Vitamin K Requirements of the Growing Pup[1]

ARMAND J. QUICK, GEORGE E. COLLENTINE, JR., AND CLARA V. HUSSEY
Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wisconsin

The recent observations that vitamin K functions in a coenzyme system for oxidative phosphorylation has stimulated a renewed interest in this vitamin (Isler and Wiss, '59; Quick, '46, '61a). The desirability of knowing its daily requirement under various conditions is of both theoretical and practical value. The only known simple means for studying the effect of vitamin K deficiency *in vivo* is the one-stage prothrombin time (Quick, '37). By producing a severe depletion of vitamin K, it is possible to determine how much is needed daily to maintain a constant normal prothrombin time.

A high degree of avitaminosis K can be produced in the chick by feeding a vitamin K-free diet, whereas in the dog the condition can be produced surgically by a cholecystnephrostomy, which consists in anastomosing the pelvis of the right kidney with the gallbladder and ligating the common duct. By this complete drainage of the bile through the urinary tract, the absorption of a fat-soluble substance such as vitamin K is severely impaired.

It was found that the newly-hatched chick requires approximately 3.75 μg of vitamin K_1 daily during the first 11 days or about 50 to 75 μg/kg of body weight. (Quick and Stefanini, '48). In marked contrast, an adult dog requires only 0.5 μg/kg of body weight daily (Quick et al., '54). In a preliminary study, it was observed that the growing pup has a much higher requirement for vitamin K than the adult animal (Quick, '52). Since this clearly suggested a relationship between growth and the requirement for vitamin K, a detailed study to determine the quantitative needs of the growing pup for vitamin K was made.

EXPERIMENTAL

Three male pups were used. Cholecystnephrostomy was performed by the technique of Kapsinow et al. ('24). Two of the pups were three months old and the other 4 months. Recovery from the operation was prompt and uneventful. The animals received ordinary postoperative care and a transfusion of glucose. The pups were fed a diet of various types of canned dog food and sometimes were also given dry pellets. During periods when the effect of vitamin K was studied, a uniformly constant diet was maintained. Every fourth week 50,000 USP units of vitamin A and 250,000 USP units of vitamin D were given parenterally.

During the intervals between studies, the prothrombin time was determined approximately twice a week and sufficient vitamin K_1 (10 to 20 μg/kg of body weight) was injected intravenously every few days to maintain a prothrombin activity level high enough to safeguard the animal against hemorrhage. The results reported constitute only about 10% of the data collected.

Prothrombin time. The method of Quick ('57) using acetone-dehydrated rabbit brain as the source of thromboplastin was used.

Vitamin K_1 preparation. A stock solution of vitamin K_1 was prepared by dissolving 0.2 gm in 50 ml of 95% ethyl alcohol. Immediately before injecting, 0.5 ml of the stock solution was mixed with 100 ml of saline (1 ml = 20 μg). The stock solution was kept in the dark, and the diluted solution was not exposed

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to strong light because vitamin K is quickly destroyed by light.

2 - Methyl - 1,4 - naphthoquinone (menadione). A stock solution was prepared exactly as for vitamin K_1.

Bile. Fresh ox bile obtained from the slaughterhouse was fed.

RESULTS

The objective of the study was to determine the maintenance dose of vitamin K_1 in growing pups. Since the results in three pups agreed so completely, it was not deemed necessary to study a larger number of animals.

The first pup was three months old at the time of operation and weighed 2.7 kg. It lived 8 months and gained 2.3 kg. This animal appeared well until shortly before death, when it became paralyzed. A veterinarian diagnosed the condition as encephalitis but an autopsy showed signs only of malnutrition.

TABLE 1

Maintenance dose of vitamin K_1 required to keep the prothrombin time normal in a pup after cholecystnephrostomy (pup 1)

Date	Vitamin K_1[1]	Prothrombin time	
	μg	sec	%[2]
May 30[3]		6	100
June 7		7	50
June 16		8.5	35
June 24		11	15
July 2		16	4.5
July 7		20	3
July 8	5	20	3
July 9		7	50
July 10		11	15
July 11	5	13	7
July 12		7	50
July 14	2.5	14.5	5.5
July 15	2.5	12	10
July 16	2.5	9	25
July 17	2.5	7.5	45
July 18	5	10	20
July 21		9	25
July 22	5	12	20
July 23	5	9	25
July 24	5	7.5	45
July 25		7	50
July 26		7	50
July 28	7.5	9.5	22
July 28		6	100
July 29		7.5	45
July 30	5	9	25
July 30		6	100
July 31		9	25

[1] Vitamin K_1 injected intravenously per kilogram of body weight.
[2] The percentage of prothrombin activity was determined from a standard table (Quick, '57).
[3] Cholecystnephrostomy was performed on this date.

