CANINE HEMOPHILIA

OBSERVATIONS ON THE COURSE, THE CLOTTING ANOMALY, AND THE EFFECT OF BLOOD TRANSFUSIONS*

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Field, Rickard, and Hutt (1, 2) have reported the occurrence of a sex-linked hemorrhagic disease in male dogs similar to hemophilia in man. The chief symptoms were due to subcutaneous hematomas and hemarthroses. Deformities frequently occurred. Most of the pups affected with the disease died during the first 12 weeks of life. Of 17 affected males described, none were reared to maturity. The female stock, heterozygous for the disease, was turned over to this laboratory so that a controlled breeding program could be instituted, and a more extensive investigation of the clotting defect could be made. Our studies of the affected male progeny indicate that the clotting defect is identical with that found in human hemophilia. Repeated transfusions with whole blood or plasma alleviate the hemorrhagic phenomena, and permit growth of affected dogs to maturity practically free of deformities.

Materials and Methods

The male bleeder dogs used in this study were born and reared in our kennel. The dams were known transmitters of the disease and of Irish setter stock. The sires were normal males selected without respect to breed.

The clotting time of whole blood was determined by two methods: (*a*) a modified Lee and White method (3), in which two 10 × 75 mm. dry glass tubes were used for each test, and (*b*) the silicone method, in which needles, syringes, and tubes coated with methylchlorsilane (4) were used. The method for determining the clotting time of platelet-rich and platelet-poor citrated plasmas has been described (5). Platelet-rich plasmas were obtained by centrifugation of citrated whole blood for 5 minutes at 450 *g*. Platelet-poor plasmas were prepared by centrifugation for 2 hours at 13,000 *g* in silicone-treated glassware.

Bleeding time was determined by a modification of Duke's method (6). The site of incision in one series of observations was the mucous membrane of the lip (1); in another series the inner hairless aspect of the pinna (tragus of the ear) was used. Incisions were approximately 2 mm. deep. On the lip the incisions were 2 to 5 mm. long, on the ear 5 to 7 mm.

The platelets in whole blood were counted by a modification of Nygaard's method (7),

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using 3.2 per cent sodium citrate as a diluent in the ratio of 4 parts to one part of whole blood. After 30 minutes of sedimentation, plasma was transferred to a counting chamber and the platelets were counted directly.

Fibrinogen was determined by the method of Jones and Smith (8).

Serum calcium was determined by the method of Kramer and Tisdall (9).

Prothrombin was determined by the two-stage method of Warner, Brinkhous, and Smith (10, 11). Fresh dog serum, as described in the original method, was used for plasma defibrination (10). The plasma prothrombin time was determined by the method of Quick (12).

For determination of thrombin clotting time, 0.4 ml. of citrated plasma was mixed with 0.1 ml. thrombin, prepared by dissolving Parke-Davis topical thrombin in 0.9 per cent NaCl. Thrombin solutions of two different strengths were used: one was diluted to give a 15 second clotting time with normal citrated plasma in this test; the other was diluted to give a 45 second clotting time.

The qualitative method of Conley, Rathbun, Morse, and Robinson (13) for demonstrating anticoagulants in plasma was used. The clotting tests were carried out at 28°C. Quantitative determination of plasma antithrombin was made by the method of Wilson (14).

For transfusions, citrated whole blood and plasmas were used. Unless otherwise indicated the whole blood was freshly prepared. The citrated plasma was stored in 40 ml. aliquots at −35°C. and used within 10 days of collection.

Course of the Malady and the Effect of Transfusions

The observations given below were made on bleeder dogs from nine separate litters. Nine of the bleeder animals were reared to maturity.

The Hemorrhagic Phenomena.—These dogs suffered from repeated hemorrhages which were occasionally massive and fatal. The hemorrhages usually appeared spontaneously, although at times observable trauma was the cause. Hemorrhages involved many tissues. The most common sites were the joints, particularly the hip and shoulder joints. The hemarthroses appeared painful. The joints were swollen and were held immobile in a flexed position. The animals refused to bear weight on an affected extremity, and they resisted passive movements of the joints. If promptly treated with transfusions, the swellings usually disappeared in 2 to 4 days, and return of function appeared complete. Involvement of a joint appears to predispose it to further hemorrhage. In a few dogs deformity with limitation of motion occurred in joints which were the site of frequent hemorrhage. Two dogs have developed scapulohumeral ankyloses. Subcutaneous hemorrhages were the second most common type. They have been observed more often in pups than in adult dogs. Several young animals have had hematomas of the scalp, resembling the cephalhematoma of infants. Large spreading hematomas, occasionally reaching a very large size, have been observed. Another site of hemorrhage has been the gums, particularly during tooth eruption. Internal hemorrhages have been observed less frequently. One adult dog has had signs of cerebral hemorrhage, followed by paraplegia. A litter mate developed a hemothorax. Another dog had signs of massive intestinal bleeding.

