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A Note on the Differences Between Histamine and Leukotaxine.

VALY MENKIN.

*From the Department of Pathology, Harvard University Medical School, Boston.**

Previous studies have demonstrated the presence in inflammatory exudates of a factor capable of increasing capillary permeability.[1] Purification of the exudate material has yielded a crystalline nitrogenous substance which is extremely active in inducing prompt increased capillary permeability as shown by the pronounced seepage of an intravenously injected vital stain into treated cutaneous tissue.[2] Furthermore, this material manifests a chemotactic effect. Within 15 to 30 minutes following its intracutaneous inoculation, polymorphonuclear leukocytes accumulate in abundance in the lumen of small vessels. These cells at first adhere closely to the endothelial wall from which they soon migrate actively into the extra-capillary spaces.[3] The chemotactic property of this active substance may be demonstrated *in vitro*. Polymorphonuclear leukocytes in a sample of exudate readily aggregate around particles of the material deposited on a slide.[3] For the sake of convenience this substance has been tentatively named *leukotaxine*.[4] The untreated inflammatory exudate contains both a permeability and a chemotactic factor. Prolonged chemical fractionation yields a relatively homogeneous crystalline end-product which still manifests both of the properties possessed by the original exudate. The analytical procedure has thus failed to dissociate the two factors. The available data warrant, for the sake of convenience, a name for this active substance which *per se* appears to behave as a definite biological unit. Leukotaxine is thus capable of rapidly reproducing the basic sequences of the inflammatory reaction, namely an initial increased capillary permeability followed by polymorphonuclear leukocytic infiltration.

In the foregoing studies, no concrete evidence has been obtained to support Lewis' view that a histamine-like substance in exudates or in their partially purified active fractions, can adequately account for the primary mechanism of increased capillary permeability in

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1 Menkin, V., *J. Exp. Med.*, 1936, **64**, 485.

2 Menkin, V., *J. Exp. Med.*, 1938, **67**, 129.

3 Menkin, V., *J. Exp. Med.*, 1938, **67**, 145.

4 Menkin, V., *Arch. Path.*, 1937, **24**, 65.

inflammation. Leukotaxine does not exhibit the properties of histamine. The evidences supporting this fact have been described in detail elsewhere.[1, 2, 5, 6]

Bier and Rocha e Silva[7] have recently repeated the earlier studies of the writer on whole exudates and have confirmed the findings of a permeability and of a chemotactic factor in inflammatory exudates. They have, however, neglected to purify the exudates in order to obtain the relatively pure material capable *per se* of reproducing the increased capillary permeability and migration of polymorphonuclear leukocytes. On the basis of only meager evidence, they have concluded that the permeability factor (but not the chemotactic factor) is histamine. Their conclusions have been reached after extracting histamine from exudates, by the method of Barsoum and Gaddum[8] in order to eliminate thus the presence of any depressing substances when the end-product is tested on the isolated strip of guinea pig intestine. They admit, as I have pointed out previously,[1, 5] that with the accumulation of trypan blue there is a difference in the staining pattern of tissue as elicited by histamine and by an inflammatory exudate.† Yet these investigators assume, without any evidence, that this is presumably referable to the loose combination of histamine with proteins or by adsorption of colloids. In brief, the observations of these investigators on the contraction of the isolated intestine of the guinea pig simply indicates that histamine probably exists in exudates, and can by appropriate extraction, be demonstrated. This has been surmised and known (Loos[9]), as I have recently pointed out elsewhere.[2, 5, 6] In other words, the possibility that histamine exists in exudates has not been wholly dispelled by the available evidence on the presence of the permeability factor;[1, 2] but the primary importance of histamine in explaining the mechanism of increased capillary permeability in inflammation has been open to serious doubt.[1, 2, 5, 6] The permeability factor (*i. e.*, leukotaxine) recovered and isolated from an exudate displays, as pointed out elsewhere, none of the specific properties of histamine. Yet leukotaxine is capable *per se* of reproducing the same type of reaction as the whole exudate. The mutual, non-specific properties of

5 Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

6 Menkin, V., and Kadish, M. A., *Am. J. Physiol.*, 1938, **124**, 524.

7 Bier, O., and Rocha e Silva, M., *Arqu. d. Inst. Biologico*, 1938, **9**, 109, 123, 129.

8 Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, 1935, **85**, 1.

