Proc. Natl. Acad. Sci. USA
Vol. 75, No. 9, pp. 4514–4518, September, 1978
Medical Sciences

Venom coagglutinin: An activator of platelet aggregation dependent on von Willebrand factor

(platelet aggregating factor/ristocetin/Factor VIII/species specificity/bioassays)

M. S. READ, R. W. SHERMER, AND K. M. BRINKHOUS

Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514

Contributed by K. M. Brinkhous, June 16, 1978

ABSTRACT **A platelet-aggregating activity was found in many snake venoms, predominantly those of the genus *Bothrops*, that is apparent only in the presence of the platelet-aggregating von Willebrand factor of plasma. It is designated "venom coagglutinin." The coagglutinin can be largely separated from the thrombin-like enzyme of the venoms by ion-exchange chromatography. The venom factor acts on formaldehyde-fixed platelets and is effective with decalcified, heparinized, and afibrinogenemic plasmas but not with severe von Willebrand disease plasmas or with normal plasmas in which the von Willebrand factor has been neutralized by specific antibodies. Use of this coagglutinin permits the assay of von Willebrand factor without the many disadvantages of the ristocetin test. The coagglutinin is active with human, dog, pig, and bovine plasmas and with platelets of any one of these species. This broad-spectrum activity without regard to species contrasts with the ristocetin-resistance of many combinations of plasma and platelets from various species. The assay provides a procedure for studying human, porcine, and canine von Willebrand disease. The lack of species specificity of the coagglutinin suggests that it may be a universal activator of the von Willebrand factor–platelet reaction.**

One of the biologic functions of plasma is the aggregation of blood platelets by the macromolecular Factor VIII complex. The platelet-aggregating activity, one of several activities of the complex (1), appears to be essential for normal hemostasis and is missing in severe von Willebrand disease (vWD) of man (1) and swine (2). In its absence, vWD subjects are prone to mucosal bleeding and have a prolonged bleeding time. vWD plasma, in specific tests, fails to aggregate or agglutinate platelets, including formaldehyde-fixed platelets (3). The missing factor in vWD related to platelet aggregation has often been designated as von Willebrand factor (vWF). However, there is a high degree of species specificity in the *in vitro* demonstration of this platelet-aggregating activity in mammalian plasmas (4). In human plasmas, ristocetin is needed to elicit this activity with human platelets; hence, the term "ristocetin cofactor" has been used for vWF. Platelets of most other species are poorly reactive or unreactive and cannot be substituted for human platelets (4). In pig plasma and that of ruminants, platelet-aggregating activity can be determined directly if human platelets are used, and the term "platelet aggregating factor (PAF)" is used. The test with animal plasmas without ristocetin is referred to as the PAF test.

In view of the apparent biologic identity of the PAF and ristocetin cofactor in the plasmas of various species, we use the term "platelet aggregating factor/von Willebrand factor (PAF/vWF)" for the platelet-aggregating function of the Factor VIII complex. This term serves to distinguish this function from other specific activities of the complex that are deficient in vWD, such as the bleeding time correction factor (5), and that may be included if the term vWF is used alone. In other plasmas, notably those of dog and rabbit, the PAF/vWF activity is not readily elicitable or quantifiable by present test procedures (4). Thus, the ristocetin and PAF tests are highly restricted in applicability and are useful for only a narrow range of species of both plasma and platelets. Most species combinations of platelets and plasma, with or without ristocetin, are inactive or nearly so.

A search was undertaken for vWF-dependent agents that would lead to platelet aggregation and that might overcome the species barrier so evident with ristocetin. Venoms of many different reptiles were found to have this property which we have termed "venom coagglutinin" activity, acting as it does only in conjunction with PAF/vWF. In this study, we have partially characterized the activity of this thromboagglutinin factor and have demonstrated its utilization in screening and bioassay procedures for determining plasma PAF/vWF with human and animal platelets. A preliminary report of some aspects of this study was presented recently (6).

