

Further Studies of Canine von Willebrand's Disease

By W. Jean Dodds

Additional characterization of von Willebrand's disease (VWD) in a family of German shepherd dogs is presented. Genetic studies of three generations of affected dogs indicate that about 50% of the progeny are affected if one parent has VWD and about 60% if both parents have the defect. Some of these progeny manifested an incomplete form of VWD, suggesting autosomal dominant inheritance with variable expressivity. The disease became progressively less severe with advancing age and repeated pregnancies. Ristocetin-induced platelet aggregation was significantly reduced in VWD dogs as compared with normal, thrombopathic, and hemophilic carrier dogs. Immuno-

diffusion and electroimmunodiffusion studies with rabbit anticanine factor VIII showed the level of factor VIII-related antigen to be low in VWD dogs but present in increased amounts in hemophilic dogs. VWD affected dogs had markedly delayed hemostatic plug formation, but their plugs appeared normal by light and electron microscopy. Their platelet nucleotides, ATP/ADP ratio, and platelet protein content were normal. Platelet and fibrinogen survival times with [⁷⁵Se] selenomethionine were also normal, although platelets from VWD dogs incorporated more radioactivity than did those from normal dogs or from dogs with incomplete VWD.

IN 1970 WE DESCRIBED a family of German shepherd dogs with a hereditary bleeding disorder analogous to human von Willebrand's disease (VWD).^{1,2} The defect was characterized by prolonged bleeding time, reduced factor VIII activity, abnormal platelet adhesiveness (retention), short prothrombin consumption time, overresponse of factor VIII levels after transfusion with normal or hemophilic plasma, autosomal inheritance with variable penetrance, and a mild to severe bleeding diathesis. The present report describes additional studies of the original family members and three successive generations of affected dogs.

FAMILY HISTORY

Our original family of VWD dogs consisted of seven mildly affected animals, five females and two males.¹ The propositus was severely affected, sterile, and died of an acute abdominal accident at 4 yr of age. Although the mildly affected dogs (28%–55% factor VIII) had not experienced bleeding episodes at the time of the original report, some of them subsequently developed large hematomas from minor trauma and required transfusion therapy.^{3,4} The most frequently encountered hemorrhagic complications have been hematoma formation, intermittent lameness from periarticular bleeding, serosanguinous otitis externa, melena or protracted bloody diarrhea, prolonged estrual bleeding, and bloody postpartum vaginal discharges. The clinical expression of the disease appears to become progressively less severe with advancing age and repeated pregnancies.

Other moderately affected or normal adult dogs, closely related to the original family, have

From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. 12201.

Submitted July 29, 1974; accepted August 27, 1974.

Supported in part by USPHS Grant HL-09902 from the National Heart and Lung Institute, Bethesda, Md.

Address for reprint requests: Dr. W. Jean Dodds, Division of Laboratories and Research, New York State Department of Health, New Scotland Avenue, Albany, N. Y. 12201.

© 1975 by Grune & Stratton, Inc.

Table 1. Results of Genetic Studies in Canine VWD

Mating Types Dam Sire	No. of Matings	Progeny (Live Births)										
		Males						Females				
		N*	Mild or Moderate†	In-complete‡	Severe§	Total	N*	Mild or Moderate†	In-complete‡	Severe§	Total	Total
N N	7	15	0	0	0	15	23	0	0	0	23	38
VWD VWD	6	10	6	2	1	19	4	8	4	0	16	35
VWD N	6	2	9	1	0	12	11	7	1	0	19	31
N VWD	3	4	3	0	0	7	5	4	0	0	9	16
Total	22					53					67	120

*N, normal hemostatic tests (factor VIII activity, platelet retention, bleeding time).

†Mild or moderate, factor VIII 20%–60%, platelet retention < 50%, bleeding time > 12 min.

‡Incomplete (see text).

§Severe, factor VIII < 20%, platelet retention < 10%, bleeding time > 30 min.

recently been studied or obtained for breeding purposes. Three additional generations of affected dogs have been raised from double-heterozygous and heterozygous-normal matings of the foundation and newly acquired stock. Three types of live progeny resulted from these matings: normal, mildly or moderately affected, and incompletely affected (Table 1). The one severely affected puppy bled to death at 10 days of age. The stillbirths were: two females from the normal-normal matings, ten males and seven females from the VWD-VWD matings, and one male and four females from the normal-VWD matings. The causes of death were unknown; no lesions were seen at autopsy.

