

**BLEEDING TENDENCY AND PROTHROMBIN DEFICIENCY  
IN BILIARY FISTULA DOGS: EFFECT OF  
FEEDING BILE AND VITAMIN K\***

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(Received for publication, March 14, 1938)

Hawkins and Whipple (1) recently described a number of abnormalities which occur commonly in dogs having long standing biliary fistulae. They observed a bleeding tendency which could be cured by feeding bile. If the animals were fed bile periodically in the earlier stages of the experiment the bleeding tendency did not develop. Subsequently, one of us in conjunction with Hawkins (2) showed that the bleeding was due to profound lowering of the plasma prothrombin level. When the bleeding was prevented by feeding bile, the prothrombin remained within normal limits.

Several years ago a hemorrhagic deficiency disease of chicks was described in several laboratories. Dam (3) and Almquist and Stokstad (4) have identified the deficiency factor as a new fat-soluble vitamin, which has been designated the antihemorrhagic vitamin, or vitamin K. Both Schönheyder (5) and Quick (6) have concluded that the bleeding in this hemorrhagic chick disease is due to a low plasma prothrombin. These findings suggested that the biliary fistula dogs also suffer from vitamin K deficiency, a result of faulty absorption when bile is excluded from the gut. That this explanation is correct is indicated by a preliminary report of Greaves and Schmidt (7) on rats. Important confirmation is also supplied by our successful treatment of jaundiced human bleeders with vitamin K (8). A preliminary report of our work with patients was followed almost at once by a similar report from the Mayo Clinic (9).

\* Aided by a grant from the John and Mary R. Markle Foundation.

Our animal work includes data on 36 fistula dogs studied during the last 3 years. Though a number of perplexing problems still exist, our experience is in general agreement with the work of Greaves and Schmidt, and in view of their preliminary publication we are presenting at this time a group of our experiments.

#### *Methods*

The gall bladder-renal type of biliary fistula described by Kapsinow, Engle, and Harvey (10) was used. The dogs used in these experiments were in good physical condition and free of jaundice.

The kennel diet consisted of mixed table scraps. It contained bread, potato, and vegetables in large quantities, with small amounts of meat and salad dressing. This diet was given unless otherwise indicated. Diet 9 was used in some experiments. It consisted of the following: 1,800 gm. dry white bread, 400 gm. cane sugar, 220 gm. corn starch, 400 gm. canned lean meat, 20 gm. McCollum's salt mixture, and 4,500 gm. water; this mixture was cooked 45 minutes in the autoclave at 15 pounds pressure; then 100 gm. cod liver oil and 100 gm. powdered yeast were added. Diet 15 consisted of dog chow.<sup>1</sup> The restricted vitamin diet (diet 31) consisted of diet 9 without addition of cod liver oil or yeast. In each experiment sufficient food was given so that the animal would maintain its weight. The plasma prothrombin was determined by the method of Warner, Brinkhous, and Smith (11).

Two preparations of vitamin K were used. The first was an extract of dry alfalfa meal. The solvent used in making the extract was a special low boiling gasoline (Skellysolve A). The dry alfalfa meal was placed in flasks and extracted in series, after which the solution was filtered and concentrated by distillation. The second preparation was a potent vitamin K concentrate, partially purified by a modification of the method of Almquist (12). His procedure was followed with these exceptions: (a) the initial adsorption steps were omitted, and (b) vitamin K was taken up from the final methyl alcohol solution into Skellysolve A by addition of a large volume of water. 1 cc. of the final solution of this concentrate was the equivalent of 100 gm. alfalfa meal. Prior to using either of these preparations, the solvent was evaporated rapidly in a current of air.