MATERIALS AND METHODS

Venoms. Dried venoms from 71 different species or subspecies of snakes were obtained from three commercial sources (Sigma Chemical Co., St. Louis, MO; Miami Serpentarium Laboratories, Miami, FL; and Ross Allen, Silver Springs, FL). Two venoms, from *Bothrops marajoensis* and *Bothrops moojeni*, were a gift from K. Stocker (Pentapharm, Basel). The 73 venoms were as follows: *Agkistrodon acutus, bilineatus, c. contortrix, c. mokason, c. pictigaster, halys, p. leucostoma,* and *rhodostoma*; *Atractaspis c. congica; Bitis arietans, caudalis, gabonica,* and *g. rhinoceros*; *Bothrops alternatus, atrox, b. bilineatus, cotiara, jararaca* (reference venom), *jararacussu, lansbergii, marajoensis, medusa, moojeni, nasuta, neummifera, neuwiedii,* and *schlegelii*; *Cerastes cerastes* and *vipera*; *Crotalus adamanteus, atrox, basiliscus, cerastes, d. durissus, d. terrificus, d. totonacus, h. atricaudatus, h. horridus, m. molossus, ruber, scutulatus, v. cerberus, v. helleri, v. lutosus, v. oreganus,* and *v. viridis*; *Dendroaspis angusticeps* and *jamesoni*; *Echis carinatus*; *Elapsoidea s. sundevallii*; *Micrurus corallinus, f. frontalis, f. tenere,* and *n. nigrocinctus*; *Naja haje, naja,* and *nivea*; *Notechis scutatus*; *Oxyuranus s. scutellatus*; *Sistrurus c. tergeminus* and *m. barbouri*; *Trimeresurus flavoviridis, mucrosquamatus, okinavensis, purpureomaculata,* and *wagleri*; *Vipera ammodytes, aspis, berus, russellii, r. siamensis,* and *xanthina palestinae*; and *Walterinnesia aegyptia.*

Stock solutions of venom (4 mg/ml) were prepared with 84

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: N, normal; vWD, von Willebrand disease; vWF, von Willebrand factor; PAF, platelet aggregating or agglutinating factor or activity.

mM imidazole (Sigma)/0.154 M NaCl buffer, pH 7.35, and stored at −20°. Unless indicated otherwise, reference venom stock solution (*B. jararaca*) diluted with buffer was used.

Ristocetin (Abbott) was dissolved in the imidazole/saline buffer to give a stock solution of 2.4 mg/ml.

Preparations of Plasmas. Normal citrated plasmas were prepared as described (4). vWD citrated plasmas were obtained from human and porcine vWD subjects, both heterozygous and homozygous (2, 7). Canine vWD plasmas were obtained from a new strain of vWD dogs maintained at the University of North Carolina.

Fixed Platelets. Human and animal platelets (800,000/mm^3) were prepared by a modification of the procedure of Allain *et al.* (3).

PAF/vWF Neutralization Tests. The antibodies used were as follows: human anti-human vWF (8); goat anti-human Factor VIII (a gift of Howard Reisner, Chapel Hill) diluted 1:20 with imidazole/saline buffer and absorbed twice with fixed human platelets to remove PAF (9); and rabbit anti-porcine factor VIII (a gift of W. P. Webster, Chapel Hill) diluted 1:8 and absorbed with fixed dog platelets (4) in the presence of venom coagglutinin (6 μg/ml). PAF/vWF neutralization studies were performed as described (10).

Assay of PAF/vWF. The bioassay for human vWF with ristocetin was performed by a modification of a described procedure (7, 8); that for porcine and bovine PAF was by a modification of the procedure of Griggs *et al.* (2) using fixed platelets. The bioassay of PAF/vWF with the venom was performed as follows. A four-part system consisting of 1 vol of plasma and 1 vol of buffer (or 2 vol of plasma), 1 vol of fixed platelets, and 1 vol of venom coagglutinin (100–250 μg/ml) was used, and the macroscopic aggregation time was determined (7). The aggregometry assay procedure, in which fixed washed platelets (0.1 ml), buffer (0.1 ml), and citrated plasma (0.1 ml) were stirred in the cuvette at 37° and 0.2 ml of venom coagglutinin was added, was an adaptation of a described method (3, 11).

