing effect of the exudate or of its dialysate on the tonus of the intestine was immediately replaced by powerful tonic contractions, showing thus that there was no evidence of any refractory state to histamine induced by preliminary treatment with the inflammatory exudate.



TEXT-FIG. 1. 1a represents the effect on the strip of intestine of the cell-free exudate. 1b represents the effect of histamine on such a segment.



TEXT-FIG. 2. The effect on the strip of intestine of an inflammatory exudate diluted 1:4 in rabbit serum.



TEXT-FIG. 3. The addition to the isolated strip of intestine of an aqueous solution of the active crystalline material recovered from the dialysate of an inflammatory exudate. This was followed by the addition of histamine, as indicated. Compare on the one hand the lack of response induced by the active crystalline material with the pronounced contractile reaction obtained by histamine.

In order, however, to establish any further differences in properties between histamine and the active capillary factor found in an inflammatory exudate, the following tests were made. Histamine is known to increase the tonus of the guinea pig intestine or of the virgin uterus. This effect is readily elicited and recorded on a revolving kymograph as a contraction of the isolated segment. Although several experiments were tried with the uterus most of the observations were made by tests on the isolated intestinal loop, comparing thus the effect of histamine, cell-free exudate, and the crystalline material obtained from the dialysis of the exudate. When histamine in the various concentrations employed for the skin tests is added drop by drop to a suspended loop of intestine, a prompt contraction of the segment ensues (Text-fig. 1 b). When, on the other hand, the cell-free exudate is added to a fresh loop the intestinal segment definitely relaxes, as indicated by a decrease in tonus and a definite drop of the recording pointer (Text-fig. 1 a). Precisely the same decrease in tonus is obtained if the exudate is diluted 1 part in 4 in rabbit serum (Text-fig. 2). In this connection it is to be recalled that dilution of the exudate produces merely peripheral localization of dye in the treated skin area. Nevertheless the depression in the tonus of the intestine persists in contrast to the contractile effect of histamine. When the crystalline material obtained by evaporating the dialysate of an exudate is tested on the loop of intestine, it likewise fails to induce contraction, whereas the subsequent addition of histamine may give rise to a powerful contraction (Text-fig. 3). Undiluted blood serum, which in itself may occasion merely peripheral staining of a treated skin area, fails, like the exudate, to produce any contraction of the isolated intestinal strip.

In order to ascertain whether a human inflammatory exudate, in contrast to histamine, elicits the same reactions as the exudates of dogs or rabbits, identical tests were repeated on a sample of fluid removed from the chest of a patient with a hemolytic streptococcus infection.[5] The prompt uniform staining reaction on the dermis of the abdomen of a rabbit and the response of the isolated guinea pig intestine were precisely the same as in the samples from experimental animals, differing thus entirely from the reactions obtained with histamine.

[5] This exudate was obtained from the Beth Israel Hospital through the courtesy of Dr. H. L. Blumgart and Mrs. D. R. Gilligan.

The above observations indicate that the active factor recovered from an inflammatory exudate, which is capable of inducing increased capillary filtration primarily by injury to the endothelial wall, does not seem to be histamine nor is it the H substance in so far as its properties are supposed to resemble closely those of histamine. This is revealed by comparing histamine and the active factor found in exudates in regard to their local effects on the capillary wall, as evidenced by the differential staining patterns produced and also by the opposite types of response which they induce on the isolated strip of intestine.

SUMMARY AND CONCLUSIONS

Various types of inflammatory exudates have been obtained either by the introduction into normal tissues of a chemical irritant, or by a burn, or by bacteria in either dogs or rabbits. A study has also been made on an exudate of human origin.

These exudates have all been found to contain a factor which induces prompt increase in the permeability of normal skin capillaries, demonstrable by the almost immediate accumulation from the circulation of trypan blue into areas of skin injected with the cell-free exudate.

The active factor may be carried down with the precipitate resulting from the interaction of the exudate with either saturated ammonium sulfate or 20 per cent sodium sulfate.

The active factor passes through a dialyzing membrane. It can be recovered from the dialysate as a protein-free crystalline material.

The active factor manifests no property in common with histamine or presumably with the hypothetical H substance assumed to be closed related to histamine.

This is indicated by the following considerations: (*a*) difference between the tissue staining pattern of the exudate or of its active fraction and that of histamine; (*b*) opposite effects by histamine and the active factor found in exudates on the tonicity of the isolated strip of guinea pig intestine.

The observations presented in this report do not substantiate Lewis' hypothesis of histamine or of its closely related H substance as the primary cause of increased capillary permeability in inflammation.

