PROTHROMBIN DEFICIENCY THE CAUSE OF BLEEDING IN BILE FISTULA DOGS

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In a recent publication Hawkins and Whipple (2) stressed spontaneous bleeding as one of the important abnormalities which develops in bile fistula dogs completely deprived of bile. After 3 to 5 months of total bile deprivation there is some lengthening of the clotting time, and finally there may be spontaneous bleeding from mucous membranes or prolonged bleeding from sites of minor trauma such as a vena puncture wound. These abnormalities may be abolished within a week or two by continued feeding of dog bile (100 cc. daily). Transfusion of whole blood also will cause cessation of the bleeding, but in this case hemorrhage may soon recur. If an adequate amount of dog bile (50 to 75 cc. daily) is given from the beginning, this bleeding tendency does not develop.

It is of great interest to know what abnormality of the blood is responsible for this bleeding. Recently Warner, Brinkhous and Smith (6, 7) have developed quantitative methods for the titration of prothrombin and of antithrombin. By the use of their methods we have found a marked reduction in the amount of plasma prothrombin in our cases of bleeding. In contrast, other clotting factors–fibrinogen, calcium, blood platelets and antithrombin—have shown no significant variation from their normal levels.

Methods

The gall bladder renal type of fistula devised by Kapsinow, Engle and Harvey (5) was used. The fistula tract remains free of infection, and these dogs, properly fed, can be maintained in excellent condition for long periods of time.

The diet consisted either of salmon bread or of stock kennel rations. The salmon bread is a complete diet and its preparation has been described previously

(8). It contains wheat flour, potato starch, bran, canned salmon, sugar, canned tomatoes, yeast, cod liver oil and a salt mixture. The bread contains 10 per cent protein, 6.5 per cent fat and 83.4 per cent carbohydrate. It is a very suitable diet for fistula dogs, since it is so rich in carbohydrates. Dogs as a rule are fed 300 to 400 gm. of this salmon bread plus 75 gm. of salmon and 30 gm. of whole milk powder (Klim). The kennel ration is composed of mixed hospital scraps and it contains much bread, potato, vegetables, a little meat and variable amounts of butter.

The prothrombin of plasma was titrated according to the method of Warner, Brinkhous and Smith (7). It is expressed in per cent of prothrombin of a normal control dog plasma. Fibrinogen was determined by the method of Jones and Smith (3). For the determination of the antithrombic activity of plasma, a known thrombin was incubated with oxalated plasma which had been dialyzed several hours against 0.9 per cent NaCl solution. The thrombin remaining was then titrated. The amount of thrombin destroyed is a measure of the antithrombin of the plasma. This method of determining antithrombin has been outlined briefly by Smith, Warner and Brinkhous (6) and will be published in detail soon. The method of Clark and Collip (1) was used for serum calcium determinations.

EXPERIMENTAL OBSERVATIONS

Most of the dogs studied had been followed in the bile pigment or anemia colonies at the University of Rochester for considerable periods of time. For purposes of brevity and clarity only the data pertinent to this problem will be given. It is well to add that the dogs were in excellent physical condition during the period of these studies. Seven dogs in all were studied. Three of these experiments, representative of the various states found, are presented in detail.

Dog. 33-90. This dog, weighing 18.6 kilos, was fed 600 gm. of kennel diet daily. It was operated upon Mar. 13, 1934, and a gall bladder renal fistula was made. The dog was continued on the kennel diet for nearly 7 months, except for a 2 week period in June when 200 gm. of pig liver and 200 gm. of salmon bread were substituted. Then, beginning Oct. 3, the kennel diet was replaced by the salmon bread mixture. This diet was fed, with the addition of pig liver (300 gm. daily) from Dec. 12 to Jan. 15, until the death of the animal.

For the first 19 weeks postoperatively, the dog was given no bile. However, during part of this time (Apr. 10 to May 20), 1 to 3 gm. of sodium taurocholate were added daily to the food. Starting July 24, ox bile (50 cc.) was given by stomach tube 6 days a week. This was continued for almost 6 months until Jan. 15, 1935. On Jan. 9, it was found that whole venous blood, placed in small clean test tubes clotted solidly in 13 to 15 minutes. 6 days later, the ox bile was replaced by 1 gm. of sodium taurocholate. Then, on Jan. 18, bleeding occurred for the first time. Blood was present in the urine, and during the next 5 days much blood was