The incomplete form of canine VWD is manifested in this family in two ways: by reduced factor VIII activity alone, or by low platelet retention with normal factor VIII levels and either a normal or prolonged bleeding time. All mildly or moderately affected dogs have a consistently prolonged bleeding time (> 10 min) regardless of their factor VIII level.

Several moderately affected dogs from this highly inbred family have recently developed rather bizarre secondary diseases. One bitch was born with VWD and a disease analogous to congenital lymphoedema (Milroy's disease) of man, cattle, and poodle dogs⁵; a 4-yr-old male with a history of chronic hypertrophic colitis and melena died of generalized miliary lymphomatosis; a 2-yr-old bitch was euthanized for acute renal failure secondary to Coombs' positive immune glomerulonephritis⁶; her brother died suddenly of intestinal volvulus; and several parasitefree affected dogs have demonstrated persistent eosinophilia, intermittent shifting lameness, and radiographic features indistinguishable from canine eosinophilic panosteitis.⁷ The case reports of these animals will be presented elsewhere.

MATERIALS AND METHODS

Coagulation assays were performed on citrated plasma samples (1 part 3.8% trisodium citrate to 9 parts whole blood). Factor VIII was assayed by using canine factor VIII-deficient (hemophilic) plasma in a partial thromboplastin assay¹; a pool of normal dog plasma from 20 random young adult dogs, ten of each sex, was assigned a value of 100% factor VIII. Bleeding times were determined on the subcutaneous tissue of the inner thigh with direct microscopic observation.⁸ Hemostatic plug formation was studied by photomicrography, histology, and electron micrography.⁸ The plugs were fixed in glutaraldehyde and postfixed in osmic acid for electron microscopy.

Platelet adhesiveness (retention) was measured by the Salzman and Bowie techniques,^{9,10} modified as follows to facilitate reproducibility with canine blood: 6 ml of whole blood was drawn rapidly by clean venipuncture into a plastic syringe. A 1-ml aliquot was immediately dispensed to and mixed in a plastic tube containing 0.1 ml of 3.8% trisodium citrate. The remaining whole blood was passed by a syringe pump at 7 ml/min over a standard 1.0-g glass-bead column and collected in successive 1-ml aliquots in a series of plastic tubes, each containing 0.1 ml of the citrate anticoagulant. The platelet counts of the last three aliquots of blood from the column were averaged and subtracted from that of the precolumn sample to obtain the percentage platelet retention of the sample. The use of heparin anticoagulant for this assay was abandoned because canine platelets spontaneously aggregated in the presence of varying amounts of heparin (4–20 NIH U/ml), thereby preventing accurate platelet counts.

Ristocetin-induced platelet aggregation was measured in suspensions of washed rabbit platelets prepared by the albumin density-gradient technique of Walsh.¹¹ The assay technique of Weiss et al.¹² was modified for canine plasma as follows: Rabbit platelets were resuspended after the last albumin wash in modified Tyrode's solution (without divalent cations) and mixed with equal volumes of serially diluted normal and test canine platelet-poor plasmas. Ristocetin (Abbott Laboratories, Chicago, Ill.) was added to a final concentration of 2 mg/ml. The length of the lag phase and the degree of platelet aggregation were proportional to the final concentration of dog plasma in the system. Ristocetin-induced aggregation was compared in normal, VWD, and thrombopathic dogs^{2,13} and in carriers of canine hemophilias A and B.^{3,8}

This modified assay has the following advantages: a reproducible platelet suspension can readily be obtained from a strain of randomly bred rabbits; ristocetin-induced aggregation can be measured with plasma or plasma fractions from many species, including man; and lower concentrations of ristocetin will elicit aggregation with animal plasmas. By contrast, ristocetin aggregation of animal platelet-rich plasma requires concentrations of ristocetin (3–5 mg/ml) which also cause precipitation of fibrinogen and other plasma proteins.

