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Plasma Antithrombin Activity: A Comparative Study in Normal and Diseased Animals (40574)[1,2]

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The syndrome of disseminated intravascular coagulation (DIC) occurs secondary to a variety of underlying acute and chronic diseases in man and in many other species, including the dog and hamster (1–4). These diseases may block the natural inhibitors of blood coagulation, especially those which act to neutralize thrombin, and may thus promote thrombosis or DIC.

Inactivation of thrombin in normal blood occurs progressively. Antithrombin III (AT III) is the most potent inhibitor; its concentration is reduced in hereditary antithrombin III deficiency, liver disease, DIC, and some cases of acute thrombosis (5, 6). Thrombin can also be inactivated progressively by α_2-macroglobulin and α_1-antitrypsin.

In 1975, Lane and co-workers described a method to measure total progressive antithrombin activity (7). Using specific antisera they determined the distribution of total activity in humans as 47% AT III, 29% α_2-macroglobulin, and 23% α_1-antitrypsin.

We have adapted this method to study plasma antithrombin activity in normal animals and in animals with hereditary and acquired hemostatic defects. We chose this method because it uses small amounts of sample, does not require defibrination, is not influenced by the presence of fibrinogen or fibrin-degradation products, and measures the total biological activity of plasma antithrombins, in contrast to immunologic assays of the individual components.

Materials and methods. Animals. Of the 61 dogs used for this study, 18 were clinically ill with DIC. The criteria used for diagnosis of DIC were thrombocytopenia, decreased coagulation activities of fibrinogen and factors V and VIII, prolonged activated partial thromboplastin time and prothrombin time, and the presence of >40 μg/ml fibrinogen–fibrin degradation products. In some cases there were also reduced levels of coagulation factors II, VII and X reflecting severe consumption coagulopathy. The primary diseases in these animals included leukemia, autoimmune hemolytic anemia, severe chronic hepatitis, various sarcomas and carcinomas and, in a few cases, the precipitating cause of DIC was undetermined. The remaining dogs were in good health. These were comprised of 12 normal dogs and 31 with inherited hemostatic defects: 19 with von Willebrand's disease (VWD), 10 with hemophilia A, and 2 with thrombasthenic thrombopathia (TT, Ref. (4)). Dogs with either inherited or acquired hemostatic defects were selected at random regardless of breed, sex, or age. Whenever possible the healthy and diseased animals were matched by age and sex.

In addition, 38 severely affected Syrian hamsters, 12 to 24 months old from a colony previously reported to have a high incidence of spontaneous atrial thrombosis and consumption coagulopathy (3) were studied. The controls were 24 healthy hamsters, 3.5 to 6 months of age, from the same colony.

Blood samples. Platelet-poor plasma was prepared from fresh citrated whole blood (1 part trisodium citrate to 9 parts whole blood) by centrifugation (3000 rpm for 10 min) at room temperature. Samples were immediately frozen at −40° until assayed. The normal dog standard was pooled from 8 normal adult dogs (4 males, 4 females). The normal hamster pool included plasma from 42 adult animals (21 males, 21 females). The antithrombin activity of each standard was defined as 100%.

Thrombin–agarose gel diffusion method.

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[2] Portions of this work were presented at the 62nd Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 11, 1978 (*Fed. Proc.* 37, 586 (1978)).

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Bovine thrombin (5 U/ml; Miles Labs, Cleveland, Ohio) was mixed with an equal volume of molten 2% Seakem agarose (Bausch and Lomb, Rochester, N.Y.) at 50° and pipetted into a 1 × 110 × 250-mm glass-plate mold. Thirty-two 3-mm wells were punched on this plate; then 5 μl of standard or test plasma was placed in each well and incubated in a moist chamber at 37° overnight.