RESULTS

Venom Survey for Coagglutinin Activity. The venoms were tested for aggregating activity with fixed human or fixed dog platelets and normal or severe vWD plasmas, either human or porcine. The venom coagglutinin activity is defined as that which will aggregate platelets with normal plasma but not with vWD plasma. A number of the 73 venoms tested caused platelet aggregation in the presence of PAF/vWF but not in its absence—i.e., with severe vWD plasma. Results with five venoms that caused rapid platelet aggregation with normal plasmas are summarized in Table 1. In further testing, serially diluted plasmas, 1:1 to 1:32, were examined with the various venoms. In the porcine plasma/dog platelet systems, some venoms such as from *B. atrox* showed good coagglutinin activity with porcine but not with human plasma.

The active venoms also contained a thrombin-like enzyme. In the aggregation tests, all plasma test samples were observed up to 120 sec during which time no clotting was observed. However, with longer observation, all of the venoms listed in Table 1, for example, caused plasma clotting, usually in 2–10 min. On mixing with human fibrinogen solutions (Kabi, 350 mg/100 ml), these same venoms caused clotting in 30–300 sec. A comparison of platelet aggregation and clotting time of citrated, EDTA–, and heparinized plasmas with venom coagglutinin is shown in Table 2. The macroscopic platelet aggregation time with venom was approximately the same with EDTA– and heparinized plasma as with citrated plasma. Also, afibrinogenemic plasma caused prompt platelet aggregation. The clotting time of normal plasma with venom was longest with EDTA as anticoagulant.

Table 1. Venom coagglutinin activity with fixed platelets (human or dog) and normal and vWD plasmas (human or porcine)

Venom		Aggregation time (sec) with Human platelets/human plasma		Dog platelets/human plasma		Dog platelets/porcine plasma	
Species	μg/ml	N	vWD	N	vWD	N	vWD
B. arietans	125	7.0	>60	6.0	>60	6.0	>60
B. alternatus	250	8.0	>60	11.0	>60	7.0	>60
B. jararaca	200	8.0	>60	11.0	>60	6.0	>60
B. medusa	250	9.5	>60	13.0	>60	8.0	>60
B. neuwiedii	125	8.0	>60	9.0	>60	7.0	60(1+)

Test mixture: 0.025 ml each of plasma, buffer, platelets, and venom. Platelet/venom mixtures without plasma did not aggregate. N, normal.

Chromatographic Separation of Venom Aggregating and Clotting Activity. Experiments were carried out with ion exchange chromatography and stepwise elution in attempts to separate the aggregating and clotting activities of venom. Fig. 1 gives data from an illustrative experiment in which these two activities were largely separated. The aggregating activity eluted with the high-molarity salt solution, whereas the clotting activity eluted with the starting buffer.

Comparison of Venom-Induced and Ristocetin-Induced Platelet Aggregation. In a series of experiments, PAF/vWF-dependent platelet aggregation with venom and with ristocetin was compared. Table 3 illustrates one set of experiments with the two aggregating agents. Normal plasmas from four species—man, dog, pig, and cow—were compared in four separate test systems in which the aggregating agent and the species of platelets were varied. All plasmas aggregated promptly with venom in tests using either human or dog platelets. The canine plasma, which is inactive with ristocetin, caused rapid platelet aggregation with venom. The two PAF-positive plasmas, porcine and bovine, reacted similarly with venom and ristocetin. A further comparison of the venom, ristocetin, and PAF tests with serially diluted porcine plasma is shown in Fig. 2. Venom and ristocetin acted similarly with PAF-positive plasmas in accelerating aggregation.

The venom was also compared with ristocetin regarding precipitation of proteins in test plasmas. Ristocetin in sufficient concentration regularly causes turbidity of plasma due to protein precipitation (12). Venom, in a wide range of concentrations up to 4 mg/ml, was tested with all plasmas listed in Table 3. In no case was protein precipitation observed.