The second pup weighed 9 kg and was 4 months old at the time of the operation. The growth curve for this animal was normal; maximal weight (23 kg) was attained at 10 months of age which was retained until the pup died 4 months later from a massive pleural and pericardial hemorrhage. Extensive liver damage was noted.

The third pup weighed 4.5 kg and was three months old when operated upon. It had a normal growth curve which reached its maximum (21 kg) at 9.5 months and then remained constant until death from pneumonia occurred at the age of 12 months. Gross liver damage was noted.

Most of the data are summarized in table 1 and in figures 1, 2 and 3. A daily intake of 5 to 10 μg or more/kg of body weight was required to maintain a normal prothrombin time in growing pups. These animals may be regarded as fairly normal except for their inability to absorb fat and fat-soluble compounds such as vitamins A, D, E and K. By giving vitamins A and D parenterally, their requirements were met. No signs of vitamin E deficiency were observed.

The normal behavior of these animals and their normal growth curve clearly suggest that their metabolism was not essentially different from that of nonoperated pups and that their vitamin K requirements as determined in this experiment are a fairly accurate measure of the normal metabolic demands for this compound.

The results in respect to the third pup are particularly informative. Shortly after operation, a normal prothrombin time could not be maintained with 5 μg/kg of body weight (fig. 2), but three months later when the growth curve was beginning to flatten, 2.5 μg were adequate to attain a 6-second prothrombin time (fig. 3). The ineffectiveness of adding bile to the diet for the absorption of vitamin K_1 is clearly illustrated.

DISCUSSION

The results of this study show that the growing pup requires 10 or more times vitamin K_1 than does the adult dog. If it were feasible to perform the cholecystnephrostomy at an earlier age, it is probable

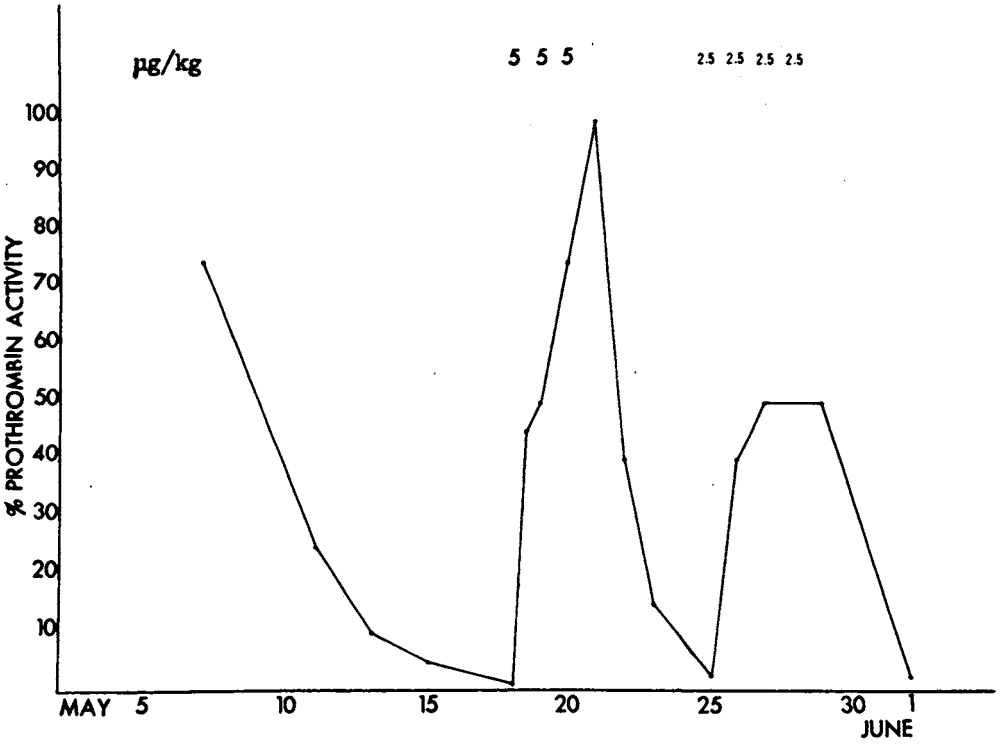

Fig. 1 Pup 2. After cholecystnephrostomy (May 7), the prothrombin time was brought to normal by daily injections of 5 µg of vitamin K_1/kg of body weight, whereas 2.5 µg brought it only to 50%.