Platelet protein content was determined on washed platelet suspensions by Miller's adaptation¹⁴ of the techniques of Lowry et al.¹⁵ Platelet nucleotides were measured on ethanol extracts by the method of Holmsen et al.¹⁶

Platelet and fibrinogen survivals were measured simultaneously with [⁷⁵Se] selenomethionine according to the method of Brodsky et al.¹⁷ Normal and VWD dogs were given 4 μ Ci of ⁷⁵Se per gram of body weight. The platelet and fibrinogen survival times were each determined as the interval (in days) between the 50% points of peak radioactivity on the ascending and descending slopes of the curve.¹⁷

Agarose gel immunodiffusion and electroimmunodiffusion were performed with rabbit anti-canine factor VIII kindly provided by Dr. B. N. Bouma (Utrecht, Netherlands).¹⁸ The antiserum was diffused against pooled normal, hemophilic, and VWD dog plasmas. Immunodiffusion utilized a template system with 2.5-mm wells, and electroimmunodiffusion was performed by the Laurell technique as described by Zimmerman et al.¹⁹ Additional studies with these techniques will be reported elsewhere.

RESULTS

The primary bleeding time was prolonged in canine VWD (Table 2), and there was frequent rebleeding through the hemostatic plug, resulting in a prolonged secondary bleeding time. By contrast, the normal dogs stopped bleeding

Table 2. Bleeding Times by Direct Microscopic Observation in Normal Dogs and Dogs With Mild and Moderate VWD

Normal Dogs			VWD Dogs		
Dog	Sex	Bleeding Time*	Dog	Sex	Bleeding Time*
Donor	M	3'57''	Jorja†	F	14'30''
Igor	M	5'35''	Prince	M	22'10''
L-29†	F	2'37''	Wilhelm‡	M	17'13''
Lady¶	F	2'56''	Corey†‡	F	5'49''
Drew	M	2'00''	Sugar§	F	8'30''
Atlas	M	2'34''	Ulyssa¶	F	5'35''
			Phillip‡	M	13'00''
			Pretty Boy (G-3)	M	14'45''
			G-2†	F	13'30''

*Average from four to six vessels ranging from 50–150 μ in diameter.

†Virgin bitch.

‡Have incomplete form of VWD.

§Bitch after one pregnancy.

¶Bitch after three pregnancies.

Table 3. Ristocetin-induced Platelet Aggregation in Normal, VWD, and Thrombopathic Dogs and in Carriers of Canine Hemophilias A and B

Dog	No.	Final Plasma Dilution *			
		1:8	1:16	1:32	1:64
Lag Time (sec)					
Normal	10	89 ± 15	132 ± 32	218 ± 32	312 ± 65
VWD	14	124 ± 30†	184 ± 71‡	307 ± 69†	455 ± 80†
VWD§	3	87 ± 11	119 ± 16	218 ± 23	279 ± 38
Hem. A	4	91 ± 23	146 ± 18	191 ± 31	275 ± 34
Hem. B	4	89 ± 12	128 ± 31	197 ± 31	277 ± 39
Thromb.	4	82 ± 32	103 ± 20	182 ± 80	274 ± 116
Degree of Aggregation (%)					
Normal	10	100	75	25	25
VWD	14	65	50	25	25
VWD§	3	75	25	25	25
Hem. A	4	67	38	25	25
Hem. B	4	88	33	25	25
Thromb.	4	100	67	25	25

VWD dogs had 25%–60% factor VIII; proven hemophilic carriers had 42%–65% factors VIII or IX.

*Ristocetin, 2 mg/ml final concentration.

† $p < 0.01$.

‡ $p < 0.05$.

§VWD bitches after two or more pregnancies.

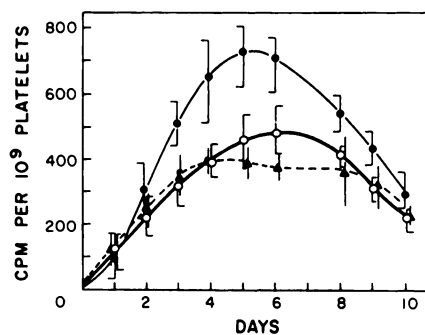
within 2–5.6 min with little or no bleeding. The small skin incision healed readily in the normal dogs by first intention, whereas in several cases the incisions in VWD dogs became badly bruised or subject to hematoma formation, and healing was delayed. Although the formation of hemostatic plugs was markedly delayed in VWD dogs, histologic and electron micrographic sections of their plugs revealed no abnormalities. In some cases, the platelets in the plugs of VWD dogs contained large amounts of glycogen.