Considerable variation in the severity of the disease has been observed in

different animals. Some bleeder animals have suffered from frequent and severe hemorrhagic episodes. In these dogs, prolonged periods of disability have resulted, despite repeated transfusions. In contrast, other dogs have developed hemorrhages less frequently, and with prompt administration of transfusions no deformities have resulted. The varied course is illustrated by case histories of two dogs, one a severe bleeder and the other a comparatively mild bleeder (see below).

Effect of Blood or Plasma Transfusions on the Hemorrhagic Phenomena and Survival Time.—Transfusions promptly alleviated the hemorrhagic phenomena. For severe hemorrhages in which the animal showed evidence of anemia or shock, repeated whole blood transfusions have been given. For the more frequent but less severe hemorrhages, transfusions with normal citrated dog

TABLE I

The Effect of Transfusions on Longevity of the Bleeder Dogs

Group	No. of bleeder dogs					
	Total	Survival time, *mos.*				
		1–3	4–6	7–9	10–12	Over 12
Treated	11	2*	1*	0	0	8
Untreated	10	8‡	1‡	0	1§	0

* Death not associated with hemorrhage; one pup, aged 2 months, died of unknown cause, no hemorrhage at autopsy; one pup, aged 2 months, died of intussusception; one pup aged 4 months died of distemper.

‡ Death following extensive hemorrhage.

§ No treatment until 10 months of age, when plasma transfusions were started.

plasma have been used in the amount of 2.5 to 5 ml. per kilo, given on alternate days as long as symptoms last. The efficacy of the transfusions is shown by the prompt improvement of treated animals. If the hemorrhage is external, cessation of bleeding is usually observed within 5 to 10 minutes after transfusion. Dogs treated promptly for each hemorrhagic episode have reached maturity, while untreated dogs have usually died early in life. This is shown in Table I. It will be noted that 90 per cent of untreated animals died of hemorrhage in the first 6 months of life, while none of the treated animals died from this cause during the 1st year of life.

Case Abstract of a Severe Hemophiliac.—Dog 4, a male, was born on Apr. 2, 1947, the first of a litter of five males. The birth weight was 377 gm. The umbilical cord was ligated because of continued oozing of blood from the stump. Artificial feeding with a cow's milk formula was started on the 1st day of life. The formula was supplemented with cereals during the 3rd week of life, and homogenized vegetables were added during the period of tooth eruption. From the age of 8 weeks, dry commercial dog food has been given. Ground and biscuit

types of food have been alternated, supplemented by brewer's yeast, wheat germ, and vitamins A and D. Maximum weight, 21.8 kilos, was attained at 14 months. One-quarter growth in terms of weight was reached at 2½ months, one-half growth at 4 months, and three-quarters growth at 6 months. Dietary intake and weight have always been less than those of the litter mates. The animal has been kept in an individual cage, but allowed to exercise daily on a runway. Distemper vaccination and treatment with a vermifuge, *n*-butyl chloride, were given first at 3 months of age, and repeated every 6 months thereafter.

Lameness has characterized this dog from puppyhood. The first symptom was on the 17th day of life, when the left front leg became lame. Frequent severe hemarthroses have recurred in many joints. During the 9th month of life the animal was almost bedridden and had to be helped to his feet. Subsequently he improved, only to have the severe joint symptoms recur during the 15th month of life. In the 16th month, ankylosis of the right shoulder joint was noted roentgenologically.

Except following transfusions, the clotting time has always been prolonged. Usually it has been between 60 and 120 minutes. However a blood sample obtained on the 18th day of life was unclotted after 4 hours, but did clot overnight. The dog has received 118 transfusions in 22 months. Approximately 4 liters of plasma and 250 ml. of whole blood have been given.

At the age of 22 months, the dog is greatly crippled and his general activity is limited. He is able to arise and walk, but cannot run. Muscular atrophy is pronounced. He shows no signs of sexual maturity; he fails to elevate his leg during urination and has no interest in females in rut. His appearance is that of an emaciated arthritic old dog.