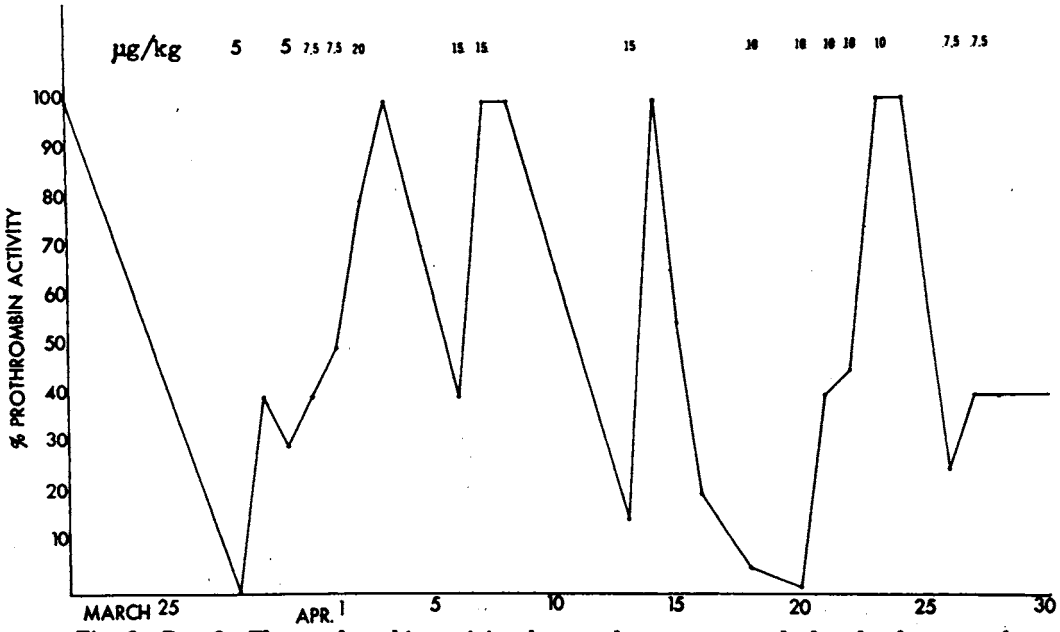

Fig. 2 Pup 3. The prothrombin activity decreased to an extremely low level one week after cholecystnephrostomy (March 21). It was brought to normal by daily injections of 15 µg of vitamin K_1/kg of body weight. Two weeks later 10 µg sufficed as a maintenance dose.