Ristocetin-induced platelet aggregation time was normal in thrombopathic dogs, carriers of canine hemophilias A and B, and three VWD bitches that had experienced two or more pregnancies (Table 3). By contrast, VWD dogs had defective ristocetin aggregation. Although the carriers of canine hemophilia A had factor VIII levels similar to those of dogs with moderately severe VWD, plasma from the VWD dogs was significantly slower to react in the ristocetin assay.

Table 4. Platelet Nucleotide and Platelet Protein Content of Random Normal Dogs, Breed-specific Normal Dogs, and Dogs With VWD

Platelet Nucleotides ($\mu\text{moles}/10^{11}$ platelets)	Normal Dogs		VWD Dogs
	Random	Breed-specific	
ATP	(n = 10) 5.3 ± 1.1	(n = 6) 5.2 ± 0.8	(n = 21) 4.4 ± 1.3
ADP	1.9 ± 0.5	1.9 ± 0.6	1.6 ± 0.6
ATP/ADP ratio	2.9 ± 0.5	2.8 ± 0.6	3.1 ± 1.1
Platelet protein (mg/ 10^9 platelets)	2.2 ± 0.6 (n = 8)	—	1.9 ± 0.5 (n = 13)

Fig. 1. Platelet survival with [^{75}Se] selenomethionine in six normal dogs (O—O), four dogs moderately affected with von Willebrand's disease (●—●), and two dogs with incomplete von Willebrand's disease (▲—▲). The data are shown as the mean \pm SD survival curve for each group.



The platelet nucleotide and platelet protein content of our inbred German shepherds with VWD are compared with those of random normal dogs and normal related German shepherds in Table 4. The breed-specific control group was included to examine the possibility that a random population of normal dogs might have a different norm than that of an inbred line of purebred dogs. There were, however, no significant differences between the groups studied. The data indicate that, because of their lower ADP content, canine platelets have a higher ATP/ADP ratio than that reported in man.¹⁶ These findings agree with those for other animal species.²⁰

The platelet survival studies with [^{75}Se] selenomethionine in normal and VWD dogs are shown in Fig. 1 and Table 5. Although platelet survival time and protein content were similar in all three groups, there was significantly greater incorporation of radioactivity into the platelets of moderate VWD dogs ($p < 0.01$). This difference may reflect a more rapid turnover of platelet proteins in dogs with VWD. The survival times obtained for ^{75}Se -labeled normal dog platelets are consistent with those reported previously²¹ and with those in studies utilizing other radioactive labels such as chromium-51.²² Plasma fibrinogen survival time was also determined during this study. The means for the six normal and six VWD dogs were 5.5 ± 0.9 and 6.3 ± 0.8 days, respectively. These results agree with those obtained for normal dogs with other labels such as sulfur-35²³ and iodine-131.²⁴

When immunodiffused against rabbit anticanine factor VIII, plasma from dogs with moderate VWD produced a very faint precipitin line by 48–72 hr, whereas plasmas from normal and hemophilic dogs consistently developed a moderately heavy precipitin line within 36 hr. Electroimmunodiffusion studies using the Laurell technique showed that hemophilic plasmas from four different

Table 5. Platelet Survival of Normal and VWD Dogs

Dog	Survival Time, Mean (days)	Peak Radioactivity (cpm/ 10^9 platelets)*	Protein Content, Mean (mg/ 10^9 platelets)
Normal (n = 6)	7.4 ± 1.0	475 ± 40	2.6 ± 0.7
VWD, moderate (n = 4)	7.3 ± 0.7	710 ± 90	2.2 ± 0.8
VWD, incomplete (n = 2)	8.4 ± 0.5	378 ± 30	1.9 ± 0.1

*Peak occurred on days 5–7 for all groups.