Case Abstract of a Mild Hemophiliac.—Dog 6, a male, and a litter mate of dog 4, was born Apr. 3, 1947. The birth weight was 472 gm. He has been reared in a manner practically identical with that of his sibling described above. From the first week of life, this dog was more vigorous and had a greater appetite than his siblings. Maximum weight, 26.5 kilos, was reached at 16½ months. One-quarter growth was reached at 2½ months, half-growth at 4½ months, and three-quarters growth at 7½ months. Except following transfusions, clotting times have usually ranged between 28 and 50 minutes.

Many episodes of lameness have occurred, but they were less frequent and of shorter duration than those of his hemophilic brother. The cervical spine and left hind leg have been affected most often. Ninety transfusions, totaling 2.8 liters of plasma and 200 ml. of whole blood have been given. Many of these transfusions have been given to minimize the possibility of hemorrhage incident to the rough sex play of mating. At the age of 22 months, he is the largest, most aggressive male in the kennel. Except during short, infrequent periods of lameness and one episode of massive intrathoracic hemorrhage, superficially he has appeared to be completely normal.

EXPERIMENTAL OBSERVATIONS

The Clotting Anomaly

Prolonged Clotting Time.—The clotting times were prolonged in all bleeder animals. One set of determinations is given in Table II. In plain glass tubes, the clotting times of individual dogs varied from day to day. Practically all clotting time determinations fell within the range of 40 to 120 minutes. Once clotting occurred clot retraction was as great in the hemophilic blood as in normal whole blood. In silicone-treated glassware, the whole blood clotting time was greatly lengthened, and varied between 9 and 28 hours. The clotting

time of recalcified plasma rich in platelets was much shorter than the whole blood clotting time, while the recalcified platelet-poor plasma did not clot in the 24 hours of observation. Similar findings have been obtained in human hemophilia.

Bleeding Time Normal or Prolonged, Depending on Site and Size of Incision.— Bleeding from small incisions of the mucous membrane of the lip stopped in the same time as in normal animals (Table III). In contrast, bleeding from slightly larger incisions of the ear was profuse, and continued for many hours

TABLE II

The Clotting Times of Whole Blood and Plasma of Bleeder Dogs, Female Transmitter Dogs, and Normal Dogs

Dog No.	Sex	Type	Clotting time*			
			Whole blood		Recalcified citrated plasma	
			Lee-White	Silicone	Platelet-rich plasma‡	Platelet-poor plasma§
			min.	*min.*	*min.*	*min.*
1	M	Hemophilic	43	1200	17	>1440
2	M	Hemophilic	66	1200	14	>1440
3	M	Hemophilic	54	1320	14	>1440
4	M	Hemophilic	71	1380	15	>1440
5	M	Hemophilic	65	1680	28	—
6	M	Hemophilic	43	1200	12	>1440
7	M	Hemophilic	73	540	18	>1440
8	M	Hemophilic	82	1140	15	>1440
9	F	Transmitter	5	37	2	17
10	F	Transmitter	8	42	2	14
11	F	Normal	6	28	2	19
12	M	Normal	7½	40	2	16

* All clotting times except in the column labeled silicone were done in ordinary glassware.

‡ Platelets per c.mm. of plasma, 125,000 to 200,000.

§ Platelets per c.mm. of plasma, less than 300.

unless controlled by transfusions or local application of thrombin. The bleeding times of normal dogs from ear incisions were 2½ to 3 minutes. The results of the bleeding time test in four bleeder dogs are given. In one animal (dog 7) bleeding stopped in 3 minutes, but oozing from under the edge of the clot developed a few minutes later and continued for 24 hours. In another animal (dog 6) temporary stoppage of bleeding occurred in 4 minutes, but oozing continued, and bleeding was again profuse at the end of 2 hours. Bleeding was controlled by the local application of thrombin and by a transfusion of 40 ml. of citrated normal dog plasma. In two other animals (dogs 4 and 8) bleeding was continuous and profuse for 15 to 20 minutes. Blood loss was at the rate of

1.5 to 2.0 ml. per minute. Hemostasis was accomplished by the application of thrombin and normal plasma transfusions.

