important point, we wish to state that it was prepared by hydrolysis of the pure diacetyl dihydrovitamin K_1. The vitamin K_1 was purified by recrystallization at —70°C.

Analysis: Found C 82.39%; H 10.37%
Calculated C 82.62%; H 10.26%

$E^{1\%}_{1\ cm}$ of a hexane solution at λ 249 mμ = 448

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Effect of Leukotaxine on Cellular Permeability and on Cleavage Development.

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Previous studies have demonstrated the presence of a nitrogenous substance in inflammatory exudates capable *per se* of increasing capillary permeability and of inducing rapid diapedesis of polymorphonuclear leukocytes. This substance has been named leukotaxine.[1-3] Its isolation has offered a reasonable explanation for two of the basic mechanisms in the development of the inflammatory reaction. Furthermore, this substance has been shown to possess none of the manifest physiological properties of histamine, thus rendering it difficult to accept the view that the latter plays a primary rôle in increasing capillary permeability at the site of injury.[4-5] Kaiser has recently reached in regard to histamine an essentially similar conclusion, *i. e.*, at least as far as a long-standing inflammatory reaction is concerned.[6]

The present series of experiments have been undertaken in an endeavor to determine whether leukotaxine exerts any direct effect

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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., *Physiol. Rev.*, 1938, **18**, 366; *Dynamics of inflammation*, 1940, Macmillan Co., New York.

5 Menkin, V., and Kadish, M. A., *Am. J. Physiol.*, 1938, **124**, 524; Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 103.

6 Kaiser, P., *Schweiz. Z. f. allg. Path. u. Bakteriol.*, 1939, **2**, 1.

on cellular permeability. The studies of Lucké and McCutcheon have demonstrated the usefulness of marine ova as effective material in the study of the living cell in its relation to permeability to water.[7] The rate of passage of water placed in ova in a hypotonic medium is computed from the rate of change of volume. The permeability is derived from the equation: Permeability $= \frac{dv}{dt}/S\ (P - Pex.)$ For a detailed description of the terms involved in the equation, the reader is referred to the various publications of Lucké and McCutcheon.[7-8]

The present studies were made on the eggs of the sea urchin, *Arbacia punctulata*. For each experiment the ova were obtained from a single specimen of *Arbacia* and placed in sea water. The diameter of a number of ova was determined with an eyepiece micrometer. The cells are spherical and therefore the diameters were readily converted into measurements of surface area and volume. The ova were then transferred to hypotonic sea water (50%), and the rate of change of volume measured from minute to minute for a period of about 6 minutes. A smooth curve was drawn from which at a given time (2 to 3 minutes) the slope of the curve was obtained by drawing a tangent. Calculation of the permeability to water was then readily computed by applying the above equation. Ova were also exposed for several minutes to sea water containing leukotaxine in concentration of about 5 mg per cubic centimeter. These ova were then transferred to hypotonic sea water (50%) and their rate of swelling immediately measured. In each case the mean volume of several ova was plotted against time. Leukotaxine suspended in sea water induced a slightly acid medium which, after a prolonged interval, seemed to inhibit any change in the permeability of ova. The same type of result was obtained when normal ova were immersed in sea water previously acidified with HCl. For this reason, several experiments were performed by adjusting to a slightly alkaline level with 0.5 N NaOH the pH of the sea water containing leukotaxine. In experiments of short duration, this precaution was found superfluous. It seemed, in brief, as if only ova exposed for several hours to an acid medium failed to swell when subsequently transferred to a hypotonic medium. The data are summarized in Table I. It is clear that leukotaxine-treated ova showed, when immersed in a hypotonic medium, a considerable aug-

[7] Lucké, B., and McCutcheon, M., *Physiol. Rev.*, 1932, **12**, 68.

[8] Lucké, B., and McCutcheon, M., *Arch. Path.*, 1930, **10**, 662.

TABLE I.
Effect of Leukotaxine on Permeability of Ova to Water When Exposed to Hypotonic Sea Water.