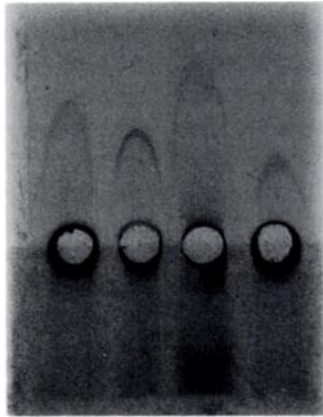


Fig. 2. A representative electroimmunodiffusion with rabbit anticanine factor VIII (0.5% of 1:10 diluted antiserum in 0.9% agarose). The antigen wells, reading from left to right, contained: undiluted pooled normal, 1:2 diluted pooled normal, hemophilic, and VWD canine plasmas. The hemophilic and VWD plasmas contained <1% and 33% factor VIII activity, respectively. Samples were electrophoresed for 24 hr at 4°C. The heights of the precipitin peaks measured from the centers of the wells were 11.0, 8.5, 13.0, and 6.5 mm, respectively.

canine families had increased amounts of factor VIII-related antigen as compared to pooled normal dog plasma, while 14 VWD plasmas had $41.5\% \pm 22.4\%$ factor VIII activity and antigen levels of $48.1\% \pm 6.5\%$ of the same normal pool (Fig. 2). These findings with respect to factor VIII-related antigen parallel those observed for normal humans and for humans with hemophilia and von Willebrand's disease.^{18,19,25,26}

DISCUSSION

The additional genetic information available from the three new generations of affected dogs (Table 1) does not clarify the problem with regard to heterozygosity and homozygosity in VWD.²⁷ As originally reported,¹ the disease appears to be expressed in the heterozygous state because mildly and moderately affected dogs demonstrate the abnormalities of VWD in laboratory tests and also manifest a bleeding tendency.

Of the 35 live puppies born to affected parents, only one was severely affected; it bled to death at 10 days of age. By contrast, none of the 47 live progeny of one affected parent was severely affected. However, there were 17 stillbirths from the six double-heterozygous matings and five from the nine heterozygous-normal matings, whereas only two puppies were born dead in the seven litters from normal parents. Some of the dead progeny of the VWD dogs may have manifested a homozygous, lethal form of the disease.

Most of the surviving affected offspring manifested mild to moderately severe VWD, and the remainder had an incomplete form of the disease. Several of the moderately affected dogs experienced clinical bleeding episodes, some of which appear to have been spontaneous.

If one considers only the live progeny of VWD dogs (Table 1), about 60% of the offspring of double-heterozygous parents, and 50% of those born to heterozygous-normal parents had VWD or incomplete VWD. However, assuming that the stillborn puppies were affected, the incidence increases to 73% for the double-heterozygous and 57% for the heterozygous-normal matings. These data from a relatively small population approach the expected 3:1 and 1:1 ratios for the double-heterozygous and heterozygous-normal crosses of an autosomal dominant trait. We cannot conclude, however, that inheritance of canine VWD

is the function of a single gene locus. The phenotypic heterogeneity expressed in the affected progeny is more compatible with a polygenic inheritance, as has been suggested for humans and swine with VWD.²⁷⁻³¹

The incomplete expression of human VWD has been recognized for many years.²⁷⁻³⁰ More recent studies in man,³² swine,³¹ and dogs¹⁻³ indicate that the so-called recessive or carrier state of VWD can be determined fairly accurately by utilizing a combination of laboratory tests for hemostasis. Veltkamp and Van Tilburg³² detected three heterozygous carriers of VWD among the asymptomatic relatives of a severely affected patient by statistically comparing their levels of factor VIII activity and factor VIII-related antigen. On the basis of their findings, these investigators suggested that factor VIII-related antigen is more directly related to the primary gene product of the von Willebrand locus than is factor VIII activity. The studies of Owen et al.,³¹ using a combined hemostatic score obtained from the factor VIII level, platelet retention, and bleeding time of VWD pigs, showed that affected swine did not overlap the range for carriers but that about 40% of carrier pigs fell within the normal range. Our studies of canine VWD suggest that the disease can be manifested in three ways: severely affected (homozygotes, usually lethal), moderately to mildly affected (heterozygotes), and incompletely affected (heterozygotes). The latter two expressions of VWD may reflect the extremes of heterozygosity or variable penetrance of the abnormal gene.²⁷