#### *Low Plasma Prothrombin Level and the Bleeding Tendency: Effect of Blood Transfusions and Whole Bile Feeding*

In biliary fistula dogs the plasma prothrombin level occasionally, though rarely, falls steadily in a very few weeks to the bleeding level. More commonly there is a latent period of several weeks before any

<sup>1</sup> Purina Mills, St. Louis.

significant fall is noticed. Sometimes this latent period is extended over many months. Dog 1 is intermediate in this respect.

Dog 1. (See Chart 1.) Mongrel female, 10.5 kilos. Biliary fistula operation Feb. 19, 1935. Bleeding time and clotting time normal for several months, but on the 214th day the bleeding time was 10 minutes, and on the 230th day the clotting time was 18 minutes. No spontaneous bleeding, however.

On the 304th day a silk stitch abscess appeared and was drained 2 days later. Considerable oozing of blood followed. Transfusion (210 cc.) failed to control the hemorrhage. The incision was infiltrated with thrombin solution and was packed with gauze, after which the bleeding ceased. During the following week gross hematuria and melena were noted. The clotting time on the 310th day was 8 minutes; the bleeding time 3 minutes.

No further bleeding until the 394th day, when moderately profuse vaginal bleeding was noted for several days. No evidence of estrum. On the 422nd day bleeding persisted several hours from a vena puncture site. Transfusion (130 cc.) given; bleeding ceased. Diet now changed from table scraps to a high protein diet, but the dog ate poorly and lost 2.6 kilos in the next 6 weeks. During this period prolonged bleeding followed vena puncture on several occasions, controlled in each case by transfusion.

On the 466th day diet changed to table scraps with which were mixed 50 cc. ox bile daily. Diet continued 7 months and was well eaten. Weight 9.6 kilos. No bleeding since bile feeding was begun. Bile feeding discontinued on the 674th day. For the next 3 months dog was fed table scraps supplemented with canned tomatoes and 4 cc. cod liver oil daily. Weight increased to 11.5 kilos during this period.

Dog 1 is a case in which the latent period was about 12 weeks. Chart 1 shows that the prothrombin level fell to 25 per cent of normal during the next 4 weeks, and to about the 10 per cent level 13 weeks after this. During this entire period of 6 months the dog showed no evidence whatever of spontaneous bleeding.

It has been our experience that bleeding rarely occurs before the plasma prothrombin reaches the 10 per cent level. This bears out our previous experiments (11) showing that the prothrombin level normally is vastly in excess of minimal requirements. Even at the 10 per cent level actual bleeding is conditioned by minor injuries. This dog remained in the danger zone for 4 months without bleeding, but on the 306th day operative drainage of a stitch abscess provided a bleeding site, and hemorrhage was controlled with difficulty. For the next 3 months no bleeding was observed, despite prothrombin levels

of 10 per cent or less. During the next 10 weeks (394th to 466th day) there were 5 episodes of bleeding, once spontaneously from the vagina, and 4 times following vena puncture.

The value of blood transfusions in controlling hemorrhage in such dogs has been shown by Hawkins and Whipple (1). This is well illustrated in this particular dog. As shown on the chart, there was only a slight concomitant rise in the plasma prothrombin. This rise, however, was sufficient to bring the prothrombin above the bleeding

