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A HEMOPHILIAC DOG COLONY: GENETIC STUDIES AND COAGULATION FINDINGS IN HEMOPHILIAC AND NORMAL DOGS

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Abstract—1. A colony of hemophiliac dogs was developed as descendants of a single affected Pomeranian. Over a 13 year period, a total of 29 hemophiliac dogs survived to the age of 6 months or longer. Eleven were female and 18 were male. All five of the possible matings in a sex-linked recessive inheritance pattern were accomplished and the offspring fell into the expected sex and disease groups.

2. Twenty-one dogs died from untreated hemorrhages which usually occurred in loose tissues or body cavities during the night. Clinically, there were no hemarthroses but 22 other hemorrhages responded promptly to treatment with dog cryoprecipitate.

3. Coagulation studies showed that the hemophiliac dogs averaged about 0.23 U/ml equivalents of human F VIII:C while normal dogs averaged 8.0 U/ml equivalents of human. F's V, VII, II, X, IX, XI and XII were higher than human in both normal and hemophiliac dogs. Dog fibrinogen fell within the human range and Fletcher F was very low.

INTRODUCTION

Almost half a century ago, Taskin (1935) described a dog with a bleeding disease which appeared similar to human hemophilia. Since that time there have been occasional reports of hereditary bleeding disease in dogs (McKenna, 1936; Merkens, 1938; Field *et al.*, 1946; Brock *et al.*, 1963), cats (Cotter *et al.*, 1978), horses (Nossel *et al.*, 1962; Hutchins *et al.*, 1967; Archer & Allen, 1972), and swine (Muhrer *et al.*, 1965). The first hemophiliac dog colony was established by Brinkhous and his associates in North Carolina using dogs described by Field *et al.* (1946). The North Carolina group has described their dogs in many publications (Brinkhous *et al.*, 1973; Parks *et al.*, 1964; Graham *et al.*, 1949; Swanton, 1957). Such dog colonies offer almost exact models of human disease and can serve as important research tools.

This paper describes such a dog colony including the inheritance pattern, the causes of hemorrhage and compares the coagulation factor levels to those of normal dogs (*Canis familiaris*).

MATERIALS AND METHODS

Coagulation methods

These have been described in previous publications (Lewis, 1976; Lewis *et al.*, 1978; Benson & Dodds, 1976). Antibody to canine factor VIII was made following the method of Benson & Dodds (1976). F VIIIR:Ag was measured using the Laurell rocket technique.

The dog colony

With meager funds a hemophiliac dog colony was gradually built following the gift of a male Pomeranian hemophiliac by Dr Paul Didisheim (Didisheim & Bunting, 1964). In order to upgrade the dog size, this Pomeranian was mated with three beagle bitches. Two of the female offspring, 1050 and 1056, were retained for breeding purposes. These two bitches were, with the aid of grant funds, moved into the University of Pittsburgh Central Animal Facilities and mated first with large normal male beagles. Figure 1 illustrates the seven matings for 1050, the first two with normal beagles, the last five with hemophiliacs. Figure 2 illustrates the results of four matings of 1056. One was with a normal male from which one hemophiliac male originated, the next three were with hemophiliacs. All puppies were tested as soon as possible and the normals or carriers given away because funds were not available to feed them. When the colony was disbanded, the seven living hemophiliacs were also given away.

Von Willebrand deficient dog

Blood samples were obtained from a champion Doberman pinscher, who had been diagnosed as suffering from von Willebrand's disease by W. Jean Dodds, D.V.M., Division of Laboratories and Research, New York State Department of Health, Albany, NY. This dog had no bleeding tendency.

RESULTS

Genetics

The five possible matings in the sex-linked recessive pattern of hemophilia are shown in Fig. 3. Each of these is illustrated in the dog colony. The three matings of the original dog are Type I—between a normal bitch and a hemophiliac male. Type II matings were utilized in the first two for 1050 and one for 1056. Type III breeding occurred eight times resulting in a total of ten hemophiliac females, ten presumably carrier females, seven normal males and ten hemophiliac males. A Type IV mating occurred once (see Fig. 2, 443 and 1099) and, as predicted, all offspring were hemophiliacs. The Type V mating was inadvertent and occurred between 449 and a male sibling. The mother, 449, bled to death and two male offspring