Von Willebrand's disease becomes progressively less severe with advancing age and during pregnancy.^{28,30,33} In our experience, the factor VIII level of puppies 2-4 mo of age is about 80% of that in adult dogs. For this reason, the most accurate means for detecting the affected or partially affected dogs in a VWD litter is to use age-matched and/or littermate controls. The status of several affected dogs of both sexes over 6 yr of age has improved to the point where their hemostatic tests, except bleeding time, overlap the normal range. Affected bitches demonstrate a rapid increase in factor VIII activity and platelet retention during gestation. These parameters revert toward their base-line level after parturition but, in contrast to man,³³ never return to the baseline. After repeated pregnancies, several older VWD bitches have hemostatic tests that overlap the normal range (Tables 2 and 3). In these instances, we cannot separate the effects of age from those of multiple pregnancies. Perhaps the changes relative to age and pregnancy are more apparent in canine VWD because of the shortened gestation period and lifespan of the dog as compared with man.

The bleeding time abnormality in affected dogs (Table 2) parallels that observed in man and swine with VWD.^{4,30,31,34} Primary and secondary bleeding times were both prolonged, and there was frequent rebleeding through the initial hemostatic plug. The frequency and duration of secondary bleeding were similar to those reported previously for canine hemophilias A and B, but in these diseases the primary bleeding time is normal.⁸ In contrast to the paucity of fibrin in the hemostatic plugs of hemophilic dogs,⁸ the plugs obtained from VWD dogs appeared normal by electron microscopy. Kahn et al.³⁵ reported quantitative and qualitative differences in the platelets of bleeder swine during viscous metamorphosis when compared with platelets from normal swine.

However, in our present studies and those of White,³⁶ human and canine VWD platelets appear to have normal ultrastructural features.

Dogs with VWD had abnormally delayed ristocetin-induced platelet aggregation (Table 3), as has been reported for man.^{12,37,38} This defect was evident despite the relatively high factor VIII procoagulant activity of affected dogs. Weiss et al.¹² have similarly shown that the ability of plasma to support ristocetin aggregation is a function of its level of von Willebrand's factor and not necessarily its factor VIII procoagulant activity. The normal lag time for ristocetin aggregation of plasma from canine hemophilic carriers, in contrast to that from VWD dogs with similar factor VIII levels, supports this concept. Although the lag time was prolonged in VWD dogs as compared to hemophilic carriers, the degree of aggregation was reduced in both groups (Table 3). These findings may differ from similar studies in man which have dealt primarily with the degree rather than the rate of aggregation.^{12,37,38}

Howard et al.³⁸ reported that they could separate human VWD patients into two groups by their ristocetin response: normal aggregation for patients with low normal platelet adhesiveness and mild to moderately reduced factor VIII activity, and reduced or no aggregation for more severely affected individuals. The moderate but significant increase in the lag time for ristocetin aggregation with VWD dog plasma may therefore reflect the relatively mild to moderate form of the canine disease. Some patients with Glanzmann's thrombasthenia also have reduced ristocetin aggregation,³⁷ but the response of four dogs from our family with thrombasthenic thrombopathia^{2,13} was normal (Table 3).

Previous reports of several VWD patients^{30,39,40} have described platelet nucleotide abnormalities and an increased ATP/ADP ratio, but the present investigation showed no abnormalities of total platelet protein, nucleotide content, or ATP/ADP ratio (Table 4). The VWD dogs also had normal platelet and fibrinogen survival times, although their platelets incorporated more radioactivity than normal dog platelets. This finding is not understood, but it suggests an increased platelet protein turnover, which may bear some relationship to the platelet function abnormalities of VWD.

The reduced level of factor VIII-related antigen in VWD dog plasma clearly establishes the analogy between this animal model and its human counterpart.^{18,19,25,26,32} In addition, plasmas of hemophilic dogs were shown to contain increased amounts of factor VIII-related antigen. These data also parallel those described for man.^{18,19,25,26}

ACKNOWLEDGMENT

The studies reported here were accomplished with the assistance of Dr. Valerie J. Shepard (bleeding time and hemostatic plug formation), Joanne E. Kull (ristocetin aggregation), Sharon L. Raymond (platelet proteins and nucleotides), and F. Stephen Blaisdell (platelet and fibrinogen survivals).