passed. On Jan. 21, in addition to the sodium taurocholate, 1 egg yolk was added to the diet to see if a combination of these substances would influence clotting. The next day whole blood clotted slowly; in 20 minutes only a fragile clot had formed, but at the end of 40 minutes there was a solid clot which later retracted well. At this time the dog's pulse and respirations were rapid, so a whole blood transfusion (175 cc.) was given to stop the bleeding. 2 days later, on Jan. 24, the urine was free from blood. However, the delay in clotting continued. On Feb. 4, the hematuria recurred, so on the next day dog bile (50 cc.) was substituted for the bile salt and egg yolk. At this time whole blood clotted in 25 minutes. Blood oozed all that day from the vena puncture wound. The blood sample was drawn at 9:00 a.m. and at 11:30 p.m. there was still oozing of blood and a small hematoma was present in the neck. A blood transfusion (140 cc.) was given, and shortly thereafter the oozing ceased. On Feb. 9, the dog bile was increased to 100 cc. daily and no further bleeding occurred while the bile was fed. On Feb. 20, recalcified oxalate plasma (equal parts of plasma and 0.6 per cent $CaCl_2$ solution) clotted in 9 minutes. On Feb. 23, the dog bile was discontinued and 1 gm. sodium taurocholate was added to the food. 3 days later blood was present in the urine and continued to be present on succeeding days with large amounts on Mar. 2. Recalcified oxalate plasma clotted solidly in 26 minutes. On Mar. 3 the dog was found lying quietly in the cage whereas the day before it had been very active. Respirations were labored and a red cell hematocrit was only 15 per cent. A whole blood transfusion (220 cc.) was given without obvious improvement, so 2 hours later more blood (270 cc.) was given. Respirations still remained labored and in the evening the dog was found dead. In the anterior mediastinum there was a large mass composed of fat infiltrated with blood, much of which was still fluid, and in the pleural cavities there were 750 cc. of fluid blood preventing expansion of the lungs. When the heart was opened well formed clots were found within the chambers. No bleeding points could be demonstrated in the urinary tract to explain the hematuria.

It is seen from this history that ox bile and sodium taurocholate in the amounts given did not prevent the abnormality in blood clotting from developing. This bleeding tendency was controlled successfully by the feeding of whole dog bile. Whole blood transfusions were also of value, but when given alone only temporary improvement resulted. The beneficial effect of transfusion was strikingly demonstrated at the time of autopsy. The blood in the mediastinal fat and pleural cavities was unclotted and after sitting 24 hours in a flask only a flimsy clot formed. This is in contrast to the solid clots formed within the heart.

This dog was carefully studied with regard to the clotting factors from January 24 to February 5. During this period there was marked lengthening of the coagulation time (20–25 minutes), with episodes of

spontaneous bleeding. Although the coagulation time was greatly lengthened, a fairly firm clot finally formed. That there was no fibrinogen deficiency was further shown by a plasma fibrin value of 322 mg. per 100 cc. In titration of antithrombin, the same rate of destruction of thrombin resulted both with this plasma and with a normal control plasma. Titration of prothrombin, however, revealed a very marked hypoprothrombinemia. During this period the prothrombin level was always below 5 per cent of the normal control. Practically identical prothrombin values were obtained on study of the globulin fraction—a fraction which normally contains nearly all the prothrombin but almost no antithrombin. In this way we showed that neither antithrombin nor heparin interfered with the ordinary prothrombin titration.

As a supplement to this experiment we added prothrombin to this abnormal whole blood.

The prothrombin was prepared by fractional precipitation of plasma globulin with $(NH_4)_2SO_4$. It was dissolved in isotonic saline and dialyzed against saline for several hours to remove the excess of salts. The preparation was calcium free and failed to clot oxalated plasma. Titration showed it to contain somewhat more than twice as much prothrombin as normal whole blood.

Tube 1.—3 cc. whole blood—fragile clot in 25 minutes.

Tube 2.—2 cc. whole blood + 1 cc. saline—fragile clot in 22 minutes.

Tube 3.—2 cc. whole blood + 1 cc. prothrombin—began to clot in 4 minutes 45 seconds; clot very solid in 5 minutes 45 seconds. In tube 3, then, the prothrombin concentration was nearly equal to that of normal whole blood.

The prompt restoration to a normal clotting time strongly suggests that prothrombin alone is responsible for the abnormal state of clotting.

Studies on other dogs of this type with a marked hypoprothrombinemia showed that both serum calcium and blood platelets are within the limits of normal.

Dog 32-74. Splenectomy was performed on Nov. 29, 1932, and a gall bladder renal fistula was made on Feb. 2, 1933. Beginning 11 days later, ox bile (50 cc.) was given by stomach tube 6 days a week and this was continued for the next 21½ months. During the earlier part of this period (Mar. 16 to Dec. 18) the ox bile was supplemented with dog bile (50 cc.). The kennel diet, which was fed during the greater part of this time, was changed to the salmon bread diet on Aug. 2, 1934.