No. of experiment	Permeability of control ova*	Permeability of leukotaxine-treated ova*	% increase
1	.12	.22	83.3
2	.09	.16	77.7
3	.15	.25	66.6
4	.11	.15	36.4
5	.13	.16	23.1
Avg	.12	.19	57.4

*The units of permeability are in terms of cubic micra of water entering per minute, per square micron of cell surface, per atmosphere of pressure.

mentation in their permeability to water. The average increase over that of control ova was about 57%.

The present observations add further support to the view that probably leukotaxine increases capillary permeability by a direct effect on the permeability of the endothelial cell. Bier and Rocha e Silva originally postulated that histamine and leukotaxine were identical substances.[9] Their view was severely criticized by the writer.[4, 5] In view of the mass of accumulated evidence Bier has recently retracted his original contention[10] (and personal communication, 1939). His present interpretation, however, that leukotaxine possibly liberates histamine which in turn is responsible for the increased capillary permeability[10] is not supported by any observations. On the contrary, a concentration of histamine (1:20,000 to 1:50,000) equal to that recovered from exudates by Bier and Rocha e Silva, fails to induce the exact pattern of reaction on capillary permeability as elicited by either the untreated exudate or by leukotaxine recovered from such exudative material. It is also of interest to note that histamine fails to augment the permeability of sea urchin ova to water (Lucké, personal communication). In view of the opposite effect obtained with leukotaxine, it would be difficult to postulate that the latter acts on these cells by first releasing histamine. Finally, Ivy has recently succeeded in showing that whereas histamine induces increased gastric secretion of free acid in a Pavlov-pouch dog, leukotaxine is wholly ineffective in inducing any such effect (personal communication). This evidence supports further the view that leukotaxine fails to induce a release of

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

10 Bier, O., *Proc. Third International Congress for Microbiol.*, New York, 1940, p. 768.

histamine, since the latter in concentration as low as 0.1 mg manifests demonstrable secretion of free acid.

Lucké and McCutcheon showed that *Arbacia* ova placed in a hypotonic solution display an increased permeability to water. When these cells were returned to ordinary sea water and inseminated, cleavage frequently failed to occur or was atypical.[8] These investigators expressed the belief that increased permeability is an expression of injury due to the rapid entrance of water. Leukotaxine not only enhances the permeability of sea urchin eggs to water but it also definitely disturbs cleavage development. This would suggest that this substance probably induces a certain amount of cellular injury.

The eggs of *Arbacia punctulata* were exposed to leukotaxine suspended in sea water in concentration of about 0.7 mg per cubic centimeter. The length of exposure to leukotaxine, prior to insemination in ordinary sea water, varied from one minute to 3 hours. In some instances, the pH of the sea water containing the leukotaxine-treated ova was adjusted approximately to that of ordinary

TABLE II.
Effect of Leukotaxine on Cleavage of *Arbacia* Ova.

	Experimental Time exposure to leukotaxine		Control Time exposure to sea water prior to fertilization	
Exp. No.	1-30 min % ova in cleavage	1-3 hr % ova in cleavage	1-30 min % ova in cleavage	1-3 hr % ova in cleavage
1	42 0	0 0	40	14
2	58 92	94 88 58	92	98
3	32	26 37 6	100	74 76
4	60	98 66 92	100	100 98
5	44 58	32	100	94
6	44 34 18 22		100	
Avg	42	49.75	88.67	79.14

sea water by the addition of 0.5 N NaOH. This precaution induced essentially no difference in the ultimate results. The effect on cleavage formation obtained in leukotaxine-treated and in control ova is summarized in Table II. In both groups similar containers with approximately the same volume of suspension of eggs were utilized. It is clear that with the exception of one experiment most of the ova in the control group displayed, within several hours, variable numbers of blastomeres. The percentage of ova in cleavage exposed 1 to 30 minutes to sea water prior to insemination averaged 88.67. When exposed for longer intervals (1 to 3 hours) the percentage of ova in various stages of cleavage was slightly reduced, namely 79.14. These two figures stand in sharp contrast with the effect obtained in the leukotaxine-treated group. The number of ova with blastomeres averaged, in the experimental ova, 42 and 49.75% respectively. In other words, about half of the eggs exposed to leukotaxine failed to divide when subsequently inseminated.