The present studies are being continued in an endeavor to free of its impurities and to identify the active crystalline-like material iso-

lated from an inflammatory exudate. The details of this investigation will form the subject of a separate future communication.

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BIBLIOGRAPHY

1. Landis, E. M., *Am. J. Physiol.*, 1930, **93**, 353.
2. Cohnheim, J., Lectures on general pathology, London, New Sydenham Society, 1889.
3. Menkin, V., *J. Exp. Med.*, 1932, **56**, 157.
4. Ricker, G., and Regendanz, P., *Virchows Arch. path. Anat.*, 1921, **231**, 1.
5. Florey, H. A., *Proc. Roy. Soc. London, Series B*, 1926, **100**, 269.
6. Landis, E. M., *Physiol. Rev.*, 1934, **14**, 404.
7. McMaster, P. D., and Hudack, S., *J. Exp. Med.*, 1932, **56**, 239.
8. Hudack, S., and McMaster, P. D., *J. Exp. Med.*, 1933, **57**, 751.
9. Menkin, V., *J. Exp. Med.*, 1931, **53**, 171.
10. Menkin, V., *Arch. Path.*, 1931, **12**, 802.
11. Menkin, V., *Am. J. Med. Sc.*, 1935, **190**, 583.
12. Menkin, V., *J. Exp. Med.*, 1929, **50**, 171.
13. Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1930, **51**, 285.
14. Menkin, V., *J. Exp. Med.*, 1931, **53**, 617.
15. Lewis, Sir T., The blood vessels of the human skin and their responses, London, Shaw & Sons Ltd., 1927.
16. Lewis, Sir T., and Grant, R. T., *Heart*, 1924, **11**, 209.
17. Krogh, A., Anatomy and physiology of capillaries, New Haven, Yale University Press, revised edition, 1929.
18. Ebbecke, U., *Klin. Woch.*, 1923, **2**, 1725.
19. Rous, P., and Gilding, H. P., *J. Exp. Med.*, 1930, **51**, 27.
20. Goldschmidt, S., and McGlone, B., *Am. J. Physiol.*, 1934, **109**, 42.
21. Menkin, V., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 570.
22. Opie, E. L., *J. Exp. Med.*, 1907, **9**, 391.
23. Menkin, V., *Am. J. Path.*, 1934, **10**, 193.
24. Morgan, J. R. E., *Arch. Path.*, 1934, **18**, 516.
25. Osterhout, W. J. V., Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1933, **1**, 125.

EXPLANATION OF PLATE 34

FIG. 1. The dermis of the abdomen of a rabbit treated with an inflammatory exudate and some of its fractions. The skin inoculations were followed by the intravenous injection of 10 cc. of 1 per cent trypan blue in saline. The effect on the permeability of the capillary wall is determined by the extent of dye accumulation in the various skin areas treated. For an explanation of the material inoculated in each area the reader is referred to Table I in the text.

FIG. 2. The dermis of the abdomen of a rabbit treated with various fractions of an inflammatory exudate and with histamine. The skin inoculations were followed by the immediate intravenous injection of 1 per cent trypan blue. The effect on capillary filtration is indicated by the extent of the local staining reactions induced by the respective fractions. These were as follows:

No. 1. Cell-free exudate treated with saturated $(NH_4)_2SO_4$ and dialyzed. The dialysate was concentrated to about 1/17th of its original volume and inoculated in the skin. Note the considerable accumulation of the dye in the area.

No. 2 a. The dialysate in area 1 was treated with 10 per cent $BaCl_2$ to precipitate out the $SO_4^{=}$ ions. The supernatant fluid was diluted with an equal volume of phosphate buffer mixture (pH 7.43) and inoculated intracutaneously. The accumulation of dye is a conspicuous feature.

No. 3. The protein material of the exudate remaining in the cellophane bag after dialysis. The dialysate of this sample was injected after concentration into area 1. Note the inactivity of the protein fractions of the exudate.

No. 3 a. The same as area 3 with the exception that the protein material was rendered alkaline by diluting one part of it with an equal volume of phosphate buffer (pH 7.43).

No. 5. Some material flocculated and remained behind in the cellophane bag after dialysis of the exudate. This precipitate probably represents the euglobulin fraction of the exudate. Its inactivity is evident.

No. 6. The cell-free exudate treated with saturated $(NH_4)_2SO_4$. The precipitate was dissolved in a phosphate buffer mixture (pH 7.43). The effect is evident, indicating that the active material was carried down with the precipitate.