Study of Various Clotting Factors.—Blood platelet counts, plasma fibrinogen levels, serum calcium values, and plasma prothrombin determinations were all within the range of normal (Table III). No difference was observed in the thrombin clotting times of plasma from bleeder and normal dogs (Table III). This would indicate that the fibrinogen reactivity was normal, and that antithrombic activity was not excessive. No excess of anticoagulants was demonstrable. In one group of experiments, native plasma from three bleeder dogs

TABLE III

Clotting Factors and Clotting Tests in Bleeder Dogs, Transmitter Female Dogs, and Normal Dogs

Dog No.	Sex	Type	Bleeding time (mucous membrane)	Platelets	Plasma fibrinogen	Serum calcium	Plasma prothrombin (two-stage)	Prothrombin time	Thrombin clotting time	
									With concentrated thrombin	With dilute thrombin
			sec.	*No./c.mm.*	*mg./100 ml.*	*mg./100 ml.*	*per cent*	*sec.*	*sec.*	*sec.*
1	M	Hemophilic	95	295,000	327	10.0	95	9.0	—	—
2	M	Hemophilic	145	294,000	451	11.2	95	9.5	17	45
3	M	Hemophilic	60	271,000	626	10.3	90	9.0	15	44
4	M	Hemophilic	110	225,000	333	10.8	104	9.0	—	—
6	M	Hemophilic	80	288,000	427	9.8	109	10.0	17	47
7	M	Hemophilic	115	402,000	406	10.9	96	9.5	15	46
8	M	Hemophilic	135	325,000	387	9.7	91	9.0	—	—
9	F	Transmitter	110	304,000	350	9.7	106	9.8	14	41
10	F	Transmitter	70	253,000	331	9.5	—	—	—	—
11	F	Normal	85	419,000	252	11.0	96	9.3	15	44
12	M	Normal	110	280,000	467	11.2	106	10.0	16	46
13	M	Normal	105	350,000	360	10.2	100	9.5	15	45

was mixed with freshly drawn normal dog blood (13) and the clotting times determined. In all tubes, the clotting times were within the same range as the normal controls (5 to 7 minutes). Determinations of plasma antithrombin (14) in four bleeder dogs gave results of 90 to 120 per cent of normal control dogs; this is within the range of normal.

Recent evidence indicates that faulty conversion of prothrombin to thrombin may be due to a deficiency of one or more cofactors which accelerate prothrombin conversion in the presence of optimal calcium and an excess of thromboplastin. This subject has been summarized recently by Seegers and Ware (15). A number of terms, as prothrombin convertibility factor, Ac globulin, factor V, and labile factor have been applied to a factor or factors involved in prothrom-

bin conversion. Several qualitative tests were done to determine whether such a factor was lacking in the bleeder dogs. In one series of experiments it was shown that with optimal Ca^{++} concentration and excess thromboplastin, thrombin formed at the same rate in bleeder and normal dog plasma. The results of one experiment are given in Table IV. In another experiment, plasma Ac globulin was added to whole hemophilic blood to determine whether it would correct the clotting defect. Bleeder dogs 4 and 7 were used. Clotting times are given in Table II. Purified bovine plasma Ac globulin (16) was added to normal and bleeder whole blood so that its final concentration in each was equivalent to 17 mg. per 100 ml. of plasma. No effect on clotting was noted. The results of the prothrombin tests (Table III) likewise indicate that prothrombin was converted to thrombin at a normal rate; if a factor V

TABLE IV

Rate of Thrombin Formation in Normal and Bleeder Dog Plasma with Optimal Recalcification and Excess Thromboplastin

Diluted plasma*	Clotting time, *sec.*						
	Prothrombin conversion time‡						
	90 sec.	100 sec.	105 sec.	110 sec.	120 sec.	130 sec.	140 sec.
Normal	20		17		16	16	16
Hemophilic	21	18		16	16	16	16

* Plasma was diluted so that after optimal recalcification and addition of thromboplastin, thrombin concentration in the clotting mixtures would be approximately 1 unit.

‡ Period of reaction of mixtures of plasma, Ca^{++}, and thromboplastin prior to the addition of fibrinogen.

deficiency existed, the plasma prothrombin time should have been prolonged (17), even if the prothrombin was normal in the two-stage test.