Furthermore, the cleavage pattern in the majority of leukotaxine-treated ova appeared abnormal, being characterized by fewer blastomeres than in the control eggs and by unequal forms of division. This is exemplified in Fig. 1 and 2. The former shows normal development of an ovum about one hour and a half following insemination. Fig. 2 illustrates the type of development encountered in an ovum exposed to leukotaxine prior to insemination. The ovum is from the same specimen of *Arbacia* as the one in Fig. 1 and the intervals following insemination are approximately identical in both

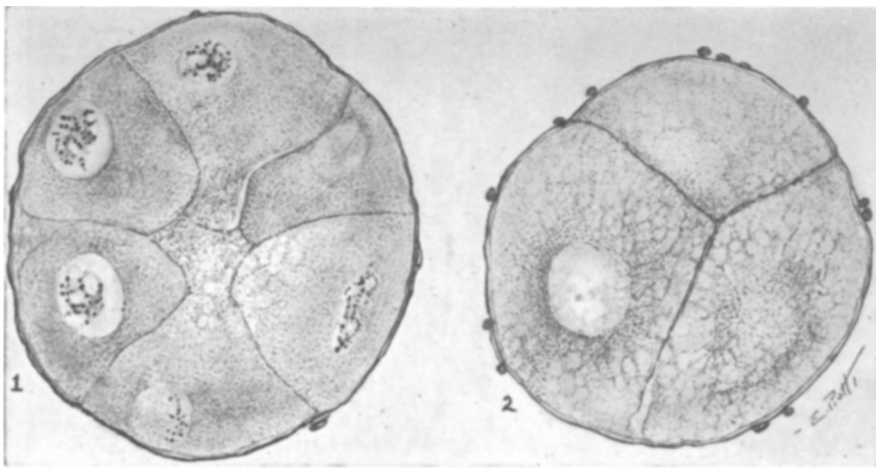

FIG. 1. FIG. 2.

cases. The illustrations represent histological sections after fixation in Bouin and acetic acid followed by 70% alcohol. This abnormal form of development in the leukotaxine-treated ova was frequently traced back to the fertilization reaction. The fertilization membrane appeared as a zone distinctly narrower than under normal circumstances. It was frequently found surrounded by sperms adhering to it (Fig. 2). Evidence of either cytolysis, absence or localization of the pigment in one area of the ovum were not of infrequent occurrence. These facts suggest that leukotaxine induces some degree of injury to the ova. It is also important to note that besides unequal cleavage, the leukotaxine-treated eggs displayed, after a given interval of time, considerably fewer blastomeres than in the untreated ova (Fig. 1 and 2). This strongly suggests that leukotaxine tends to retard the rate of cleavage.

Finally, sperms exposed for only a few minutes to leukotaxine induced fertilization of the ova of *Arbacia* and the subsequent cleavage pattern appeared to be unaltered. When, however, sperms were placed in contact with leukotaxine for about one hour, there was a sharp reduction in their fertilizing capacity. In some cases 98% of the ova failed to segment. This indicates that leukotaxine is evidently likewise injurious to sperms provided the latter are exposed to this substance for a sufficiently long interval.

Conclusions. Leukotaxine, the substance obtained from inflammatory exudates which is capable *per se* of increasing capillary permeability and of inducing leukocytic migration, markedly augments the permeability of sea urchin ova to water. Furthermore, a considerable number of ova exposed to this substance manifest abnormal cleavage development following their insemination. This appears in the form both of unequal cleavage and of an appreciable retardation in the rate of cell division. Sperms are also inactivated after prolonged exposure to leukotaxine. These various manifestations indicate that leukotaxine induces a certain degree of cellular injury when in contact with the ova or sperms of *Arbacia punctulata.* These effects on invertebrate eggs coupled with its rôle in inflammation suggest that leukotaxine may prove of biological significance in the study of cell division and permeability.

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