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Vitamin K Storage and Prothrombin Levels in Chicks Obtained from Injected Eggs.*

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It has been shown[1] that hatching eggs obtained from hens maintained on a diet rich in vitamin K yield chicks which appear to contain stores of the vitamin. Such chicks, when placed on a vitamin K-free diet show evidence of some protection against the development of "hemorrhagic chick disease." Also, seasonal variations in the severity of this disorder have been noted.[2] Such variation might well be due to seasonal variation in the amount of vitamin K in the diet of the laying hens.

In an attempt to study storage of vitamin K in greater detail, we have conducted a number of experiments in which vitamin K was injected into the eggs prior to their incubation. Certain of the synthetic compounds, now readily available, can be injected into the eggs without seriously interfering with the hatching yield or with the vigor of the newly-hatched chicks. In the present paper we wish to present data obtained by injecting potassium 2-methyl-1,4-naphthohydroquinone disulfate into the eggs.

The vitamin was dissolved in phosphate buffer (pH 7.05) and heated to the boiling point briefly to reduce the likelihood of bacterial contamination. In each instance, the dose injected was contained in 0.2 cc of the buffer solution. The eggs, immediately prior to their incubation, were cleansed with alcohol and the vitamin was injected into the albumin portion. The opening in the shell, made with a diamond point pencil, was sealed with celloidin. The two vitamin K levels used in the present experiment were 200 μg and 1000 μg. The hatch yielded 15 chicks from eggs injected with 1000 μg, 10 chicks from the eggs injected with 200 μg, and 18 uninjected controls. At the time of hatching, all of the chicks were

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1 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

2 Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194.

placed on a vitamin K-free diet, previously described.[3] The blood samples were taken from the jugular vein with a syringe and hypodermic needle. Individual chicks were bled at 3-6-day intervals, so spaced as to give maximal coverage of the entire experimental period and at the same time avoid severe anemia. The prothrombin determinations were made by the two-stage method of Warner, Brinkhous and Smith.[4-6] A total of 152 determinations was made during this experiment.

The results obtained are shown graphically in Fig. 1. For simplicity in charting, the prothrombin values obtained were grouped into 5-day periods. The average prothrombin level and the average age of the chicks at the time tested were computed for each 5-day interval.

At the time of hatching, the prothrombin level of the control chicks is only slightly lower than that of the chicks obtained from the injected eggs. From statistical analysis and from unpublished data, we doubt that this difference is significant.

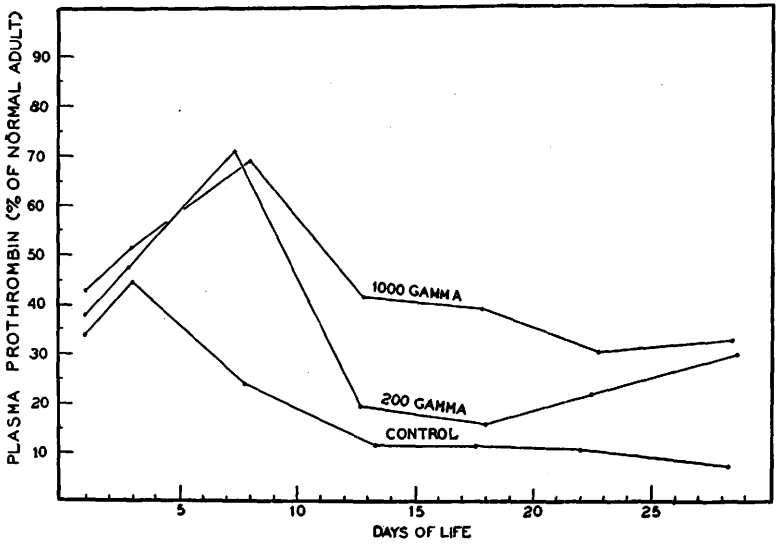

FIG. 1.
Prothrombin levels in chicks from injected eggs and from uninjected control eggs.

[3] Tidrick, R. T., Joyce, F. T., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 853.

[4] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

[5] Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

[6] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296.

There is a small initial rise in the prothrombin level of the control group. This suggests the existence of small amounts of stored vitamin. This reserve is soon exhausted, however, and the prothrombin level falls markedly within the next 10 days. The chicks from the injected eggs, in contrast, contain sufficient stores that for a time the prothrombin level continues to rise toward adult levels which, by the two-stage prothrombin method, are somewhat greater than twice the hatching level. After about 10 days, this reserve is no longer adequate to maintain the rise but does prevent the prothrombin from rapidly falling to the bleeding level.

Statistical analysis of the results showed remarkably little individual variation in the prothrombin values of the control group. In the injected groups, there was considerable variation from chick to chick. Also, there was considerable overlapping between the two injected groups during the first 10 days of life. The prothrombin values of the injected chicks were, however, consistently higher than those of the controls, and after the first 10 days of life, there was a conspicuous difference between the two injection levels. For the 10-15-day period, the observed difference in the average prothrombin values between the 200 μg and the 1000 μg groups is approximately 3⅓ times the calculated standard error of the difference. During the next 5-day period, the observed difference between the average prothrombin values of these two groups is approximately 6 times the standard error of the difference. During these two age periods, the observed difference between the control group and the 200 μg group is slightly greater than twice the standard error of the difference. Also, it is significant that during the entire 30-day period, none of the chicks from the injected eggs showed any evidence of spontaneous hemorrhage. In sharp contrast, all of the control group showed bleeding by the end of the third week of life and 7 of the 18 had died of hemorrhage by this time. All died of hemorrhage by the thirtieth day.

Summary. Vitamin K (potassium 2-methyl-1,4-naphthohydroquinone disulfate), injected into eggs prior to their incubation, produces vitamin K stores in newly-hatched chicks. The injected vitamin protects against the rapid and extreme fall of the prothrombin level which occurs when chicks are placed on a vitamin K-free diet.