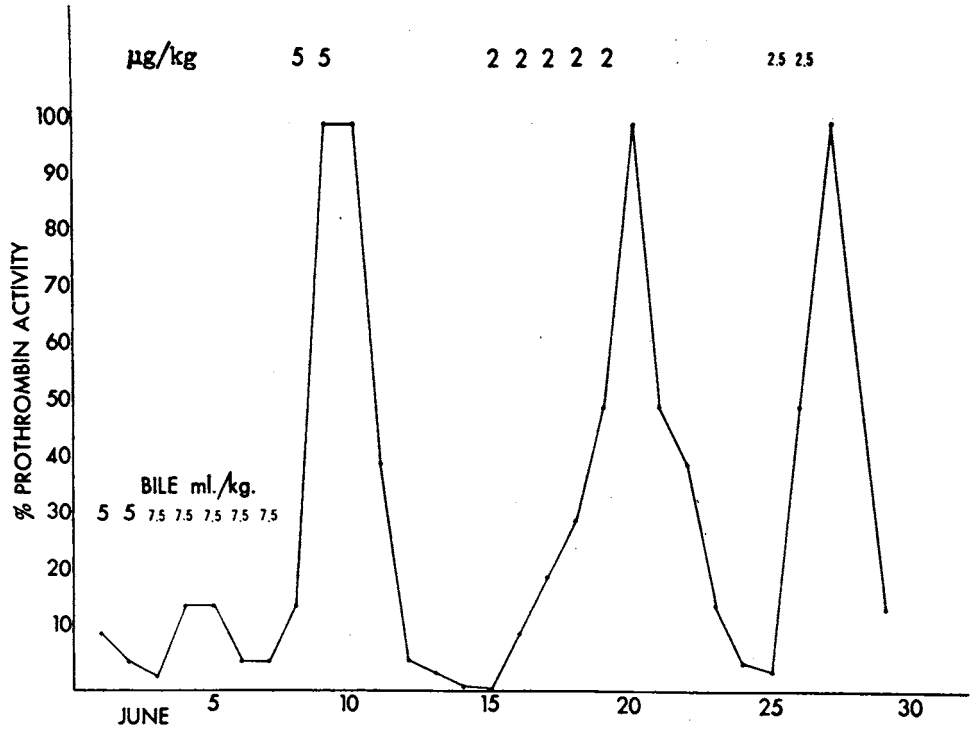

Fig. 3 Pup 3. Three months after the cholecystnephrostomy and after constant body weight had been attained, the prothrombin time was brought to normal by daily injections of 2 µg of vitamin K_1/kg of body weight. The oral administration of large doses of bile had no significant beneficial effect on vitamin K absorption.

that the requirement for vitamin K would be found even higher. In the chick it has been found as high as 75 µg/kg of body weight.

The experimental data strongly suggest that the requirement for vitamin K is related to growth. Recent studies initiated by Martius ('54) showed that vitamin K is utilized by the organism in an oxidative phosphorylative system. However, the only effect detected in *in vivo* vitamin K deficiency is a prolongation of the one-stage prothrombin time. Nevertheless, it seems unlikely that a vitamin which appears to be closely related to growth should function only in prothrombin activity.

Current studies on vitamin K depression by antivitamin K drugs such as Dicumarol indicate that vitamin K functions in the production of factor VII (also known as stable factor or proconvertin) and that the prothrombin decreases only after factor VII becomes depressed. (Quick, '61b; Quick and Hussey, '61). This suggests the probability that the formation of factor VII may constitute an intermediary step in the synthesis of prothrombin. It is probable that the organism may utilize factor VII for other purposes but that these are not detectable because the animal succumbs to hemorrhage before these can become manifest. This would be analogous to a marked depression of the calcium concentration of the blood. Although calcium is indispensable for the clotting of the blood, the animal dies from tetany long before the coagulation reaction is affected.

It is certain that the vitamin K need of young growing animals is much higher than that of the adult. This appears especially true in man. Because the absorption of this vitamin from the intestines is very poor, even when bile is supplied, the danger of vitamin K deficiency, especially when the demands are high, is understandable. In two of the pups the pro-

thrombin time became greatly prolonged within a week after the operation. In adult dogs a month or more was required before the test showed a depletion of vitamin K. This is easily explainable. The adult dog has a fairly high amount of stored vitamin K, whereas the store in the growing animal is low.

The rat is more susceptible to vitamin K deficiency than the dog. Greaves and Schmidt ('37) promptly produced hypoprothrombinemic activity in the rat by means of a biliary fistula and Flynn and Warner ('40) by ligating the common duct. The recent work on irradiated beef is particularly significant (Metta et al., '59). Apparently, the vitamin K contained in the beef is destroyed and, when fed to rats, produces a prolonged prothrombin time and a severe hemorrhagic state. In dogs and cats, the feeding of irradiated beef causes no detectable effect (Reber and Malhotra, '61).

SUMMARY

The vitamin K requirement of growing pups was studied by inducing a severe deficiency by means of a cholecystnephrostomy and testing the amount of vitamin K that had to be injected intravenously daily to obtain and maintain a normal prothrombin time.

Two pups were operated at the age of three months and one at 4 months. The vitamin K_1 requirement shortly after the operation when the pups were still in the active growing stage was 10 μg or greater/kg of body weight. As the animals matured and attained a constant body weight, the requirement decreased to less than 5 μg. The greater need for vitamin K of the growing pup than that of the adult dog suggests a relationship to growth.

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