Impaired Prothrombin Utilization; Correction by Thromboplastin, Normal Plasma, or Fraction I.—During and after clotting of normal whole blood, the prothrombin rapidly disappears. Within 2 hours after collecting the blood, little prothrombin remains. In hemophilia, on the other hand, prothrombin utilization is delayed, and relatively large amounts of prothrombin remain in the serum hours and even days after clotting occurs. Periodic determination of the residual prothrombin in serum gives a measure of the rate of conversion of prothrombin to thrombin and provides a delicate test of the course of clotting. This procedure (18), devised 10 years ago, is a sensitive indicator of the hemophilic state, and of changes resulting from the use of corrective agents. This technique was used to follow the rate of prothrombin conversion in blood samples obtained from bleeder dogs (Table V, tests 1 to 3). It will be observed that very little prothrombin was consumed in 4 hours. Thereafter the pro-

TABLE V

Delayed Prothrombin Utilization in Hemophilic Blood; Corrective Effect of Thromboplastin, Normal Plasma, and Fraction I

Test No.	Blood	Material added		Residual prothrombin content* (*per cent* of original plasma)					
		Type	Amount per 100 ml. whole blood	Time after collection of blood					
				15 min.	30 min.	60 min.	4 hrs.	24 hrs.	48 hrs.
			ml.						
1	Hemophilic	None	—	100	—	96	96	69	22
2	Hemophilic	None	—	100	—	100	96	61	27
3	Hemophilic	None	—	100	—	97	96	57	20
4	Normal	None	—	59	30	<10	<10	<10	<10
5	Normal	None	—	48	16	12	<10	<10	<10
6	Normal	None	—	46	30	<10	<10	<10	<10
7	Hemophilic	Thrombo-plastin‡	0.1	<10	<10	<10	<10	<10	<10
8	Hemophilic	Thrombo-plastin‡	0.01	76	59	<10	<10	<10	<10
9	Hemophilic	Thrombo-plastin‡	0.001	100	85	83	44	31	16
10	Hemophilic	Thrombo-plastin‡	0.00001	100	100	100	54	22	22
11	Hemophilic	Thrombo-plastin‡	0.000001	100	100	92	100	54	24
12	Hemophilic	Normal dog plasma	10.0	18	<10	<10	<10	<10	<10
13	Hemophilic	Normal dog plasma	1.0	22	<10	<10	<10	<10	<10
14	Hemophilic	Normal dog plasma	0.1	62	42	22	<10	<10	<10
15	Hemophilic	Normal dog plasma	0.01	74	62	25	18	<10	<10
16	Hemophilic	Normal dog plasma	0.001	91	91	62	—	20	19
17	Hemophilic	Normal dog plasma	0.0001	100	93	93	65	23	20
18	Hemophilic	Normal dog plasma	0.00001	100	100	91	68	23	23
19	Hemophilic	Fraction I (human)	10.0 (0.3 gm.)	78	43	11	<10	<10	<10
20	Hemophilic	Fraction I (bovine)	10.0 (0.3 gm.)	71	27	<10	<10	<10	<10

* At the intervals indicated, the samples were mixed with approximately one-seventh their volume of 1.85 per cent potassium oxalate, centrifugalized at 2800 *g* for 15 min., and the residual prothrombin determined by the two-stage test.

‡ Thromboplastic lung extract (11); other extracts, similarly prepared, contained 3.3 gm. per cent organic solids.

thrombin slowly disappeared. Serum obtained 48 hours after clotting still contained approximately the same amount of prothrombin as did normal serum obtained 30 minutes after clotting.

The delayed utilization of prothrombin in the blood of these animals can be corrected *in vitro* by the addition of small amounts of thromboplastin, normal dog plasma, or a protein fraction (fraction I) prepared from either bovine or human plasma (Table V, tests 7 to 20). Addition of a small amount of thromboplastic lung extract (equivalent to 0.01 ml. per 100 ml. hemophilic blood) accelerated prothrombin utilization to a nearly normal rate. From data given in the table, it can be calculated that 0.33 mg. of solids was contained in this amount of thromboplastin added to 100 ml. of whole hemophilic blood. This is close to the amount of thromboplastin previously found necessary for a normal clotting rate in human hemophilia (18). In these experiments, a noticeable effect was observed with as little as 0.001 ml. of thromboplastic extract per 100 ml. of whole blood.

Addition of normal plasma in amounts of 1 to 10 ml. per 100 ml. of whole hemophilic blood greatly accelerated the prothrombin consumption. Addition of only 0.01 ml. of normal plasma per 100 ml. of hemophilic blood had an appreciable effect, but with lesser amounts of plasma, changes from the control values were slight.

Both human and bovine fraction I were effective in accelerating prothrombin consumption. However, their corrective action appears to be considerably less than that of equivalent amounts of normal plasma. Part of this difference may be due to species specificity of the antihemophilic factor.