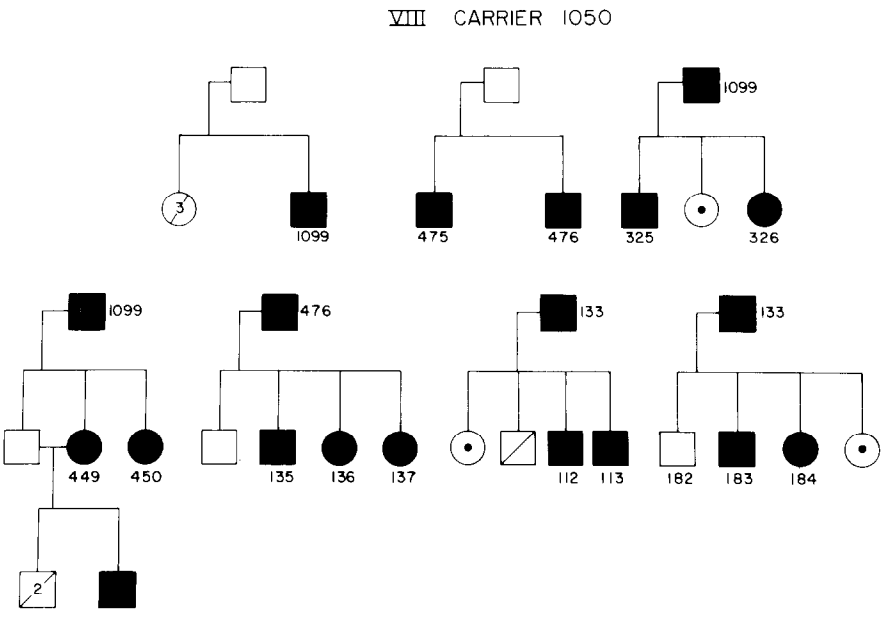

Fig. 1. Matings of carrier 1050.

died during delivery, but the remaining male, a hemophiliac, prospered as a pet. Carrier studies were not done because we could not afford to raise the female puppies.

Hemorrhages and deaths

The dogs were frisky and healthy without visible deformities of the joints. Each dog was observed in the morning and late afternoon and treated with canine plasma cryoprecipitate if indicated. In the 29 hemophiliac dogs who lived at least six months, there were no evidences of hemarthroses. Only 22 non-fatal, treatable hemorrhages were seen in six years. These included subcutaneous and scalp hematomata, epistaxis, hematuria, melena and one episode of postpartum bleeding. Response was prompt following infusion of one to six units of canine cryoprecipitate. Each unit was prepared from 200 ml of plasma obtained by plasmapheresis from a donor dog who was pheresed at regular intervals.

There were 21 deaths due to hemorrhage (see Table 1) in these hemophiliac animals. Most of these dogs were observed and thought to be well on an afternoon check, then found to be dead the following morning.

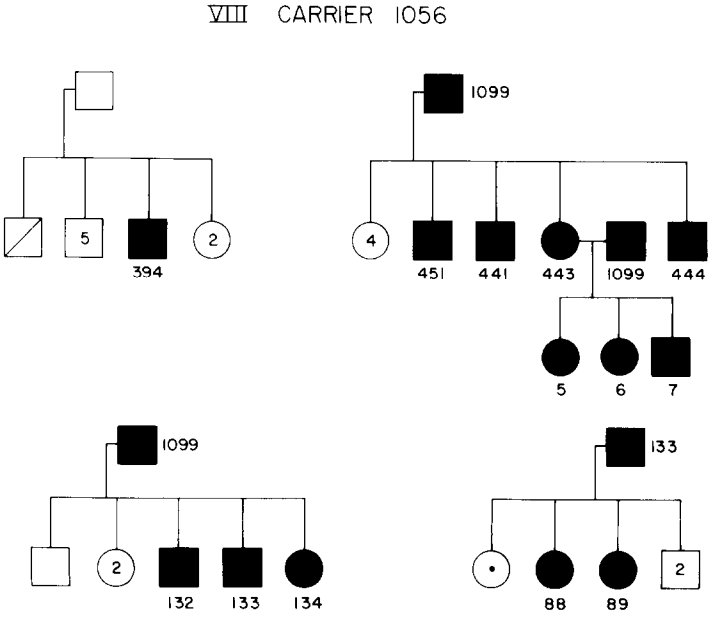

Fig. 2. Matings of carrier 1056.

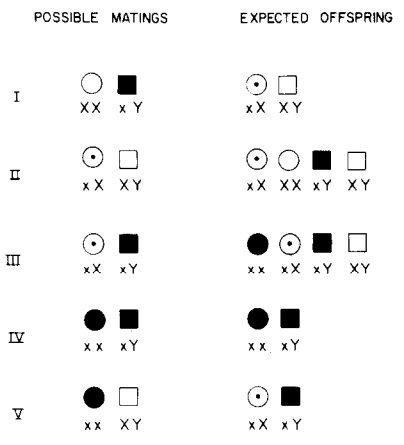

Fig. 3. Possible matings and offspring in a sex-linked disorder.

Autopsy examinations were made on all deceased dogs. Characteristically the hemorrhages were in loose tissues or cavities where a great deal of blood could be lost before back pressure would slow the flow. The most common site was intra-abdominal. The two post-coital hemorrhages pointed the way for later pre-coitus treatment with cryoprecipitate. One other dog died in anaphylactic shock following treatment with human factor VIII.