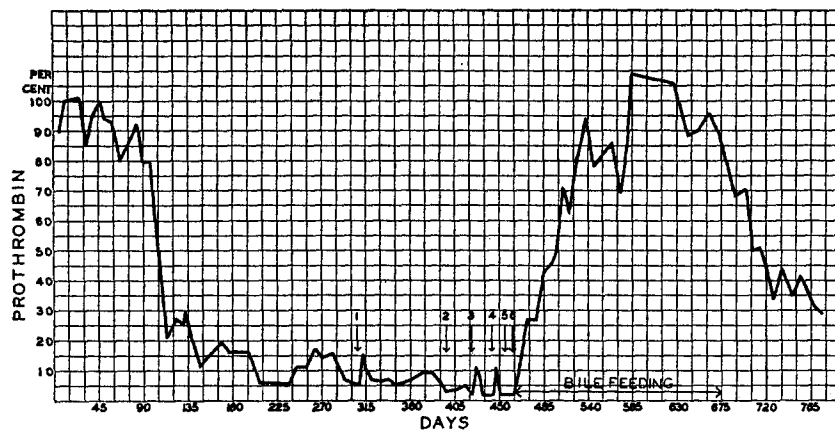


CHART 1. Dog 1. Arrows indicate occasions when prolonged bleeding occurred; (1) bleeding from operative site (stitch abscess), transfusion 210 cc.; (2) vaginal bleeding; (3) bleeding from vena puncture site, transfusion 130 cc.; (4) bleeding from vena puncture site, transfusion 110 cc.; (5) bleeding from vena puncture site, transfusion 160 cc.; (6) bleeding from vena puncture site, transfusion 75 cc.

level. The increased level could not be maintained, however, and in a short time bleeding recurred. The rapidity with which the prothrombin again fell to the extremely low levels gives an indication as to the rate of prothrombin utilization in these animals.

Beginning on the 466th day, 50 cc. ox bile were added daily to the diet of this dog. From this point on the prothrombin level rose steadily, reaching normal levels 4 months later. For the next 3 months, continued bile feeding maintained the prothrombin at an essentially normal level. On discontinuing bile feeding the prothrombin level fell again, reaching the 29 per cent level in 14 weeks. It is

worthy of note that no latent period preceded this fall. One might postulate that the reserves concerned in prothrombin manufacture were not as great as originally.

*Effect of Bile Salt Feeding on the Prothrombin Level*

Dog 2 showed a dangerously low prothrombin level in about 4 months after operation (Table I). Beginning on the 156th day of the

TABLE I

*Effect of Bile Salt Feeding on the Prothrombin Level*

Dog 2; gall bladder-renal fistula, Feb. 20, 1935; weight 18 kg.

Day of experiment	Bile salt feeding daily	Prothrombin*
	<i>gm.</i>	<i>per cent</i>
0	0	110
84	0	61
106	0	24
126	0	8
155	0	7
156-158	1.0	7
159-165	2.5	17
166-172	2.5	34
173-179	2.5	72
180-186	2.5	70
187-193	2.5	78
194-200	4.0	70
201-206	4.0	57
207-211	4.0	51
212-218	0	51

\* Prothrombin determinations at end of each period.

experiment, bile salt was added daily to the diet over a period of approximately 2 months. The dry salt was thoroughly mixed with the food, which was promptly eaten. Within 10 days a noticeable rise in plasma prothrombin had occurred, and in 24 days it had reached the 72 per cent level. This level was maintained for the next 3 weeks after which a small decline was evident. The failure to reach normal levels occurred despite an increase in the amount of bile salt added to the diet.

*Effect on the Prothrombin Level of Feeding Antihemorrhagic Vitamin Preparations*

Chart 2 shows the effect of feeding the antihemorrhagic vitamin to chronic biliary fistula dogs. In each case a number of months had elapsed since operation and gradually the plasma prothrombin level had fallen to 37 per cent or less. In no case had the diet been enriched in regard to the antihemorrhagic vitamin. In dogs 4 and 5

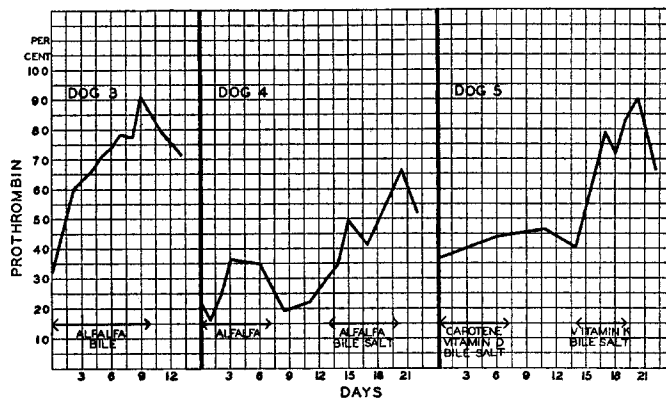


CHART 2. Dog 3, female, 10 kilos. Fistula operation 29 weeks previously. Table scrap diet throughout. Alfalfa extract (150 gm. equivalent) + 75 cc. ox bile mixed with food daily for 10 days, as shown above.