Nearly 22 months after the fistula was established, bleeding from the jugular vein was done repeatedly and thus in the next 26 days the hemoglobin percentage was brought down to 50 per cent (13.8 gm. hemoglobin = 100 per cent). During the first 9 days of the anemia period the bile feeding was continued, but on Dec. 1, 1934, it was replaced with 1 gm. sodium taurocholate daily. On Dec. 12, there was oozing of blood from a vena puncture wound. Blood transfusion of 40 cc. was given and the dog bile (50 cc.) was restored to the diet. Again, 4 days later, bleeding was noted following a vena puncture. Another transfusion (50 cc.) was given and the bile was increased to 70 cc. daily. The spontaneous bleeding did not recur, so on Jan. 2, 1935, the bile feeding was reduced to 50 cc. Beginning Jan. 9 the sodium taurocholate was increased from 1 gm. to 2 gm. daily. The anemia was maintained by carefully spaced bleedings. On this regimen, no further spontaneous bleeding occurred. The animal was followed in this experiment until Feb. 4, 1935.

This dog, a bile renal fistula of 2 years' duration, showed no bleeding tendency as long as the diet included adequate amounts of bile. When the dog was made anemic and no bile was given, prolonged bleeding from vena puncture wounds occurred. Two small blood transfusions and restoration of bile feeding checked the bleeding tendency during the final 7 weeks of the experiment. Unfortunately, analysis of the clotting factors was not made until after the period of spontaneous bleeding. But even after disappearance of the clinical tendency to bleed, the prothrombin level was still low, 16 per cent of normal. No doubt it was much lower than this at the time of bleeding. Other clotting factors were found to be normal at the time of our analysis (February 1 to February 4). Titration of antithrombin showed that both this plasma and a normal control plasma gave the same rates of thrombin destruction. Plasma fibrinogen was 369 mg. per cent. At this time whole blood clotted solidly in 9 minutes.

Dog 31-359. A gall bladder renal fistula and splenectomy were done Sept. 27, 1932. Beginning immediately, dog bile (50 cc.) was given 6 days a week by stomach tube. This was continued for nearly 15 months, with a supplement of ox bile (50 cc.) during the last 37 days of this period. Then, beginning Dec. 21, 1933, only the ox bile (50 cc.) was given. This was continued until the death of the animal 14 months later. The dog was fed the kennel diet, except for a period early in its course (Oct. 12, 1932, to Apr. 30, 1933), when salmon bread was substituted. The dog was killed with ether anesthesia on Feb. 25, 1935, since marked osteoporosis with multiple fractures had developed because of long continued partial bile deprivation. No spontaneous bleeding had occurred, however.

The above dog was fed either dog bile or ox bile or both during the entire time, nearly 2½ years, it had a bile renal fistula. No clotting abnormality was noted at any time. Thus, in this respect, the bile feeding was quite adequate to protect the animal. A short time before the dog was killed a normal prothrombin content of the plasma was found. Thus, on Jan. 25, 1935, it was 92 per cent of normal. We conclude that when bile is fed in suitable amounts, a normal prothrombin level may exist in the presence of a biliary fistula.

DISCUSSION

The abnormal blood clotting in bile fistula dogs is related without doubt to the bile deprivation. If an adequate amount of bile (50 to 75 cc.) is fed daily the blood continues to clot normally. But if the bile feeding is inadequate, the dogs gradually develop a bleeding tendency. This bleeding can then be controlled successfully by feeding whole dog bile (50 to 100 cc.). Ox bile appears to be less effective than dog bile in protecting these animals. Studies are now in progress to determine what factor in bile is responsible for its protective action.

In cases showing a bleeding tendency, analysis of the clotting factors reveals that the fibrinogen, calcium and platelets are normal, and that no excessive antithrombic activity is present. There is, however, a very marked diminution in the amount of prothrombin. This deficiency undoubtedly is responsible for the delay in clotting and hemorrhage. A hemorrhagic tendency does not develop, however, unless the prothrombin level is very low. This indicates that a wide margin of safety exists between the normal level of prothrombin and the critical level at which a disturbance in clotting results. This is in accord with the analysis of the prothrombin level of normal plasma recently made by Warner, Brinkhous and Smith. They have shown that plasma contains a great excess of prothrombin and that only a small fraction of it is required to give a normal clot. This would explain the absence of spontaneous bleeding in cases showing a relatively low prothrombin level. Likewise, it would explain the temporary benefit derived from blood transfusions. Here there is probably a transitory elevation of the prothrombin to a level at which blood will clot promptly.

In connection with this study, it is of interest to note that Judd,

Snell and Hoerner (4) have referred to a hemorrhagic tendency in patients with a biliary fistula, but without jaundice. This picture is analogous to that produced in these experiments. It seems likely that a hypoprothrombinemia associated with the bile deprivation is also responsible for the bleeding in these cases.

SUMMARY

The bleeding tendency that develops in bile fistula dogs, completely or partially deprived of bile, is due to a prothrombin deficiency of the blood plasma. However, a relatively low prothrombin level may exist in these animals without the occurrence of spontaneous hemorrhage. The prothrombin level may remain within the limits of normal if sufficient bile has been fed. In these cases no disturbance in blood clotting occurs.

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