Table 2. Comparison of venom-induced platelet aggregation and clotting of anticoagulated normal and afibrinogenemic human plasmas

Plasma	Macroscopic aggregation time, sec	Clotting time, sec
Normal, citrated	7.2	120
Normal, EDTA*	8.4	330
Normal, heparinized*	8.0	120
Afibrinogenemic, citrated	8.0	No clotting, 24 hr

* EDTA (1%) or heparin (10 international units/ml) substituted for citrate as anticoagulant. The test used 2 vol of plasma and 1 vol of venom coagglutinin at 200 μg/ml.

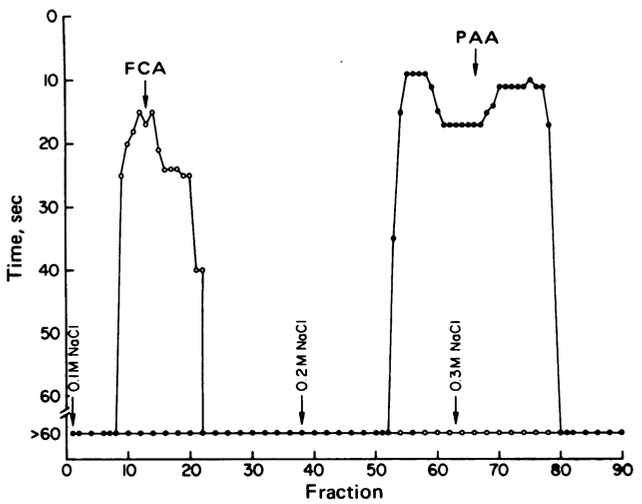

FIG. 1. Venom chromatography on DEAE-cellulose column, 1.6 × 22 cm, equilibrated with imidazole/saline buffer, pH 7.35, at 25°. Venom solution (1 ml, 20 mg/ml, dialyzed against buffer for 18 hr, 4°) was pumped onto column, one drop every 5 sec. Column was eluted with stepwise increases in molarity of NaCl in imidazole buffer. Ordinate refers to either clotting time (○) or aggregation time (●). FCA, fibrinogen clotting activity; PAA, platelet-aggregating activity.

Antibody Neutralization of PAF/vWF and Effect on Platelet Aggregation with Venom Coagglutinin. Three antibodies with PAF/vWF-neutralizing activity were examined for their effect on the venom coagglutinin reaction in several test systems. Illustrative data are shown in Table 4. The first antibody tested, an anti-human vWF, had developed in a severe multitransfused vWD patient (8, 10). This antibody, as has previously been shown, prevented ristocetin-induced platelet aggregation with normal human plasma and human platelets, due to neutralization of PAF/vWF. Data with ristocetin are included as controls. Venom coagglutinin activity was likewise not expressed after antibody neutralization of PAF/vWF. The effect of PAF/vWF neutralization with this antibody was also tested with dog platelets. After neutralization of its PAF/vWF, the plasma did not support platelet aggregation with venom. Similar experiments were performed with a goat anti-human antibody. The results were similar to those obtained with the human antibody.

A rabbit anti-porcine antibody was allowed to react with porcine and canine plasmas to test its effect on the platelet aggregating system with venom (Table 4). Both human and dog platelets were used in the tests. After neutralization of plasma PAF/vWF with the antibody, the venom was relatively inactive with either of the plasmas. Thus, the venom platelet aggregation activity is again shown to be dependent on the presence of PAF/vWF in plasma.

Table 3. Comparison of reactivity of venom coagglutinin and ristocetin in aggregation of fixed human and dog platelets with plasmas of different species

	Aggregation time (sec) with			
	Human platelets		Canine platelets	
Plasma	Venom	Ristocetin	Venom	Ristocetin
Human	13.2	11.2	11.2	Neg.
Canine	6.0	Neg.	5.6	Neg.
Porcine	6.0*	7.0*	6.8	Neg.
Bovine	18.0*	16.2*	19.0	Neg.