The next day a fibrinogen–agarose gel was prepared by mixing equal volumes of bovine fibrinogen (2.5 g/liter, 65% clottable) and 0.6% Seakem agarose at 50°. The fibrinogen had been prepared from oxalated bovine plasma by the method of Brakman and Astrup (8). This fibrinogen–agarose gel was pipetted over the thrombin–agarose gel layer. After a 15-min incubation at 37°, clear zones representing inactivated thrombin could be seen around the wells, while fibrin formation produced an opaque background.

Dilutions of the same species-specific, pooled normal plasma with assigned values of 100, 50, and 25% antithrombin activity were incubated in duplicate or triplicate with every plate. Test samples were run in triplicate.

A standard curve for each plate was constructed by averaging the diameters of the clear zones for the replicates of each standard dilution and plotting its area against the concentration of plasma. The average area of the zones for each test plasma was then calculated, and the percentage of total progressive antithrombin activity was read from the standard curve.

Results. Dogs. Mean plasma antithrombin activity exceeded 100% for all groups of healthy dogs, including those with inherited hemorrhagic disorders (Fig. 1). Of the 43 healthy dogs only 1, a dog with VWD, had less than 90% antithrombin activity. In contrast, antithrombin activities were significantly lower ($P < 0.01$, Student's t test) in plasmas from dogs with DIC (mean ± SD = 69 ± 19%). Eleven of these eighteen dogs had activities within the 60 and 90% range.

Five dogs had less than 60% activity. The primary disease in two of these animals was leukemia; one had chronic hepatitis; one had pronounced autoimmune hemolytic anemia; and in one the precipitating cause of DIC was undetermined.

Hamsters. All 24 healthy control hamsters had total progressive antithrombin activities greater than 90%; the mean was 105% (Table I). For the 38 aged, thrombosed animals the mean activities were 80% for males and 81% for females. Females are affected much earlier in life (12–16 months) than males (19–24 months) (3). Ten aged, sick hamsters *without* atrial thrombi had similar antithrombin activities with a mean of 79%. These hamsters had amyloidosis, nephrosis, or tumors, as confirmed by histopathologic examination.

The original control population was much younger than the aged, sick animals. This was necessitated by the limited availability of healthy, aged hamsters to serve as age-matched controls. A high incidence (72%) of atrial thrombosis exists in this hamster colony (3) and those that survive to old age, in good health, are few and may represent a thrombosis-resistant population. To determine

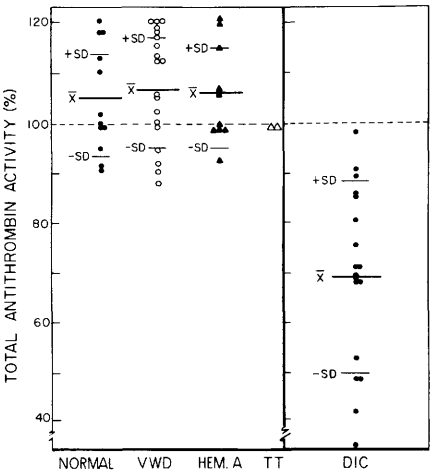

FIG. 1. Total progressive antithrombin levels in healthy dogs, including those with VWD, hemophilia A, and TT, and in dogs with DIC.

TABLE I. TOTAL PROGRESSIVE ANTITHROMBIN ACTIVITY IN HEALTHY AND THROMBOSED SYRIAN HAMSTERS

Hamsters	*n*	Percentage activity		
		>90	60–90	<60
Healthy (♂, ♀)	24	24		
Thrombosed				
Male	16	4	9	3
Female	22	6	14	2
Total	38	10	23	5

whether aging per se effects antithrombin levels, we also measured these activities in 27 healthy hamsters ranging in age from 3.5 months to 2 years. Their total antithrombin activity (mean ± SD) was 103 ± 13%. Seven 1-year-old females and four 2-year-old males, apparently healthy at the time of sacrifice and without atrial thrombi at necropsy, had antithrombin activities of 99 ± 12%.

