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Isolation and Properties of the Factor Responsible for Increased Capillary Permeability in Inflammation.*

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The previous studies of the writer have indicated the presence of a factor in various types of exudates capable of promptly increasing the permeability of the capillary wall.[1, 2] The liberation of this substance by injured tissue and its consequent recovery in the exudate has offered a reasonable explanation for the main mechanism of increased plasma filtration into an inflamed area. The factor exhibited none of the properties of histamine or of its presumably closely related H-substance; thereby rendering it difficult to accept the views of Sir Thomas Lewis on the subject.[2, 3] The active substance was shown to be dialyzable and was recovered as a crystalline protein-free material.

An attempt was made to isolate the active factor relatively free of any gross impurities. The method adopted for extracting and recovering the crystalline substance was as follows: The exudate containing the factor, is as a rule obtained in abundance, as described in previous communications, from dogs after intrapleural injection with turpentine.[4, 2] The exudate, separated of its cell content by centrifugalization, is treated with an equal volume of pyridine. The precipitated mixture is placed in a stirring apparatus for several hours and then centrifuged. The supernatant fluid contains the active fraction. It is treated with 3 times its volume of acetone. A heavy white flocculating mass precipitates out which is discarded after centrifugalization. The active factor seems to be contained exclusively in the supernatant fluid. This is evaporated to dryness *in vacuo* at a temperature ranging between 40° to 50°C. This dried acetone residue may contain some brownish tarry material interspersed in a mass of white crystalline material. In this material there may be discerned numerous "branched" or "notch-like" crystals. These crystals are characterized by the presence of a main shaft with short

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[1] Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 570.

[2] Menkin, V., *J. Exp. Med.*, 1936, **64**, 485.

[3] Lewis, T., and Grant, R. T., *Heart*, 1924, **11**, 209.

[4] Menkin, V., *Am. J. Path.*, 1934, **10**, 193.

lateral projections on either side. The residue is only partially soluble in water. Further purification is attained by filtering the aqueous suspension and evaporating *in vacuo* the filtrate. The resulting crystalline material is characterized by practically only one type of structure. These are the characteristic "branched" or sometimes "cross-like" crystals having essentially the same pattern as the crystals observed in the acetone residual fraction. There may be a moderate amount of tarry material admixed to the crystalline residue recovered after drying the acetone fraction. Some of this may be removed by shaking the aqueous suspension with butyl alcohol and subsequently by separating off the clear layer. Evaporation of this fraction yields the active crystalline substance which manifests essentially a similar structural pattern as the crystals recovered from the aqueous or the acetone fractions. Finally, the supernatant fluid after precipitation with acetone may be dialyzed for several hours against distilled water. Evaporation of both the dialyzed and the dialyzate fractions reveals the presence of the characteristic crystals. This indicates that the substance diffuses readily through a cellophane membrane.

The typical crystals recovered, as described above, are extremely active in increasing capillary permeability. This is indicated by a method previously described.[2] The crystals are readily dissolved in distilled water or saline. The solution is injected intracutaneously in the dermis of the abdomen of a normal rabbit. Trypan blue is immediately injected intravenously. This dye accumulates abundantly within several minutes in the treated cutaneous area, indicating thus a local increase in the filtration capacity of the small vessels, precisely as is the state of affairs when the untreated cell-free exudate is used. Distilled water or saline induce no accumulation of dye. Pyridine and acetone mixtures were evaporated to dryness to control for the reagents employed in isolating the active factor. None of the typical crystals were recovered and the dried mixture, treated with distilled water, failed to induce the passage of dye from the blood stream when injected intracutaneously. The isolated typical crystalline substance seems therefore to represent the active factor present in exudates and capable *per se* of increasing capillary permeability. Similarly to the untreated exudate and unlike histamine, this crystalline substance depresses the tonus of the isolated strip of guinea pig intestine.