Coagulation findings (*Table 2*)

Both hemophiliac and normal dogs were tested in systems used for testing human plasma and results expressed in human units (IU = amount of clotting factor activity in 1 ml of pooled human plasma). The normal dogs showed rapid APTT and PT's and very high levels of F's V, VII and VIII. F's II, X, IX and XI were also well above human levels. Fibrinogen and F XII fell within the human range. Fletcher F was very low compared to the level in humans. Hemophilic dog blood showed long clotting times and APTT's. The level of F VIII:C was very low, varying between 0.2 and 0.27 (mean 0.23 U/ml) when compared with normal dog at 8.0 U/ml. F VIII:C level of the von Willebrand dog was 4.0 U/ml (50% normal dog).

No F VIIIR:Ag could be detected in the plasma from the dog with von Willebrand's. The size of the rocket (level of antigen) formed by hemophilic plasma was greater than that of the normal dog. The third component of the F VIII complex, F VIIIR:vW, is measured in human plasma by the rate of aggregation of fixed human platelets by ristocetin in the presence of plasma dilutions. Dog plasma appeared to have some inhibitory effects in this system. As the plasma was diluted, the platelet aggregation became faster. If we took only the fastest time in calculation of F VIIIR:vW, normal and hemophiliac dogs were similar with means of 0.57 and 0.49 U/ml respectively. The dog with asymptomatic von Willebrand's disease assayed at 0.2 U/ml (human equivalent).

Table 1. Fatal hemorrhages in 21 hemophiliac dogs

Dog Number	Sex	Age	Site of Hemorrhage
1	M	7 Y	Intra-abdominal
5	F	11 M	Subcutaneous, Post-coital
88	F	11 M	Lung
89	F	3 Y	Sublingual
113	M	2 Y	Intracranial
134	F	6 M	Subdural
136	F	7 M	Subarachnoid
137	F	5 Y	Intra-abdominal, Post-coital
183	M	2 Y	Throat (asphyxiation)
184	F	9 M	Lung
325	M	1 ½ Y	Intra-abdominal
326	F	1 ½ Y	Subcutaneous
394	M	9 M	Bilat. thorax + mediastinum
440	M	2 ½ Y	Thorax + mediastinum
441	M	3 Y	Myocardium + mediastinum
443	F	4 Y	Intra-abdominal
444	M	4 M	Subdural
449	F	1 Y	Uterine, Post-whelping
450	F	7 Y	Retroperitoneal
475	M	6 M	Scalp
1051	M	1 Y	Intra-abdominal

Table 2. Clotting tests and factor assays in hemophiliac and normal dogs

Test		Hemophilic No.	Hemophilic Mean	Hemophilic ± S.D.	Normal No.	Normal Mean	Normal ± S.D.
Clotting time—glass - min.		8	18.1	7.7	7	5.4	0.8
—silicone - min.		8	> 240.0	> 240.0	7	27.1	13.2
Recalcification time—glass - sec.		8	370	120	7	96.7	18.7
Activated partial thromboplastin time (APTT) - sec.		8	30-50	-	15	15-21	-
Prothrombin time (PT) - sec.		8	7.8	0.6	15	7.5	0.8
I (mg/dl)		8	254	60	15	236	60
II	U/ml	8	2.86	0.9	12	2.09	0.47
V	U/ml	8	6.7	1.6	15	6.2	1.4
VII	U/ml	8	5.1	1.1	15	4.7	2.7
X	U/ml	8	2.2	0.9	12	3.5	0.9
VIII:C	U/ml	8	0.23	.02	15	8.0	1.5
IX	U/ml	8	2.3	0.5	12	2.6	0.9
XI	U/ml	8	2.1	0.4	15	2.7	0.4
XII	U/ml	8	1.1	0.2	15	1.6	0.4
F.F.	U/ml	8	-	-	12	0.16	0.03

A human pooled plasma standard was assigned a value of 1.0 U/ml.
APTT has varied with reagent and instrument.

DISCUSSION

Clinically, the most noteworthy finding is the lack of hemarthroses in these dogs. Perhaps this only reflects the low weight–joint ratio and the lack of use of the joints. The dogs were confined in large cages for about 23 1/2 hr per day. Even when they were taken out of the cages, they were not allowed to run and play for fear that a hemorrhage might occur. Over two-thirds of the dogs died of hemorrhage. Almost all of the hemorrhages occurred into cavities or loose tissues, thus large amounts of blood could be lost in relatively short time periods.

Coagulation studies on both normal and hemophiliac dogs showed high levels, as compared to human, of F's II, V, VII, IX, X and XI. Fibrinogen and F XII values fell within the human range. Fletcher factor was very low. F VIII:C values in the normal dogs were about eight times those of humans. In the hemophiliac dogs F VIII:C was, of course, very low.

The one hemophiliac dog tested for F VIIIR:Ag showed a high normal level while the von Willebrand deficient dog's plasma showed a lack of any rocket formation with our anti-dog-F VIII. The F VIIIR:vW level appeared low in this dog but must be considered questionable in view of the very variable results in normal and hemophiliac dogs. This variability appeared to be due to an inhibitor in both normal and hemophiliac dog plasmas.

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