The Formed Elements and the Corrective Action of Normal Plasma.—As shown above, the clotting defect of whole blood was corrected by the addition of normal plasma. However, the corrective action of plasma was dependent upon the presence of platelets and perhaps other formed elements (Table VI). It will be noted in the table that whole hemophilic blood quickly clotted in the presence of normal blood or plasma, and that its prothrombin promptly disappeared (tests 1 to 3). When the number of platelets in the mixtures was reduced (tests 4 to 5), clotting was accelerated but the acceleration was considerably less than that observed in mixtures having a full complement of formed elements. In the absence of formed elements (test 6), the normal plasma failed to induce clotting in the course of the experiment. Yet this same plasma was highly effective when mixed with whole blood. These data would indicate that a factor in normal plasma interacts with the formed elements, particularly platelets, to initiate clotting, and that this factor is deficient in the bleeder dog plasma. Also, if an adequate number of platelets are not present, the full effectiveness of the antihemophilic principle does not manifest itself.

Effect of Transfusions on the Clotting Anomaly

Various blood and plasma preparations were transfused into the bleeder animals in a series of twenty-six experiments. At intervals after each transfusion, the clotting time and the rate of prothrombin utilization were determined.

TABLE VI

Effect of Addition of Normal Blood or Plasma on Clotting Time and Prothrombin Utilization of Hemophilic Blood or Plasma

Test No.	Hemophilic sample	Material added	Clotting time of recalcified mixture	Residual prothrombin content* (*per cent* of original plasma)		
				Time after recalcification		
				30 min.	60 min.	120 min.
			min.			
1	Whole blood	Whole blood	4½	<10	<10	<10
2	Whole blood	Platelet-rich plasma‡	5	<10	<10	<10
3	Whole blood	Platelet-poor plasma§	3½	<10	<10	<10
4	Platelet-poor plasma	Whole blood	8	39	32	<10
5	Platelet-poor plasma	Platelet-rich plasma‡	10	51	—	8
6	Platelet-poor plasma	Platelet-poor plasma§	>120	100	100	100
7	Platelet-poor plasma	Saline	>120	100	100	100

* All blood and plasma samples were obtained by the silicone technique using 3.2 per cent sodium citrate as the anticoagulant. Volumes were adjusted in each test so that the mixtures contained the equivalent of 10 parts native hemophilic plasma and 1 part native normal plasma or saline. For determination of clotting times and residual prothrombin content (18), the mixtures, in plain glassware, were recalcified with optimal amounts of 1.2 per cent $CaCl_2$.

‡ Contained approximately 150,000 platelets per c.mm.

§ Contained less than 300 platelets per c.mm.

By comparison with pretransfusion values, any improvement in the clotting process resulting from the transfusion was readily detected.

Transfusions with Normal Blood and Plasma.—In one group of ten experiments, freshly prepared citrated whole blood or plasma was used. Both of these preparations promptly corrected the clotting defect. The clotting time immediately after transfusion was reduced to the range 5½ to 9½ minutes, and the prothrombin disappeared from shed blood at a nearly normal rate. Fig. 1 shows the course of prothrombin conversion in one experiment, typical of the group. The corrective effect was independent of the amount of plasma or blood used in different experiments; from 2.3 to 4.7 ml. citrated plasma or its

equivalent of whole blood were given per kilo of body weight. The maximum effect was noted in the first blood sample collected a few minutes after transfusion. Even at that time, prothrombin utilization was somewhat less rapid than in normal blood. Thereafter the course of clotting was progressively retarded. Pretransfusion values were reached in 4 to 7 days.

Three experiments similar to those described above were done except that the normal blood or plasma was aged for 24 hours at 28°C. prior to transfusion. The results of one experiment are given in Fig. 2. The aged preparations appear to be less potent than those freshly prepared. There was less reduction

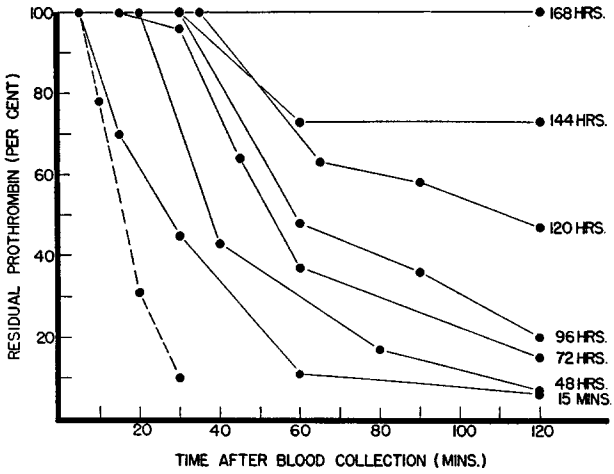

FIG. 1. A chart showing the effect of a fresh normal citrated plasma transfusion on prothrombin utilization in a hemophilic dog. The dose of plasma was 2.7 ml. per kilo of body weight. The solid lines indicate the prothrombin conversion rate at intervals after transfusion. The interval is indicated on each curve. The broken line indicates the conversion rate of whole blood of the donor animal.

