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Prothrombin Levels and Synthetic Vitamin K in Obstructive Jaundice of Rats.*

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Through dietary studies, the chick has helped to solve a number of problems concerning the chemical nature of vitamin K and its relation to the manufacture of prothrombin. However, there still exist many important questions concerning the minimal vitamin requirements, the absorption of the vitamin, the mechanisms by which it is utilized, and the rôle of the liver in the production of prothrombin. Clinical problems in man involve not merely the normal dietary requirements, but also the question of absorption from the intestine, and the problem of having adequate amounts of bile in the intestine to aid in this absorption.

To analyze a number of the problems it is desirable that an experimental deficiency be created in animals by excluding bile from the intestine, and not by dietary procedures alone. It is also desirable that certain of these studies be made on mammals, rather than on birds in order to approach more closely the physiological conditions obtaining in man.

Greaves[1] has shown that a bleeding tendency can be produced in the rat by a combination of dietary regulation and bile duct ligation and that this disorder can be cured by administration of crude concentrates of vitamin K. We have found that the prothrombin level falls both rapidly and regularly to low levels, and the response to vitamin K is sufficiently uniform that such an animal represents a valuable test animal for study of the problems outlined above. We wish to present data concerning the prothrombin levels before and after treatment with vitamin K.

Experimental Procedure. In some cases, the rats were selected at the time of weaning; in other cases young adult rats were used. In order to deplete the reserves of vitamin K in the body, the animals

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The phthiocol and the 2-methyl-1,4-naphthoquinone used in these experiments were prepared through the courtesy of Dr. George H. Coleman and Dr. Donald W. Kaiser, Department of Chemistry, State University of Iowa.

1 Greaves, Joseph D., *Am. J. Physiol.*, 1939, **125**, 429.

were placed on a diet known to be low in this vitamin. The diet was prepared as follows: cane sugar 472 g, casein 480 g, brewer's yeast 120 g, salt mixture[2] 68 g, lard 330 g, cod liver oil 30 g.

After the rats had been on this diet for 7 to 8 weeks, the common bile duct was ligated, under ether anesthesia, and the incision was closed with silk sutures. The rats weighed between 250 and 350 g at the time of operation in most instances. A few animals operated before they reached a weight of 250 g gave similar results.

Blood samples (0.8 cc) were drawn from the jugular vein into a 1 cc syringe containing 0.23 cc potassium oxalate (1.85%). The oxalated blood was centrifugalized in a small tube, and the prothrombin content of the plasma was determined by the 2-stage method of Warner, Brinkhous and Smith.[3,4] By this microtechnic, prothrombin determinations can be made several times on the same animal without producing a significant degree of anemia. We prefer jugular puncture to cardiac puncture, because the latter is far more likely to be followed by fatal hemorrhage, especially in case the prothrombin level is low.

Both phthiocol (0.2%) and 2-methyl-1,4-naphthoquinone (200 gamma per 100 cc) were dissolved in isotonic phosphate buffer solutions (pH 7.2).[5] Phthiocol solutions were not injured by sterilization in the autoclave, but 2-methyl-1,4-naphthoquinone suffered marked loss of activity. In our earlier experiments we injected the vitamin preparations without sterilization. Recently, we have found that sterilization of solutions of 2-methyl-1,4-naphthoquinone can be effected with the use of "fritted" glass filters.

Results. With deficient diet alone, it is possible to deplete the vitamin K reserves, as Greaves[1] has pointed out, but rarely does the prothrombin level fall far below the normal. It would appear that in mammals, the vitamin K synthesized in the intestine by bacteria is sufficient to prevent a marked fall in the prothrombin level. This preliminary depletion of vitamin K reserves is essential if one is to bring about a bleeding tendency promptly by ligation of the bile duct. In the undepleted rat, bile duct ligation results in a very gradual decrease in the plasma prothrombin level, and even after 3 weeks, the prothrombin level is still 20% to 40% of normal. By this time, we

[2] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

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

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

[5] Smith, H. P., Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Flynn, Joseph E., *J. Iowa State Med. Soc.*, 1939, **29**, 377.

TABLE I.
Decrease in Prothrombin After Bile Duct Ligation.

Hr post-operative	Prothrombin % of normal Rat No. 1	2	3	4
6	82	100	100	84
24	73	57	72	42
48	35	21	61	5
72	10		27	5

find that general debility and cirrhosis of the liver render the animal unsuitable for these studies.

Table I shows the rate at which the prothrombin level falls in the depleted rat following ligation of the bile duct. During the first 24 hours, the prothrombin level falls only moderately in the majority of animals. By the end of 72 hours the level is typically 10% to 25% of normal. At the 10% level the rats invariably bleed unduly from venipuncture wounds or from small scratches, and at the level of 20% to 25% this is frequently the case.

