

Effect of Heparin and of Vitamin K on the Life Span of *Daphnia magna*.^{*} (18035)

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The experiments here presented are a contribution to the pool of evidence tending to show that viscosity is related to fundamental life properties—such as parthenogenesis(1), mitosis(2), aging of egg cells(3), stimulation(4), etc. While no direct proof is presented that viscosity changes actually occurred in the presence of vitamin K and of heparin—which are the substances found in the present series of experiments to influence life span of *Daphnia*—the known relation of these substances to protoplasmic coagulation suggests the possibility that viscosity changes may be involved.

It has been previously reported that variations in the concentration of calcium and colligative ions are effective in altering the span of fertilizable life of egg cells like those of *Arbacia*(5) and *Mactra*(6). Inasmuch as the action of coagulants and anti-coagulants would appear to involve the calcium balance of protoplasm, it seemed likely that this type of substance should also influence the span of life.

For the experimental investigation of the above possibility the fresh water cladoceran, *Daphnia magna*, was utilized. Batches of 10 or, more usually, 20 specimens were placed in 50 cc of spring water to which various amounts of heparin (Hynson, Westcott and Dunning) or vitamin K (2-methyl-1,4-naphthoquinone, Eastman Kodak Company) had

been added. Controls were retained in unaltered spring water. The solutions of vitamin K were made up with 1/50, 1/25, 1/10, 1/5, 1, 2, and 4 mg per 100 ml of water; percentages amounting to 1/50,000, 1/25,000, 1/10,000, 1/5000, 1/1000, 1/500 and 1/250 of 1%, respectively. Heparin was found effective in concentrations of 10, 20, 40, and 100 mg per 100 ml; or, 1/100, 1/50, 1/25, and 1/10 of 1%, respectively. Glass electrode pH measurements showed no significant differences in any of these solutions as compared with spring water. From March 13 to June 13, 1950 seven series of experiments were performed under a temperature range of 21 to 30°C. In any single experiment the temperature did not usually vary more than 3 or 4 degrees. Including the initial exploratory experiments, about 2000 specimens were employed, but the data given below is based on about half this number. The results were clear and consistent in all cases, and can be presented simply in tabular form. Since *Daphnia* is normally an actively swimming form, mobility is taken as a criterion of condition. It was found in the experiments that immobility is followed shortly by death and disintegration.

In Table I are given the results of a typical experiment. Twenty specimens were placed in each solution; a total of 180 for the experiment. In some cases specimens were transferred to water or heparin solutions after an initial exposure to vitamin K, and in others both substances were used simultaneously, as shown in the tables. Table II is a summation of all of the experiments.

It will be noted from the data that heparin, an anticoagulant, has a prolonging effect on the life span of *Daphnia magna* under the experimental conditions employed; while vitamin K sharply reduces the life span in concentrations down to 1/25 mg/100 cc. At

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1. Heilbrunn, L. V., *Biol. Bull.*, 1915, v29, 149.
2. Heilbrunn, L. V., *J. Exp. Zool.*, 1921, v34, 417.
3. Goldforb, A. J., *Biol. Bull.*, 1935, v68, 180, 191.
4. Heilbrunn, L. V., *Outline of General Physiology*, 1937, pp. 436-9.
5. Schechter, V., *Biol. Bull.*, 1937, v72, 366.
6. Schechter, V., *J. Exp. Zool.*, 1941, v86, 461.

TABLE I.
Survival of *Daphnia* in Heparin and in Vitamin K Solutions.
Percentage Immobilized.

Time, hr	K/5	K/5 for 2½ hr then into water	K/5 for 2½ hr then into 20H	K/5 and 20H	K/5 and 40H	K/50	Control	20H	40H
2½	0	0	0	0	0	0	0	0	0
8½	95	0	0	100	100	0	0	0	0
24	—	25	25	—	—	0	0	0	0
46	—	55	40	—	—	0	0	0	0
70	—	100	65	—	—	50	45	20	0

Note: Symbol such as K/5 indicates a concentration of 1/5 mg of vitamin K per 100 ml. 20H indicates 20 mg of heparin per 100 ml of solution, etc.

TABLE II.
Survival of *Daphnia*.
Percentage Immobilized.

Solution	No. of specimens	Time in hr						
		½	2½	8½	24-28	36-44	70-100	
K/50	40	0	0	0	0	0	48	
K, 25	80	0	0	0	26	55	100	
K, 10	110	0	0	65	82	100	—	
K, 5	80	0	0	95	95	100	—	
1K	60	0	100	—	—	—	—	
2K	20	50	100	—	—	—	—	
4K	20	100	—	—	—	—	—	
Controls	150	0	0	0	7	60	73	
10H	40	0	0	0	0	35	30*	
20H	80	0	0	0	0	0	40	
40H	20	0	0	0	0	0	0	
100H	80	0	0	0	0	10	60	
100H and K/10	40	0	0	27	67	100	—	

* Drop in percentage due to discontinuation of some experiments before this time in hours.
Note: Meaning of symbols as in Table I.

1/50 mg/100 cc there appears to be some reversal in the effect, that is, vitamin K in 1/50,000 of 1% concentration was somewhat stimulating as compared with the controls. It is of interest to point out that no food was provided, and that food availability is said to have, within limits, an inverse relationship to longevity of *Daphnia* (7). Also, since heparin promotes motility, as observed during the course of the experiments, it may well be—particularly at higher temperatures where oxygen solubility in water decreases—that oxygen availability becomes limiting upon the effect of heparin. This may account for the falling off of the effect at 100H as compared with 40H, which is noted in Table II. In this connection it should be pointed out that deepness of color in *Daphnia* seems to be related to the oxygen content of the water, but that haemoglobin is not regarded as an oxygen carrier in this animal (8).

By comparing the first 3 columns of Table I, especially at 8½ hours, it will be seen that the deleterious effect of vitamin K may be reduced or delayed by transfer to spring water or heparin solutions after an initial exposure to vitamin K. The simultaneous use of heparin and vitamin K does not seem to influence the effect of the latter except perhaps to a slight extent with very high comparative concentrations of heparin. See the effect of K/10 as compared with 100H & K/10 in Table II.

Summary. The life span of the common fresh water cladoceran, *Daphnia magna*, is shortened in the presence of vitamin K in the medium, and lengthened with heparin. The possibility of relationship to protoplasmic coagulation phenomena is pointed out.

8. Fox, D. M., *Proc. Roy. Soc. London, Series B*, 1948, v135, 195; Fox, D. M., Hardecastle, S. M., and Dresel, E. I. B., *idem.*, 1949, v136, 388.