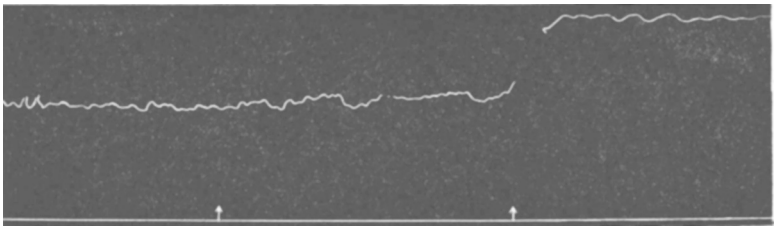
† This is generally found to be the case even when the concentration of histamine is as low as 1:500,000.

9 Loos, H. O., *Arch. f. Dermat. u. Syph.*, 1931, **164**, 199.

leukotaxine and histamine, (*e. g.*, dialyzing property, etc.) listed by Rocha e Silva and Bier, seem quite insignificant for the most part, inasmuch as they are true of numerous other unrelated substances. These investigators have failed to repeat the work on the chemical extraction of leukotaxine from exudate. They have worked solely with whole exudates. They have thus been unable to establish the fact that leukotaxine is histamine; whereas the writer has definitely demonstrated that leukotaxine has a great many physiological and chemical properties differing from those of histamine.[5, 6]

A single very important concrete evidence which nullifies the contention of these investigators is indicated by the following observation: A sample of leukotaxine, capable *per se* of actively inducing increased capillary permeability and leukocytic migration is extracted for the presence of histamine by the method of Barsoum and Gaddum.[8] According to the contention of the Brazilian workers,[7] by this procedure, the presence of any depressing impurities in leukotaxine, capable *per se* of overshadowing the contractile effect on the intestine of histamine would thus be eliminated. The final extracted material not only fails to produce a positive Zimmerman reaction for histamine; but it fails to induce an enhanced contraction of the isolated segment of guinea pig intestine (Fig. 1). Subsequent application of histamine to the same segment is followed by a powerful contraction, indicating thus the absence of any refractory effect[8] (Fig. 1). These additional observations on leukotaxine render it difficult even to consider the possibility of its identity with histamine.

Furthermore, the contention that hydrolysis with sodium hydrox-



A B

FIG. 1.

A—100 mg of an active fraction of leukotaxine extracted for histamine by the method of Barsoum and Gaddum.[8] The final product was added to a tyrode bath in which there was suspended an isolated strip of guinea pig intestine. Note the absence of any contractile effect.

B—After a few minutes, histamine dihydrochloride in a dilution of 1:5,000 was added to the tyrode bath the capacity of which was 20 cc. Note the immediate sharp contractile effect in contrast to the absence of effect with leukotaxine. It is also interesting to note the absence of any refractory effect. This indicates that leukotaxine evidently contains no histamine.[8]

ide dissociates the permeability factor from the chemotactic factor[7] is not substantiated when such a hydrolysis is carried on the purified active substance, leukotaxine. It is true that in earlier studies it was found that exposure to normal NaOH for several hours seemed to inactivate the partially purified material.[2] However, the claim of Rocha e Silva and Bier[7] of inactivating, in whole exudates, the permeability factor by alkaline hydrolysis without apparently affecting its chemotactic property is not necessarily an evidence for the presence of 2 different substances. An active group in a single substance responsible for one physiological property may well be inactivated without affecting other properties displayed by the particular substance. In order to establish whether alkaline hydrolysis is capable of dissociating the two biological properties of leukotaxine, the writer hydrolyzed for 16 to 22 hours samples of purified leukotaxine, in 1.1 N NaOH. This procedure failed completely to dissociate the two properties. The hydrolyzed product, when approximately neutralized immediately preceding its intracutaneous injection in rabbits, induced both the characteristic increased capillary permeability and leukocytic migration. Alkaline hydrolysis thus had failed to dissociate the two properties of leukotaxine.‡

Conclusions. The permeability and the chemotactic factors found in inflammatory exudates exist as well in the crystalline nitrogenous substance, leukotaxine, recovered from such exudates by chemical fractionation. There is no evidence to support the contention that the permeability factor is histamine. Extraction by the method of Barsoum and Gaddum has failed to reveal the presence of histamine in leukotaxine. Furthermore, alkaline hydrolysis of leukotaxine does not dissociate the permeability from the chemotactic factor. The foregoing observations add further support to the view that leukotaxine is the substance liberated from injured tissue which is primarily responsible for the basic sequences of the inflammatory reaction, namely, the initial increase in capillary permeability and the prompt migration of polymorphonuclear leukocytes.

‡ In regard to the statement[7] that various products of protein metabolism, such as amino acids, are capable of inducing leukocytic migration, it is interesting to note that observations with a number of representative amino acids (as well as histamine) have failed to reveal leukocytic migration.