The author wishes to thank Ann C. Moynihan for her technical assistance, Dr. Rodrigo Urizar (Kidney Disease Institute, Albany, N.Y.) for kindly preparing the electron micrographs, Roger E. Benson for performing the immunoassays, and Dr. B. N. Bouma (University Hospital, Utrecht, Netherlands) for his advice and for the rabbit anticanine factor VIII used in the immunologic studies. The assistance of the Hudson Valley German Shepherd Dog Club in the genetic studies is gratefully acknowledged.

REFERENCES

1. Dodds WJ: Canine von Willebrand's disease. *J Lab Clin Med* 76:713, 1970
2. Dodds WJ: Congenital thrombopathies and related coagulation disorders in dogs, in Sabourdy M (ed): Proceedings of "Les Mutants Pathologiques chez l'animal, leur intérêt dans la recherche bio-médicale," Orléans-la-Source. Paris, CNRS, 1970, p 317
3. Dodds WJ: Hereditary and acquired hemorrhagic disorders in animals, in Spaet TH (ed): Progress in Hemostasis and Thrombosis, vol. II. New York, Grune & Stratton, 1974, p 226
4. Dodds WJ, Webster WP, Brinkhous KM, Owen CA Jr, Bowie EJW: Porcine and canine von Willebrand's disease, in Brinkhous KM, Britten AFH (eds): The Handbook of Hemophilia, section XIII. Amsterdam, Excerpta Medica (in press)
5. Patterson DF, Medway W, Luginbühl H, Chacko S: Congenital hereditary lymphoedema in the dog. Part I. Clinical and genetic studies. *J Med Genet* 4:145, 1967
6. Lewis RM, Borel Y: Canine rheumatoid arthritis; a case report. *Arthritis Rheum* 14:67, 1971
7. Barrett RB, Schall WD, Lewis RE: Clinical and radiographic features of canine eosinophilic panosteitis. *J Am Animal Hosp Assoc* 4:94, 1968
8. Hovig T, Rowsell HC, Dodds WJ, Jørgensen L, Mustard JF: Experimental hemostasis in normal dogs and dogs with congenital disorders of blood coagulation. *Blood* 30:636, 1967
9. Salzman EW: Measurement of platelet adhesiveness. *J Lab Clin Med* 62:724, 1963
10. Bowie EJW, Owen CA Jr, Thompson JH Jr, Didisheim P: Platelet adhesiveness in von Willebrand's disease. *Am J Clin Pathol* 52:69, 1969
11. Walsh PN: Albumin density gradient separation and washing of platelets and the study of platelet coagulant activities. *Br J Haematol* 22:205, 1972
12. Weiss HJ, Hoyer LW, Rickles FR, Varma A, Rogers J: Quantitative assay of a plasma factor deficient in von Willebrand's disease that is necessary for platelet aggregation. Relationship to factor VIII procoagulant activity and antigen content. *J Clin Invest* 52:2708, 1973
13. Dodds WJ: Familial canine thrombocytopeny. *Thromb Diath Haemorrh (suppl)* 26:241, 1967
14. Miller GL: Protein determination for large numbers of samples. *Anal Chem* 31:964, 1959
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265, 1951
16. Holmsen H, Storm E, Day HJ: Determination of ATP and ADP in blood platelets: A modification of the firefly luciferase assay for plasma. *Anal Biochem* 46:489, 1972
17. Brodsky I, Siegel NH, Kahn SB, Ross EM, Petkov G: Simultaneous fibrinogen and platelet survival with [⁷⁵Se] selenomethionine in man. *Br J Haematol* 18:341, 1970
18. Bouma BN, Wiegerinck Y, Sixma JJ, van Mourik JA, Mochtar IA: Immunological characterization of purified anti-haemophilic factor A (factor VIII) which corrects abnormal platelet retention in von Willebrand's disease. *Nature (New Biol)* 236:104, 1972
19. Zimmerman TS, Ratnoff OD, Powell AE: Immunological differentiation of classic hemophilia (Factor VIII deficiency) and von Willebrand's disease: with observations on combined deficiencies of antihemophilic factor and proaccelerin (Factor V) and on an acquired circulating anticoagulant against antihemophilic factor. *J Clin Invest* 50:244, 1971
20. Mills DCB, Thomas DP: Blood platelet nucleotides in man and other species. *Nature* 222:991, 1969
21. Cohen P, Cooley MH, Gardner FH: The use of selenomethionine (Se⁷⁵) as a label for canine and human platelets (abstract). *J Clin Invest* 44:1036, 1965
22. Morrison FS, Baldini MG: Antigenic relationship between blood platelets and vascular endothelium. *Blood* 23:46, 1969
23. Madden RE, Gould RG: The turnover rate of fibrinogen in the dog. *J Biol Chem* 196:641, 1952
24. Lewis JH, Ferguson EE, Schoenfeld C: Studies concerning the turnover of fibrinogen [¹³¹I] in the dog. *J Lab Clin Med* 58:247, 1961
25. Gralnick HR, Collier BS, Marchesi SL: Immunological studies of factor VIII in haemophilia and von Willebrand's disease. *Nature (New Biol)* 244:281, 1973
26. Stites DP, Hershgold EJ, Perlman JD, Fudenberg HH: Factor VIII detection by hemagglutination inhibition: hemophilia A and von Willebrand's disease. *Science* 171:196, 1971
27. Barrow EM, Heindel CC, Roberts HR, Graham JB: Heterozygosity and homozygosity