No. 7. The injection of 0.55 mg. of histamine dissolved in distilled water failed to cause the accumulation of trypan blue in the treated skin area. Compare this effect with that in area 1.

No. 8. Phosphate buffer mixture (pH 7.43).

No. 9. Distilled water.

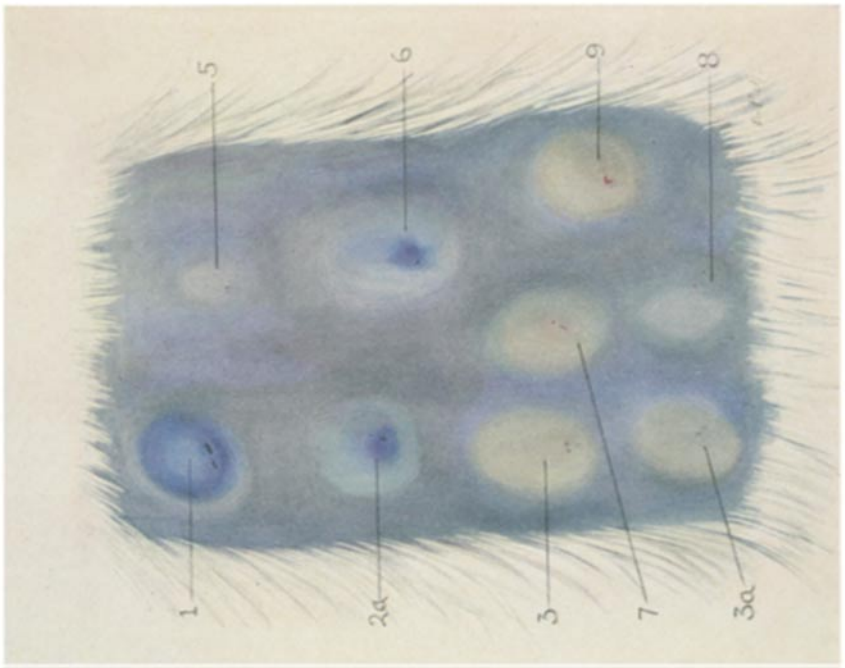

FIG. 2

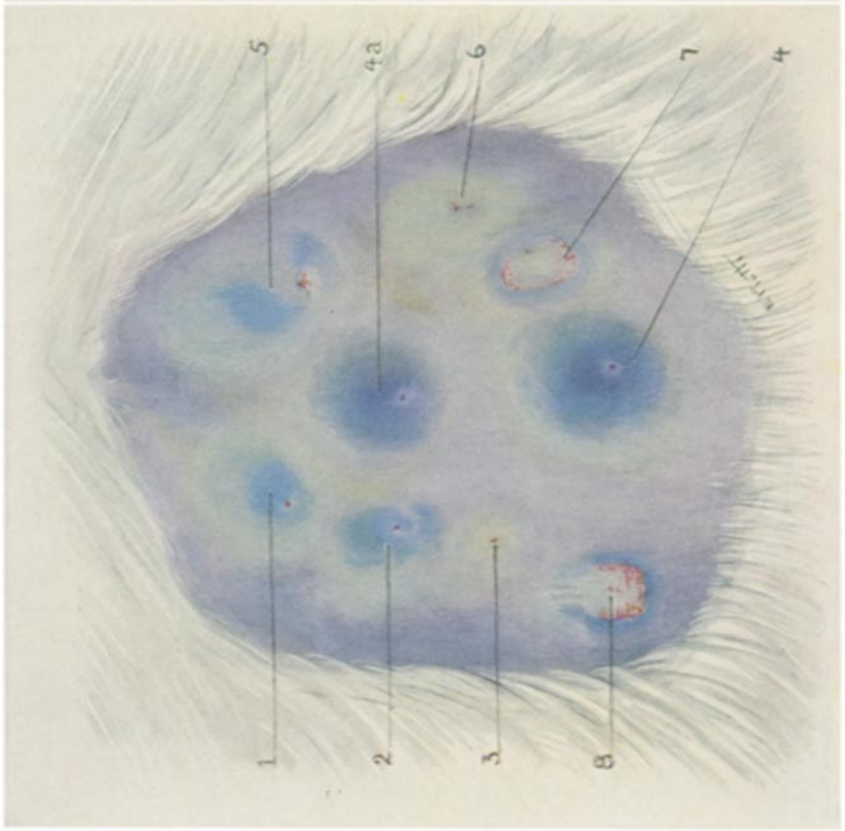

FIG. 1

(Menkin: Studies on inflammation. XII)