in the clotting times following transfusion with these preparations. The shortest clotting times after transfusion ranged from 8 to 13 minutes. Similarly, there was less acceleration in the rate of prothrombin utilization. These results indicate that the corrective principle in normal plasma is only partially stable for 1 day at room temperature.

Transfusions with Hemophilic Blood and Plasma.—Transfusions with fresh hemophilic preparations were tested in two experiments. They had no effect on the clotting anomaly. After transfusion, the clotting times and the prothrombin utilization curves were unchanged from the pretransfusion values.

In human hemophilia, citrated whole blood, aged for 24 hours and then recalcified, has a normal clotting time and a normal prothrombin consumption rate (18). Hemophilic plasma largely freed of its formed elements, does not

exhibit this phenomenon. It has been postulated that transfusions with normally clotting aged whole hemophilic blood would correct the clotting defect, just as normal blood does. This hypothesis was tested in three experiments on our animals. Two of these experiments are described.

In one experiment, citrated whole blood, obtained from bleeder dog 8, was aged for 24 hours at 28°C. On recalcification, its clotting time was 3½ minutes. Sixty ml. of this blood (approximately 3.7 ml. per kilo) was transfused back to the donor. This autotransfusion caused no change in the clotting defect in the subsequent 48 hours. At the end of this time a transfusion of 60 ml. of normal blood caused a prompt improvement in clotting, similar to that seen in Fig. 1.

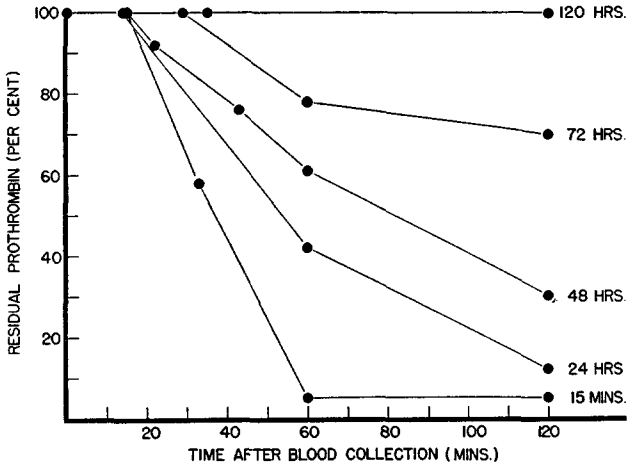

FIG. 2. A chart showing the effect of an aged normal citrated plasma transfusion on prothrombin utilization in a hemophilic dog. The dose of plasma was 2.8 ml. per kilo of body weight. The lines show the prothrombin conversion rate after transfusion, at intervals as indicated on the chart.

In another experiment, aged citrated whole blood, obtained from bleeder dog 6, had a clotting time of 2½ minutes. The blood (100 ml. or 5.6 ml. per kilo of body weight) was transfused into another bleeder animal, dog 2. This transfusion was ineffective.

Transfusions with Normal and Hemophilic Sera.—Normal and hemophilic sera were used for transfusions in eight experiments. Neither of these preparations was effective.

In one experiment, fresh serum was obtained from normal dog blood. Within 60 minutes after collection of the blood the serum was transfused into bleeder dog 1 (4.4 ml. per kilo of body weight). No change in clotting time or in the rate of prothrombin utilization occurred in the recipient. In a similar experiment, the normal serum was aged for 24 hours at 28°C. before transfusion. It likewise was ineffective. In a third experiment, hemophilic serum was obtained from whole hemophilic blood which had stood at room temperature for 24 hours. Sixty ml. of the serum was transfused back into dog 6 (2.4 ml. per kilo of body weight). No effect was observed.

In summary, only normal whole blood and plasma transfusions had a corrective effect on the delayed clotting in the bleeder dogs.