When normal blood serum containing no trace of hemolyzed red blood corpuscles is treated precisely as an exudate with pyridine and acetone, a superficially similarly appearing crystalline substance, but apparently in reduced concentration, may be recovered. The crystals,

however, seem to be more soluble in water than those isolated from an exudate. The solution of crystals obtained from blood serum are wholly or practically inactive when injected intracutaneously. Trypan blue barely accumulates in such treated skin areas. This had likewise been found to be true of the unfractionated blood serum.[2] On the other hand, blood serum, exhibiting evidence of considerable amounts of hemolyzed red blood corpuscles (or in some cases apparently extensive lipemia), when extracted as outlined above, tends to yield typical crystals which are indistinguishable from those recovered from exudates. Such crystalline material may display definite potency in the skin as evidenced by pronounced accumulation of dye from the circulating blood. This suggests that the active factor is probably liberated from injured or disintegrated tissue cells.

The active crystals recovered from exudates are water soluble. They dissolve in acetone and alcohol; but are apparently insoluble in ether or absolute alcohol. The material is heat stable. Its solution yields a negative Biuret test for proteins. The Molisch and Fehling tests indicate that the material is not a carbohydrate. Unlike histamine, which has a melting point of about 130°C., it shows no sharp melting point. At about 175°C. it chars and at 265°C. to 298°C. it still has not melted. The available evidence from proteolytic studies of total protein nitrogen and amino acid nitrogen of exudates show a reciprocal relationship when compared with their level in blood serum. The average protein nitrogen of dog blood serum was found to be 6.1 gm./100 cc. compared with a value of 4.75 gm./100 cc. in exudates. On the other hand the average figure of serum amino acid nitrogen was found to be 5.55 mg./100 cc. as compared with 8.04 mg./100 cc. in exudates. The increase in the amino acid nitrogen of exudates was correlated by the activity of the latter on increasing the permeability of capillaries. This suggests that the active substance may well be an intermediary product of protein catabolism. Its nitrogenous content averages 2.3%. The pH of its aqueous solution may be slightly acid (6.5) or slightly alkaline to phenol red. It is precipitated out by saturated $(NH_4)_2SO_4$. It is readily dialyzable. It appears unlikely on the basis of accumulated observations that it is either a proteose, a peptone, or an amino acid. The present available data seem to indicate that this active substance probably belongs to the group of polypeptides. Further studies are being conducted in an attempt to free completely of all impurities the active substance and to identify it chemically.

It is important to note that the crystalline material is evidently capable of initiating the sequences of the inflammatory reaction. Its

intracutaneous injection is not merely followed by a prompt increase in capillary permeability; but within a surprisingly short interval of 25 to 30 minutes there is considerable evidence of leucocytic migration into the treated areas. The small vessels are packed with polymorphonuclears closely adhering to the endothelial wall in the act of migrating into the extra-capillary spaces which reveal already moderate infiltration of these cells. Furthermore, the substance is positively chemotactic when placed in capillary glass tubes sealed at one end and introduced into an inflamed peritoneal cavity, the tubes soon become filled with leucocytes. The inactive crystalline material obtained from blood serum and injected intracutaneously, distilled water, or saline are all totally devoid of inducing such prompt accumulation and migration of leucocytes through the endothelial wall. Turpentine, which in the skin causes a rapid increase in capillary filtration as indicated by the pronounced seepage of dye in the affected area, is equally incapable of exhibiting as rapid a migration of leucocytes as the active crystalline material. The implications of these observations are obvious. The studies are being continued and will be reported *in extenso* elsewhere.

Conclusions. For the sake of convenience the name *leukotaxine* is tentatively proposed for this active crystalline nitrogenous substance which is evidently released by injured tissue and is readily recovered in inflammatory exudates. "Leukotaxine" *per se* appears to be capable of rapidly initiating the usual sequences of the inflammatory reaction first by inducing a prompt increase in capillary permeability and secondly by causing an extremely rapid aggregation and migration of leucocytes through the endothelial wall.

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Cane Molasses versus Beet Molasses as a Source of Vitamin B_6 and Lactoflavin.

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Regardless of the fact that Nelson, Heller and Fulmer[1] published experimental evidence concerning the value of cane molasses as a source of the whole vitamin B complex, molasses is still generally looked upon as a qualitatively inert food constituent.

In 1935 the experimental data of Nelson, Heller and Fulmer re-

[1] Nelson, V. E., Heller, V. G., and Fulmer, E. I., *Indust. and Engin. Chem.*, 1925, **17**, 199.