of blood and mammogenic lobule-alveolar growth hormone available to the growing gland tissue.

Summary. Pregneninolone alone and injected subcutaneously with estrone into castrate virgin female mice caused the development of the lobule-alveolar system of their mammary glands, having a property similar to progesterone in this respect. The injection of estrone with the pregneninolone enhanced this activity of the pregneninolone by five times. Pregneninolone has one-half the activity of progesterone in stimulating mammary lobule-alveolar growth when both are injected with estrone.

13173

Note Concerning the Mechanism of Increased Capillary Permeability in Inflammation.

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The writer has demonstrated the presence and isolated a crystalline nitrogenous substance in inflammatory exudates which offers a reasonable explanation for the mechanism of both increased capillary permeability and migration of polymorphonuclear leukocytes at the site of inflammation.[1, 2] This substance, termed leukotaxine, seems to belong to the group of relatively simple polypeptides. The possibility, however, of an additional prosthetic group is not yet precluded by the available data. Further studies on its chemical nature, including molecular weight, amino nitrogen content before and after hydrolysis, indole nucleus or perhaps tryptophane concentration, and a simpler method of purification will all form the subject of a separate future report.

Leukotaxine has been shown to have none of the physiological or chemical properties of histamine thus rendering difficult to accept the view that the latter plays a primary rôle in the mechanism of increased capillary permeability in inflammation.[3-5] The various

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

2 Menkin, V., *J. Exp. Med.*, 1938, **67**, 129, 145.

3 Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 103.

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

5 Menkin, V., *Dynamics of Inflammation*, 1940, Macmillan Co., New York.

reasons and tests for arriving at this conclusion have been enumerated at length in previous reports and will therefore not be reiterated here.[1-5]

Rocha e Silva and Dragstedt have again recently raised the issue by re-asserting the original view of Bier and Rocha e Silva that histamine seems to be the principal factor involved.[6, 7] The type of argumentation employed is unconvincing and the data brought forward in support of their view fails to substantiate the claim of these investigators.[7] Their findings of histamine in various tissue extracts, the content of which is more or less paralleled with the capacity for trypan blue to accumulate in cutaneous areas treated with these extracts merely indicates that histamine is liberated alongside with countless other substances by grinding various tissues in saline. The fact that leukotaxine is also presumably formed by injuring normal tissue in the procedure of extraction is wholly ignored. As the writer has frequently pointed out, Bier, Rocha e Silva and Dragstedt consistently have abstained from isolating leukotaxine from exudates and thus have failed to repeat his studies.[3-5] These authors have merely worked with either untreated exudates or unpurified extracts. By this means they have been able to demonstrate the presence of histamine in their material. The writer, on the other hand, has clearly shown that the effect of the whole exudate can be completely duplicated by the nitrogenous substance, leukotaxine, recovered in turn from exudates. Minami and Inugami have recently confirmed the studies of the writer on the isolation of leukotaxine from inflammatory exudates.[8] Leukotaxine has now definitely been admitted by Bier not to be histamine.[9] The subsequent claim advanced, namely that leukotaxine releases histamine which in turn is responsible for the increased capillary permeability has not been substantiated. In the first place the writer has demonstrated that leukotaxine directly increases cellular permeability in Arbacia ova whereas histamine fails to do so.[10] In the second place, Rocha e Silva has been unable to demonstrate that kallikrein, which induces the cutaneous accumulation of trypan blue can liberate histamine.[11] The original type of argumentation employed by these investigators

6 Bier, O., and Rocha e Silva, M., *Arqu. d. Inst. Biol.*, 1938, **9**, 109.

7 Rocha e Silva, M., and Dragstedt, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 303.

8 Minami, G., and Inugami, K., *Transact. Soc. Path. Japonicae*, 1940, **30**, 389.

9 Bier, O., and Rocha e Silva, M., *Proc. Third Internat. Congress for Microbiology*, 1939, published 1940, 767.

10 Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 588.

11 Rocha e Silva, M., *Nature*, 1940, **145**, 591.

on the basis of these new findings can, therefore, be now reversed. One might just as well assert that histamine acting as any other irritant in optimum concentration releases leukotaxine, which in turn is responsible for increased capillary permeability. Finally, it is interesting to note that Rocha e Silva and Dragstedt cite the studies of Duthie and Chain in an endeavor to explain in their experiments the discrepancy occasionally encountered between the positive trypan blue test and the histamine equivalent. Duthie and Chain have confirmed the earlier studies of the writer by showing that protein degradation products obtained by enzymatic hydrolysis yield biological effects similar to leukotaxine.[12, 13] Since leukotaxine seems precisely to be such a by-product of protein catabolism, the fact that a positive trypan blue test has been elicited from protein breakdown products obtained by enzymatic hydrolysis is not in the least surprising. The facts merely indicate that the argument advanced on the basis of Duthie and Chain's work in no way supports a non-specific interpretation for the positive trypan blue test whenever the desired histamine equivalent in the material studied is absent.