DISCUSSION

The foregoing observations on canine hemophilia indicate that it is very similar to human hemophilia. The sex-linked inheritance (2), the course of the malady, the type of clotting defect, and the beneficial effects of blood transfusions are very similar, if not identical, in the two diseases. The canine disease differs from the human disease mainly in the higher mortality early in life, and in the prolonged bleeding from small ear incisions. One can only speculate whether the higher mortality in untreated bleeder pups is due to a more severe clotting defect, or whether it is related to a higher incidence of serious accidents and infections in these dogs. Comparison of our data on prothrombin utilization, and on the amount of thromboplastin required to cause a normal clotting rate, with similar data in human hemophilia (18) does not reveal any difference in the severity of the clotting defect. Perhaps the severe bleeding from ear incisions is due to the character of the tissue at the site of puncture, since bleeding stopped promptly from mucous membrane incisions. Minimal thromboplastin liberation and inadequate capillary contraction in the dense cartilaginous tissues of the ear may be responsible. It has been observed that the bleeding time may be prolonged in human hemophilia, particularly if the puncture is more than 2 to 3 mm. in depth.

Further testing of the inheritance of the canine disease is now possible, since bleeder animals have been reared to maturity. The mating of bleeder males with females heterozygous for hemophilia will be of particular interest. This union should determine whether the homozygous female can actually occur, or whether the genic combination hh is lethal, as has often been suggested.

The nature of the clotting anomaly in hemophilia has been the subject of much study. The older views are discussed in a comprehensive monograph by Fonio (19). Recent studies point to an impaired mobilization of thromboplastin which prevents conversion of adequate amounts of prothrombin to thrombin. As a result, fibrin formation is slow and incomplete. Thromboplastic tissue extracts, added in small quantities to hemophilic blood, promptly increase prothrombin utilization and thrombin formation. Under these circumstances, the hemophilic blood clots promptly. Normal plasma, added to hemophilic blood, accomplishes within limits the same result. However, the plasma does not furnish preformed thromboplastin. It is seen in Table VI that its corrective action is dependent upon the presence of platelets. Thus it appears that normally thromboplastin becomes available in shed blood as a result of the interaction of platelets and the corrective antihemophilic principle in plasma. The exact nature of the platelet-plasma reaction is in doubt. One of us has suggested that the plasma factor is a thrombocytolysin, and that in

the presence of a wettable surface and Ca^{++}, it lyses platelets, thus liberating thromboplastin (5). Alternate explanations for this reaction have been given by Macfarlane (20) and by Quick (21). Regardless of the nature of the reaction, the data in Table VI would indicate that the mechanism by which adequate thromboplastin is mobilized may fail, either because of a deficiency of the antihemophilic factor, or from lack of a sufficient number of platelets.

The experiments with aged hemophilic blood are pertinent to the discussion of the clotting anomaly. On aging, the blood develops normal clotting properties, due apparently to release of thromboplastin from platelets and perhaps other formed elements. Although the clotting time of the hemophilic blood is the same as fresh normal blood, similarly treated except for the aging, the blood is not effective *in vivo*. These results would indicate that thromboplastin as such is not responsible for the effectiveness of normal transfusions. Rather they suggest the need of thromboplastin mobilization at the time the blood is shed. Also, these experiments indicate that there is available in hemophilic blood a source of thromboplastin, but that it is very slowly mobilized. These findings lend no support to the hypothesis recently proposed by Quick (21) that in hemophilic blood almost no thromboplastin is available from either the plasma or the platelets.

The failure of normal serum transfusions to correct the clotting defect of the hemophilic blood appears to be significant. It would indicate that during the clotting of normal blood the principle responsible for the corrective action in hemophilia disappears. This suggests that normally the plasma principle plays a fundamental rôle in the initiation of the clotting process, and that it is consumed in the process of clotting.

SUMMARY

A study was made of the clotting defect and the course of the malady in a group of male dogs with an inherited, sex-linked bleeding disease. The clotting defect is characterized by a prolonged clotting time and a delayed prothrombin utilization, and is corrected by the addition either of thromboplastin or of normal plasma. A plasma protein fraction, fraction I, also corrects the defect. The defect appears to be due to a deficiency of a plasma factor, which normally, in the presence of platelets, makes thromboplastin available in shed blood. The clotting anomaly appears to be identical with that found in human hemophilia.

The hemostatic defect is characterized by repeated hemorrhages, usually without obvious relationship to trauma. Hemarthroses occur frequently and may result in permanent joint deformity. The animals usually die early in life from massive hemorrhage.

Transfusions with normal blood or plasma correct the clotting defect and readily control the hemorrhagic phenomena. By the use of transfusions, these dogs have been reared to maturity.

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