Test mixture: 0.05 ml of plasma diluted 1:4 (except bovine, 1:128), 0.025 ml each of fixed platelets and venom (200 μg/ml) or ristocetin (1.6 mg/ml).

* Porcine and bovine plasma, without venom or ristocetin, caused aggregation of human platelets in 11 and 24.2 sec, respectively.

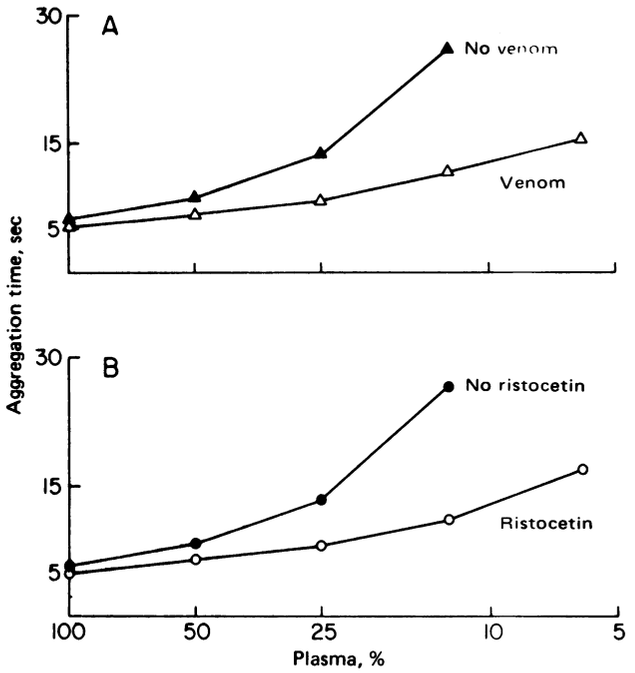

FIG. 2. Comparison of venom (100 μg/ml) (*A*) and ristocetin (2.4 mg/ml) (*B*) in aggregation of fixed human platelets with serially diluted normal porcine plasma.

Bioassay of PAF/vWF with Venom Thromboagglutinin. Fig. 3 illustrates the use of venom coagglutinin for the bioassay of PAF/vWF in human normal and vWD plasmas. The use and limitation of ristocetin are also illustrated. The results obtained with venom and the two species of platelets and with ristocetin and venom all are similar. The PAF/vWF value obtained for the mild or heterozygous vWD subject was 27% with venom and human platelets, 29% with dog platelets, and 28% with ristocetin (Table 5). Percentage PAF/vWF was calculated from interpolated values of the percentage of test plasma that would give the same aggregation time as known amounts of normal reference plasma (7). No PAF/vWF activity was detectable in the plasma of the severe vWD subject with either venom or ristocetin. In other studies, aggregometry assays with venom also reflected the PAF/vWF levels of plasma. The initial increased optical density noted with ristocetin and fixed platelets (3) did not occur.

The bioassay was also used to compare PAF/vWF activity in animal vWD subjects, both porcine and canine. One set of assay data is presented in Table 5. With porcine vWD plasmas, the results obtained with venom coagglutinin and dog platelets were similar to those obtained in the PAF test with human platelets. With the heterozygous canine vWD plasma, the PAF/vWF level as determined with venom was similar with both dog and human platelets.

DISCUSSION

A platelet-aggregating or -agglutinating activity, found in the venom of several species of reptiles and particularly in the genus *Bothrops*, was manifest with fixed platelets only in the presence of the plasma platelet-aggregating factor, PAF/vWF, missing in severe vWD. This new activity is called venom coagglutinin because it acts only conjointly with PAF/vWF. In this respect

Table 4. Antibody neutralization of PAF/vWF: Comparison of platelet aggregation with venom coagglutinin and ristocetin