The present series of experiments were devised in order to determine whether leukotaxine or histamine is the essential constituent which imparts to the whole exudate the property of inducing in rabbits a positive cutaneous trypan blue test. Leukotaxine or the permeability factor diffuses through a cellophane membrane by dialyzing the whole exudate against distilled water. Whatever histamine is present in exudates presumably also diffuses outward by such procedure. Exudates were therefore dialyzed for about 18 to 20 hours against distilled water. The cellophane bag during dialysis was continuously rotated by having it attached to a stirring apparatus. The protein material which remained behind in the cellophane tube was found, when introduced into the skin of a rabbit, incapable of inducing the characteristic pattern of accumulation of trypan blue from the circulation. This is in complete agreement with earlier studies.[1] To this inactive residual fraction of the dialyzed exudate, leukotaxine is now added in concentration ranging from 1 to 3 mg. The mixture is then injected into the skin of a rabbit. The dye accumulates rapidly from the blood stream in such cutaneous areas. The pattern is in general no different from that elicited by the undialyzed exudate. Thus, by the addition of leukotaxine, the capacity of the dialyzed residual exu-

[12] Duthie, E. S., and Chain, E., *Brit. J. Exp. Path.*, 1939, **20**, 417.

[13] Menkin, V., *J. Exp. Med.*, 1938, **67**, 153.

TABLE I.

Comparison of the Effect on Capillary Permeability of Leukotaxine and Histamine Added to Exudates after Dialysis.*

		Nature of the fractions Tested						
Rabbit No.	Exudate	Residual exudate after dialysis	Leukotaxine in saline, or 0.2% saline, or distilled H_2O	Leukotaxine + residual exudate after dialysis	Histamine 1:20,000-1:25,000 in .85% saline, or 0.2% saline, or distilled H_2O	Histamine 1:20,000-1:25,000 + residual exudate after dialysis	Histamine 1:40,000-1:50,000 in .85% saline, or 0.2% saline, or distilled H_2O	Histamine 1:40,000-1:50,000 + residual exudate after dialysis
21-05	+++ throughout	0 in center, zone ++ at periphery	+++ throughout	++ to +++ throughout	—	—	0 in center, zone ++ at periphery	0 in center, zone ++ at periphery
21-22	++ throughout	0 in center, zone + at periphery	+++ to ++++ throughout	++ almost throughout	—	—	0 in center, zone ++ at periphery	0 in center, zone + at periphery
21-43	trace throughout	0 in center, zone of trace at periphery	++ throughout	++ throughout	trace throughout	0 in center, zone of trace at periphery	trace throughout	0 in center, zone of + at periphery
21-45	++ throughout	0 in center, zone of faint trace at periphery	+++ throughout	+++ throughout	0 throughout	0 in center, zone of + at periphery	trace throughout	0 in center, zone of + at periphery

*The exudates were obtained either from dogs one day after an intrapleural injection of 1.5 cc of turpentine, or from rabbits a day after injecting 0.25 cc of 5% croton oil in olive oil into the pleural cavity. The degree of accumulation of trypan blue in treated cutaneous areas of rabbits is gauged by the number of plus signs. About 15 cc of approximately 1% trypan blue in saline was injected into the ear vein immediately after introducing the various fractions into the skin of the abdomen.[1,2] Final readings as indicated in the table were recorded after an interval of approximately 30 minutes to 1 hour. In the case of rabbits 21-43 and 21-45 the exudate and the dialyzed exudate were diluted 1:1 with physiological saline in order to control for the dilution factor which occurred when either leukotaxine or histamine were added 1:1 to the dialyzed exudate fraction. This, however, as indicated failed to alter the results.

dative material to induce an increased capillary permeability has been fully restored. When the same procedure is repeated with histamine dihydrochloride in concentrations of 1 :20,000 to 1 :50,000 which, incidentally, according to Bier and Rocha e Silva,[6] are the concentrations of histamine recovered from exudates, the dye usually fails to accumulate homogeneously in the skin as it does in the case of the original exudate or of leukotaxine. Thus, the exact effect on capillary permeability of the exudate is not restored. Histamine has failed to reconstitute the capacity of the exudate when added to the inactive residual exudate fraction obtained after dialysis. This, however, is accomplished perfectly well by leukotaxine or even by the dried diffusate fraction from the dialyzed exudate.[1] The observations are conveniently assembled in Table I.

Conclusions. An inflammatory exudate readily induces an increased capillary permeability as evidenced by the rapid accumulation of trypan blue from the circulation into cutaneous areas treated with the exudative material. Dialysis of the exudate allows the outward diffusion of leukotaxine. The indiffusible residual material remaining after dialysis of the exudate is essentially incapable of increasing capillary permeability with the characteristic homogeneous pattern. The exact original effect of the exudate, however, can be readily reconstituted by merely adding leukotaxine to the indiffusible fraction of the dialyzed exudate. This cannot be obtained by adding histamine in the concentration in which the latter is recovered from exudates. These facts therefore add further support to previous observations that leukotaxine and not histamine is the primary factor concerned in the mechanism of increased capillary permeability in inflammation.

13174

Effect of Posture on Circulating Blood Volume in a Case of Orthostatic Hypotension and Tachycardia.

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It has been shown[1] that when an individual assumes the erect posture, a decrease in plasma volume follows. This has been explained as due to the augmented hydrostatic pressure in the vascular