Dog 4, female, 12 kilos. Fistula operation 27 weeks previously. Diet 15 for last 16 weeks. Supplement of alfalfa extract (200 gm. equivalent) daily for 7 days; for second 7 day period, supplement of alfalfa (200 gm. equivalent) + 0.6 gm. bile salt daily, shown above.

Dog 5, female, 9 kilos. Fistula operation 57 weeks previously. Diet 31 for last 15 weeks. Carotene (5 mg.) + vitamin D (drisdol, 15,000 U. S. P. units) + 0.6 gm. bile salt daily for 7 days. Vitamin K concentrate (500 gm. alfalfa equivalent) + 0.6 gm. bile salt daily during second 7 day period.

standard diets were used which are low in the known sources of the vitamin. Dog 3, on a variable diet of table scraps, followed approximately the same downhill course.

Dog 3, Chart 2, shows the response to appropriate dietary supplements. A daily supplement of vitamin extracted from 150 gm. alfalfa meal was given emulsified in ox bile and mixed with the food. The response was immediate. Within 2 days the plasma prothrombin

level had risen from 32 per cent to the 60 per cent level. The rise continued, and a level of 91 per cent was reached on the 9th day of treatment. This abrupt 9 day rise should be contrasted with the slow rise (4 months) shown in Chart 1 when bile alone is fed. The slow rise in the latter case indicates that our stock diet when mixed with bile yields but small amounts of the vitamin. This sluggish rise with bile alone is also in accord with our human cases (8, 13), which indicate a recovery period of weeks with bile alone in contrast to a recovery period of days when the bile is supplemented with the vitamin. These findings differ somewhat from those of Greaves and Schmidt (7). They state that in the bile fistula rat the plasma prothrombin shows a marked rise after the brief period of 2 to 4 days of simple bile feeding. One must assume either that their diet contains far more naturally occurring vitamin than in our experiments, or that a small animal like the rat has proportionately smaller vitamin requirements or has a strikingly shorter recovery period. Then, too, one must consider the different technique for the determination of prothrombin. Our method is a two-stage titration procedure which permits complete control of all variables.

Dog 4, at the 22 per cent prothrombin level, shows an initial 7 day period of treatment with large quantities of the vitamin, but without adding bile to aid in its utilization. There appeared for several days to be a slight response, but this gain was lost, suggesting that the entire fluctuation may not have been related to the change in diet. At any rate, the vitamin, administered alone, has little curative effect. The second experimental period, shown several days later, involved feeding a similar quantity of the vitamin, along with bile salt. It is evident that the bile salt enables the animal to use the vitamin supplement, for within the brief period of 7 days the prothrombin rose from the 25 per cent to the 66 per cent level.

Dog 5, Chart 2, was given a brief period of treatment with carotene and vitamin D. This experiment was prompted by the reports of McNealy, Shapiro, and Melnick (14) and Boys (15) that jaundiced human bleeders are to some extent relieved of their bleeding tendency by adding vitamin D to the diet. In accord with their recommendation, we included a bile salt supplement to aid absorption. The results, however, were very disappointing, for the plasma prothrombin

values remained constant within the limits of experimental error. That this dog was capable of reacting promptly to vitamin K supplements was shown in the second experimental period indicated in the chart. With vitamin K concentrate plus bile salt the prothrombin rose in 6 days from the 40 per cent to the 90 per cent level.

