10464

Inactivation of Vitamin K by Light.*

D. W. MacCorquodale, S. B. Binkley, R. W. McKee, Sidney A. Thayer and Edward A. Doisy.

From the Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis, Mo.

In an earlier note,[1] we reported the isolation of crystalline material from alfalfa which after 4 recrystallizations still had the activity ascribed to Vitamin K. Since our observations were still incomplete it was impossible to state with certainty that we had isolated Vitamin K. Some time after this work, it was found that although we could obtain crystals with approximately the same melting point, solutions of these crystals did not restore the coagulation time to nomal values. Frankly, we admit that we have been unable to duplicate the work reported last summer and, moreover, thus far do not have any explanation which appears to be satisfactory.

In seeking an explanation of our unexpected results, we recalled that Almquist[2, 3] had reported briefly on the loss of activity of preparations exposed to sunlight and on the stability of the vitamin exposed to artificial illumination. The effect of sunlight has been confirmed but in addition we have found that highly purified preparations dissolved in various solvents rapidly lose activity on exposure to the illumination from ordinary daylight bulbs. On the other hand, our crude extracts of alfalfa have been found to be quite stable, no special precautions regarding light being necessary. However, as the potency is increased to 500 or 1000 units (Thayer, *et al.*[4]) per milligram, the lability is such that decomposition has frequently occurred in spite of our precautions.

Experimental. Two preparations were used in our experiments on the effect of light: (a) a non-crystalline product obtained from alfalfa having a potency of approximately 1000 units per milligram and (b) a crystalline product (M.P. 50.5-52.0°) obtained from putrefied fish meal. The latter had been recrystallized 10 times from

* We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

1 Thayer, S. A., MacCorquodale, D. W., Binkley, S. B., and Doisy, E. A., *Science*, 1938, **88**, 243.

2 Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

3 Almquist, H. J., *J. Biol. Chem.*, 1937, **117**, 517.

4 Thayer, Sidney A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, Edward A., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 478.

various solvents without detectable alteration of its potency, which was approximately 600 units per milligram.

With both preparations dissolved in either ethyl alcohol or benzene, exposure to direct sunlight for 5 hours caused a destruction of more than 75% of the vitamin.

Exposure for 20 hours to light from a 300 watt daylight bulb at a distance of 4 feet produced loss of activity of solutions of the alfalfa extract dissolved in acetone or benzene. Solutions of the material in benzene, alcohol, and acetone were exposed to white light for 96 hours. In all cases destruction was extensive (Table I). In another experiment the fish meal preparation was dissolved in benzene and the solution was divided into 2 portions, one being kept in the dark and the other exposed during 3 working days (20 hours) to the artificial illumination used in the laboratory. The preparation kept in the dark was 10 times as active as the one exposed to light.

TABLE I.
Effect of Exposure to White Light, Prep. No. T3a (Prepared from Alfalfa).

	Solution kept in the dark				Solution exposed to the light				
			Response No. of chicks				Response No. of chicks		
Solvent	Chicks (15 days old) No.	Micrograms of material administered	Pos. +	Neg. —	Chicks (15 days old) No.	Micrograms of material administered	Pos. +	Neg. —	% loss of Vitamin K Potency
Benzene	6	1.0	3	3	10	6.0	1	9	>90
Ethyl Alcohol	5	1.0	2	3	5	2.0	1	4	>65
Acetone	5	1.0	3	2	10	4.5	1	8	>85

Control Chicks—(10)—clotting time >30 minutes.

Since in these experiments we were attempting to ascertain the cause of inactivation in our routine work of purification, oxygen was not excluded from the flasks containing the vitamin preparations. It is possible that oxygen may play a part in the destruction, but the same solutions kept in the dark did not show loss of activity.