The response to treatment with vitamin K is shown in Table II. Two daily intraperitoneal doses of phthiocol, 1 mg each, brought the prothrombin level, first from 16% to 47%, and then to 91% of normal. We have administered daily doses of 0.5 mg, and in some cases a definite rise occurred; in others it did not. This would indicate that 0.5 mg represents roughly the daily maintenance requirement, and that the prothrombin level falls into the danger zone if the intake is much less than this.

A typical response to 2-methyl-1,4-naphthoquinone is shown in

TABLE II.
Prothrombin Response to Vitamin K.
(Intraperitoneal Injection.)

Days post-operative	Prothrombin % of normal	Vitamin K
Response to phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone)		
3	16	1 mg
4	47	1 "
5	91	—
Response to 2-methyl-1,4-naphthoquinone		
3	10	2 γ
4	61	2 γ
5	82	—
Response to a single large dose of 2-methyl-1,4-naphthoquinone		
3	5	20 γ
4	81	—
5	78	—
6	59	—
7	20	—

the second section of Table II. Other experiments, not recorded here, agree with this one in that 2 daily doses of 2 gamma each bring about almost complete recovery in the prothrombin level within 48 hours.

In a number of experiments the vitamin preparations were injected intravenously rather than intraperitoneally. In these experiments the response to both the phthiocol and the 2-methyl-1,4-naphthoquinone was essentially the same as that obtained by the intraperitoneal route of administration.

In comparing the results obtained with the two vitamin preparations, it is evident that under the conditions obtaining in our experiments, the 2-methyl-1,4-naphthoquinone has approximately 500 times the activity of phthiocol. This differs moderately from the ratio of 1000 to 1 which Ansbacher[6] found on using the deficient chick as an assay animal.

We have conducted a number of experiments in which the same rat was used for several successive test doses of vitamin K. This permits more accurate control than when different animals are employed. It is especially valuable in comparing different doses of a compound or different modes of administration.

Table II shows that when a single 20 gamma dose of 2-methyl-1,4-naphthoquinone was given by the intraperitoneal route, the prothrombin level rose in 24 hours to 81% of normal. It is of particular interest that this level was not long maintained, for within 3 days the prothrombin was reduced to 20% of normal. In another case we gave a much larger dose of vitamin on each of 2 successive days and found that the normal level was maintained for a number of days. It would appear that, although the avitaminous animal can efficiently utilize very small amounts of the vitamin to reëstablish the integrity of the disordered clotting mechanism, storage of the vitamin is far more difficult to accomplish. Thus the depleted animal can build up normal stores only when the vitamin is assimilated in large amounts, or perhaps with a smaller excess over daily needs, if continued over a long period of time. This observation helps to explain the clinical fact that in cases of biliary tract disease the rise in prothrombin on giving vitamin K is often quite transient, making it necessary to give the vitamin repeatedly during the period immediately preceding and immediately following operation. It is probable that the dosage ordinarily given to patients is not adequate to replenish the much-needed stores.

Summary. Rats maintained on a diet low in vitamin K, and having obstructive jaundice, were used to indicate the vitamin K

[6] Ansbacher, S., *Science*, 1939, **90**, 215.

potency of phthiocol and of 2-methyl-1,4-naphthoquinone. Data also are given to indicate the approximate maintenance requirement of this animal. Evidence at hand indicates that considerable amounts of vitamin K can be stored, provided the deficient animal is given large doses of vitamin K, or perhaps with smaller doses over a long period of time. The clinical implications of this are pointed out.

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Active and Passive Immunization Against the Virus of Malignant Panleucopenia of Cats.

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A common, acute infectious disease of cats characterized by intranuclear inclusion bodies, profound depression of the number of lymphocytes and granulocytes of the blood and an extensive aplasia of lymphoid tissue and of the bone marrow including erythropoietic elements has recently been described by Hammon and Enders[1, 2, 3] who showed that the etiologic agent was filterable.* The disease is probably identical with that previously reported by Lawrence and Syverton.[4] The experiments recorded here in detail† had as their objectives the determination of whether or not active immunization against subsequent inoculation of the virus could be effected in the natural host by means of formalinized suspensions of organs from infected cats and whether or not the immunity which had been found to result from an attack of panleucopenia could be passively transferred to susceptible animals by means of the blood serum.

Active Immunization. The data obtained in 2 experiments are pre-

1 Hammon, W. D., and Enders, J. F., *J. Exp. Med.*, 1939, **69**, 327.

2 Hammon, W. D., and Enders, J. F., *Ibid.*, 1939, **70**, 563.

3 Enders, J. F., Third International Congress for Microbiology, New York, 1939, Abstracts of Communications.

* Communications which we have received from a number of investigators indicate that the disease is present in Canada, Germany, Russia, and possibly South America.

4 Lawrence, J. S., and Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 914.

† Certain of the data included here were briefly described at the meeting of the Fourth International Congress for Microbiology held at New York, September 9-14, 1939.