- in von Willebrand's disease. *Proc Soc Exp Biol Med* 118:684, 1965
28. Barrow EM, Graham JB: von Willebrand's disease, in Brown EB, Moore CV (eds): *Progress in Hematology*, vol. IV. New York, Grune & Stratton, 1964, p 203
29. Meyer D, Larrieu MJ, Maroteaux P, Caen JP: Biological findings in von Willebrand's pedigrees: implications for inheritance. *J Clin Pathol* 20:190, 1967
30. Larrieu MJ, Caen JP, Meyer DO, Vainer H, Sultan Y, Bernard J: Congenital bleeding disorders with long bleeding time and normal platelet count. II. Von Willebrand's disease (report of thirty-seven patients). *Am J Med* 45:354, 1968
31. Owen CA Jr, Bowie EJW, Zollman PE, Fass DN, Gordon H: The carrier of porcine von Willebrand's disease. *Am J Vet Res* 35:245, 1974
32. Veltkamp JJ, Van Tilburg NH: Detection of heterozygotes for recessive von Willebrand's disease by the assay of anti-hemophilic-factor-like antigen. *N Engl J Med* 289:882, 1973
33. Noller KN, Bowie EJW, Kempers RD, Owen CA Jr: Von Willebrand's disease in pregnancy. *Obstet Gynecol* 41:865, 1973
34. Ratnoff OD, Bennett B: Clues to the pathogenesis of bleeding in von Willebrand's disease. *N Engl J Med* 289:1182, 1973
35. Kahn RA, Cooper RG, Cornell CN, Muhrer ME: Electron microscopy of bleeder swine platelets. *Am J Vet Res* 31:679, 1970
36. White JG: Ultrastructural defects in congenital disorders of platelet function. *Ann NY Acad Sci* 201:205, 1972
37. Howard MA, Firkin BG: Ristocetin—a new tool in the investigation of platelet aggregation. *Thromb Diath Haemorrh* 26:362, 1971
38. Howard MA, Sawers RJ, Firkin BG: Ristocetin: A means of differentiating von Willebrand's disease into two groups. *Blood* 41:687, 1973
39. Caen JP: Ratio adenosine triphosphate/adenosine diphosphate in platelet-rich plasma in haemorrhagic disorders (von Willebrand and Glanzmann disease). *Nature* 197:504, 1963
40. Valente A, Volpe E, Gandini M, Buonanno G: Von Willebrand's disease: platelet nucleotide alterations in a case with marked platelet adhesiveness and aggregation defects. *Acta Haematol* 47:182, 1972