

in Table I and find that for the male subjects the correlation§ between high B.M.R. and low S.D.A. is +.92. For the female subjects the correlation is +.80, and for the entire unselected group, +.86.

9703 P

Path of Escape of Vital Dyes from the Lymphatics into the Tissues.

PHILIP D. McMASTER AND ROBERT J. PARSONS.

From Rockefeller Institute for Medical Research, New York City.

The way in which substances of large molecule and fluids move through the tissue is unknown. To learn something of this we have studied the passage of vital dyes through living tissues, under varying physiological and pathological conditions.

Two dyes, pontamine sky blue and patent blue V, of widely different diffusibility, when introduced directly into the lymphatics of the ears of living mice^{1, 2, 3} eventually pass out in greater or less part from the channels at situations far from the site of the injection. Their escape and subsequent spread through the tissues have been watched at high magnification with the Ultropak microscope. The dyes do not pass out in a colored cloud from the lymphatics of normal ears, but first appear as very fine, irregular, bristling or wavy lines of color, extending outward from the wall of the vessel. With a micro-probe these colored lines can be bent and twisted and with the removal of pressure they spring back to their original positions. The dye appears to be fixed either between or upon preformed tissue elements. Within a few minutes of this first evidence of escape the extravascular coloration becomes more intense, the lines wider, and their margins less sharp, as though dye were diffusing from them. Manipulation still demonstrates that the dye cannot be dislodged. But soon, with the onset of the mild edema which these dyes induce in the tissues, the coloration becomes diffuse and pressure with the micro-probe modifies its intensity and forces it from

$$\S \rho = 1 - \frac{6(\Sigma d^2)}{N(N^2 - 1)}$$

¹ Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1932, **56**, 223.

² McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1932, **56**, 239.

³ McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1934, **60**, 479.

one region to another. It is no longer associated with the hair-like tissue elements, but has stained the edema fluid.

These are the findings in tissues previously normal. Where, however, edema had been produced prior to the introduction of the dye, by painting the ears with xylol, the character of its escape was wholly different. Now no colored, bristling lines radiated from the vessel, but the dye first appeared in a colored cloud. The color was freely movable with the micro-probe as soon as it appeared, being obviously present in the edema fluid.

When the dye, injected into normal ears, was of a sort to elicit prompt and marked edema, findings intermediate between those described were obtained. Aqueous, isotonic solutions of brom-cresol-purple (4%) and brom-thymol-blue (4%) have this effect when they are brought to pH 6.3 and 7.0 respectively. In all instances the passage of the dye from the lymph channels took the form of bristly, colored lines which could not be obliterated with pressure; but rapidly the coloration became cloud-like and freely movable, and after a minute or two the purple or blue cloud changed to buff, becoming almost invisible in the tissues. Reinjection of either indicator in the blue or purple form into the same lymphatic did not result as formerly in the appearance of discrete, bristly or wavy blue lines, but instead there appeared at once outside the channels a freely movable blue or purple cloud of dye present in free fluid.

Injections of pontamine sky blue and patent blue V into the ear lymphatics of dead mice and mice partly dehydrated by withdrawal of 40 to 50% of the blood just beforehand disclosed again the bristly and wavy lines. They developed more slowly than in normal animals but remained sharply delimited for a much longer time. In mice just injected intravenously with 1 to 1.5 cc. of Tyrode's solution, on the other hand, the bristly or wavy lines of coloration appeared seldom and transiently. There occurred instead a homogeneous coloration in the tissues.

Each of the experiments was repeated many times. The results were consistent.

What are the lines of color? Histological preparations stained for connective tissue show fine connective tissue fibers extending outward from the lymphatics in a bristling formation identical with that disclosed by the dyes in living mice. The findings strongly suggest that the dyes first extend along the interface present between the surfaces of contiguous connective tissue fibers or along that between connective tissue fibers and an interstitial gel. How-

ever this may be, it is certain that the dyes move through the tissues along tracks, as it were, determined by the form and situation of the connective tissue fibers.

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Role of Histamine in Circulatory Effects of Rattlesnake Venom (Crotalin).

CARL A. DRAGSTEDT, FRANKLIN B. MEAD AND SIMON W. EYER.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Essex and Markowitz¹ have made an extensive study of the physiologic action of crotalin and have pointed out a number of similarities to the action of histamine. Feldberg and Kellaway² have recently shown that crotalin has the property of liberating histamine into the perfusate when it is added to the perfusion fluid with which the lungs of cats or guinea pigs are being perfused. Their studies demonstrate a mechanism whereby the characteristic action of histamine can be superimposed upon the direct effects of the venom. The extent to which the added action of histamine influences the action of the venom in intact animals is not indicated by these experiments. In a series of studies on anaphylactic shock³ and peptone shock⁴ in the dog, we have been able to show that the degree of the vascular reaction is correlated with and dependent upon the amount of histamine liberated from the dog's tissues in these reactions. We were, therefore interested in determining whether histamine is liberated when venom is administered to the intact dog and whether the amount of histamine liberated is adequate to account for the degree of the vascular effect produced.

Crotalin* (from *Crotalus atrox*) was injected intravenously into 5 anesthetized dogs, in doses of 0.5 to 1.3 mg. per kilo, and samples of blood and thoracic duct lymph were collected during the ensuing reaction and tested for histamine. All of the reactions were severe, 2 being fatal within 20 minutes. Histamine was found

¹ Essex, H. E., and Markowitz, J., *Am. J. Physiol.*, 1930, **92**, 705.

² Feldberg, W., and Kellaway, C. H., *J. Physiol.*, 1937, **90**, 257.

³ Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1936, **57**, 419.

⁴ Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Therap.*, 1937, **59**, 429.

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