It will be noted that in both dog 4 and in dog 5 we used bile salt in place of whole bile. Under these conditions the bile salt appears to be highly effective, indicating that all the necessary factors for prothrombin manufacture are present. The incomplete recovery of dog 2 (Table I) on bile salt alone may merely imply that difficulty is encountered when the vitamin is present in minimal quantities, as in the stock diet given to that dog.

The rapid rise in prothrombin levels shown in Chart 2 ceased in all cases as soon as the dietary supplement was discontinued. Evidently the vitamin was used promptly, and little or none was stored. This is also shown by the fall in prothrombin which commenced as soon as the vitamin concentrate was withheld. Data, not given on this chart, show that this fall continues steadily, reaching the former low levels in several weeks.

#### DISCUSSION

Our earlier experiments with fistula dogs, like those of Hawkins and Whipple, indicated that prothrombin reaches the bleeding level in 3 or 4 months. Human cases with bile excluded from the intestine often continue for even longer periods without hemorrhages, though at times they bleed after a few weeks of biliary obstruction. This tendency to earlier bleeding in human adults is in good part due, we believe, to the fact that in man the danger zone is at a higher prothrombin level than in dogs. In some of our more recent experiments on dogs the fall in prothrombin was unaccountably slow in making its appearance. Several dogs were still above the 45 per cent level at the end of 6 months. In these cases the feces remained free of bile pigment, which excluded the possibility that the biliary tract had spontaneously reformed. To rule out the possibility that the kennel diet had varied in its vitamin content we substituted diets containing restricted amounts of vitamin K (diets 9 and 31). Even then, the prothrombin remained for long periods at about the 50 per cent level.



This extreme variability among dogs leads us to conclude that unknown factors modify the rate at which the prothrombin level falls. One might postulate the existence of large vitamin K reserves in such animals. Again, it is thought that in chicks the vitamin is manufactured by bacteria in the bowel, though mainly in the lower portion of the bowel where it may be poorly absorbed. Bacterial synthesis can be assumed to occur in mammals also, and a variable amount of absorption may occur. The factor of resorption is difficult to control. We have attempted, however, to minimize coprophagy in several of our dogs, but without manifest influence on the rate at which the prothrombin fell.

The mechanism by which vitamin K aids in the manufacture of prothrombin is still obscure. We have supplied evidence to the effect that the liver is vitally concerned in the manufacturing process (16). Among the various possible rôles one must consider the possibility that it is an important building stone for prothrombin, or that it is needed for proper function of the liver cell.

We have not as yet determined in fistula dogs the minimal effective dosage of vitamin K. It may well be that maximal prothrombin response would be obtained with much smaller amounts than we have used. Chick studies have shown that the vitamin is fairly widely distributed in nature, and there can be no doubt that our standard mixed diets do contain the vitamin in moderate amounts. This diet supplemented with bile gives a very slow response. It thus seems likely that the vitamin dosage added by way of supplement is not enormously in excess of optimal requirements. This is a matter which requires further study both in fistula animals and in human jaundiced bleeders. Standardization studies must also include a critical survey of the chick assay technique, for obviously the dosage employed in both man and animals should be based upon accurate assay of the vitamin concentrates employed.

#### SUMMARY

In biliary fistula dogs the plasma prothrombin falls eventually to low levels and bleeding commonly occurs. Faulty absorption of vitamin K from the intestine in these animals is an important causative factor. Feeding bile permits absorption of the traces of this

vitamin normally present in mixed diets, and as a result a slow rise in prothrombin level is observed. If a standard diet is supplemented with large amounts of vitamin K concentrate the prothrombin rise is rapid, provided bile or bile salt is supplied to aid in the absorption.

Variations in the rate of prothrombin depletion in biliary fistula dogs kept on constant diet indicate the existence of additional factors which require further study. Our experience indicates that vitamin A and vitamin D supplements do not correct the prothrombin deficiency in biliary fistula animals.

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