Discussion. Hereditary AT III deficiency has been associated with a high susceptibility to venous thrombi (5, 6). Subnormal AT III activity has also been reported in some patients with malignancy, liver disease, and DIC during the acute stage of venous thrombosis (6, 9). Most of this information has been obtained from studies of human plasma. Our institution examines a large number of animals which have clinical diseases and have been referred for consultation hematology. In addition, we maintain a colony of Syrian hamsters prone to age-associated intravascular coagulation. The availability of these animals prompted a comparative study of total progressive antithrombin activities in health and disease.

In comparison to healthy animals, plasma antithrombin activities were usually low in animals with acquired hemostatic defects. This was true for 17 of 18 dogs with DIC and for 28 of 38 aged, thrombosed hamsters with no hereditary antithrombin deficiency. Since healthy control hamsters, both young and old, have normal antithrombin activity, the reduced levels in sick hamsters and those with atrial thrombi appears to be secondary phenomenon rather than a predisposing factor. The large reserve of antithrombin available in extravascular spaces (10) may account for the normal plasma antithrombin activity observed in other hamsters with atrial thrombi and in some patients with ongoing thrombosis (6).

Aged, sick hamsters without atrial thrombi had low antithrombin activities similar to those of the thrombosed group. Studies of hemostasis in these animals indicated an earlier stage of DIC (3). Healthy hamsters from the same colony had normal antithrombin activity regardless of age.

Antithrombin activity was also measured in healthy dogs with three types of inherited hemostatic defects. Although high AT III levels have been reported in patients with hemophilia A (9), normal total progressive antithrombin activity has recently been reported (11). This is in agreement with our finding of normal antithrombin activity in canine hemophilia A. The relationship between AT III concentration and the progressive antithrombin activity of plasma has yet to be clearly defined.

Patients with VWD have been reported to have very high (approximately 130%) progressive antithrombin activity (11). The activities for the 19 VWD dogs in our study ranged from 86 to 120%. The mean activity (107%) was not significantly elevated.

Both thrombasthenic thrombopathic dogs had activities of 100%.

These data parallel those reported for man reemphasizing that these animals are invaluable resources as models for study of human thrombotic and hemorrhagic disorders.

Summary. Total progressive antithrombin activity was significantly below normal in dogs with DIC and in aged Syrian hamsters with atrial thrombosis and consumption coagulopathy. It was normal in healthy dogs with three types of inherited hemostatic defects: hemophilia A, VWD, and TT. These findings parallel those reported for humans with chronic diseases such as malignancy, thromboembolism, and DIC, and exemplify the potential of spontaneous diseases in animals as models for the study of human diseases.

1. Greene, C. E., J. Amer. Animal Hosp. Ass. **11,** 674 (1975).
2. Kociba, G. J., *in* "Current Veterinary Therapy" (R. W. Kirk, ed.), Vol. 6, p 448 Saunders, Philadelphia (1977).
3. Dodds, W. J., Raymond, S. L., Moynihan, A. C., and McMartin, D. N., Thromb. Haemost. **38,** 457 (1977).
4. Dodds, W. J., ILAR News **21,** A2 (1977).
5. Egeberg, O., Thromb. Diath. Haemorrh. **13,** 516 (1965).
6. Ødegord, O. E., and Abilgaard, U., Haemostasis **7,** 127 (1978).
7. Lane, J. L., Bird, P., and Rizza, C. R., Brit. J. Haematol. **30,** 103 (1975).
8. Brakman, P., and Astrup, T., *in* "Thrombosis and Bleeding Disorders" (N. U. Bang, F. K. Beller, E.

Deutsch, and E. F. Mammen, eds.), p. 332, Academic Press, New York (1971).
9. Von Kaulla, E., and von Kaulla, K., Amer. J. Clin. Pathol. **48**, 69 (1967).
10. Hensen, A., and Loeliger, E. A., Thromb. Diath. Haemorrh. **9**, Suppl. 1 (1963).
11. Barrow, E. S., and Graham, J. B., Thromb. Res. **13**, 61 (1978).

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