Antibody (Ab)	Species (normal plasma)	Aggregation time (sec) with Human platelets: Venom (V) Ab	Venom (V) No Ab	Ristocetin (R) Ab	Ristocetin (R) No Ab	No V or R Ab	No V or R No Ab	Dog platelets: Venom Ab	Dog platelets: Venom No Ab	No V*
Human anti-human vWF	Human (1:2)	>120	11.0	>120	11.0	Neg.	Neg.	>120	12.5	Neg.
Goat anti-human Factor VIII (1:20)	Human (1:4)	120(1+)	15.0	120(1+)	15.0	Neg.	Neg.	120(1+)	15.0	Neg.
Rabbit anti-porcine Factor VIII (1:8)	Porcine (1:4)	120(1+)	10.0	120(1+)	12.6	>120	20.0	>120	11.0	Neg.
Rabbit anti-porcine Factor VIII (1:8)	Canine (1:16)	120(1+)	20.0(3+)	Neg.	Neg.	Neg.	Neg.	120(1+)	21.0(3+)	Neg.

Test system: 0.025 ml of diluted plasma and 0.025 ml of diluted antiplasma or imidazole buffer, incubated 5 min at 28°; then, 0.025 ml of platelet suspension and 0.025 ml of venom (200 μg/ml) or ristocetin (1.2 mg/ml) were added. Dilutions of porcine and canine plasmas were made with diluted rabbit antiplasma.

* Tested both with and without antibody.

it is similar to ristocetin, but its action contrasts with that of ristocetin in many ways. Although this new factor was found in a large number of venoms, the five listed in Table 1 were found to be highly active.

In the survey of venoms, human plasmas and fixed human platelets were originally used in the testing. Dog platelets, which are inactive in the other test systems for PAF/vWF (the ristocetin and the PAF tests), were soon found to be as active as human platelets when the venom factor was used. It was thus possible to extend the survey to include two species of platelets, human and dog, and two species of vWD plasmas, human and porcine. For venoms with a low titer of coagglutinin activity, the porcine plasma test with dog platelets appeared to be somewhat more sensitive than tests with human plasma.

Plasmas anticoagulated with citrate, EDTA, or heparin were equally suitable for demonstrating PAF/vWF activity with the coagglutinin (Table 2). Also, afibrinogenemic plasma was as active as normal plasma. Thus, ionic calcium and fibrinogen are not needed for the reaction. The coagglutinin resembles ristocetin in these respects, as it does in aggregating fixed platelets and not requiring the platelet release reaction.

The coagglutinin-positive venoms also contain a thrombin-like enzyme that clots plasma (Table 2) and fibrinogen. The coagglutinin factor can largely be dissociated from the thrombin-like enzyme by ion exchange chromatography (Fig. 1). The thrombin-like enzyme did not aggregate fixed platelets. Thus, these two activities are distinct.

The venom coagglutinin activity was compared with ristocetin in several additional experiments. A number of similarities as well as dissimilarities were observed. Like ristocetin, the venom factor caused rapid platelet aggregation of human platelets with human plasma and caused accelerated aggregation of diluted PAF-positive pig and cow plasmas (Table 3; Fig. 2). Unlike ristocetin, the coagglutinin aggregated human platelets with canine plasma rapidly (Table 3) and also aggregated canine platelets with all of the species of plasmas.

The venom, unlike ristocetin in sufficient concentrations, does not precipitate plasma proteins. Also, the venom factor does not cause the initial increased optical density observed in the aggregometer with fixed platelets and ristocetin (3). Aggregometry assays of PAF/vWF can be carried out with venom without the disadvantages of ristocetin.

Another comparison of coagglutinin with ristocetin was made by using human plasmas in which the PAF/vWF activity had been neutralized by two separate antibodies. Like severe vWD plasmas, these plasmas were largely unreactive with venom (Table 4) as has been shown with ristocetin (10). These data provide additional evidence for the PAF/vWF dependence of the coagglutinin activity. With canine plasma it is of interest that the same antibody that neutralizes the PAF in the porcine plasma/human platelet system also neutralizes the canine "plasma factor" required for venom aggregation of human platelets (Table 4). These and other data support the thesis that canine PAF/vWF, although insensitive to the ristocetin probe, is fully reactive with venom coagglutinin.

The venom thromboagglutinin was found to be an effective agent for the assay of PAF/vWF in all of the species of plasmas used. Detailed data from illustrative assays of human PAF/

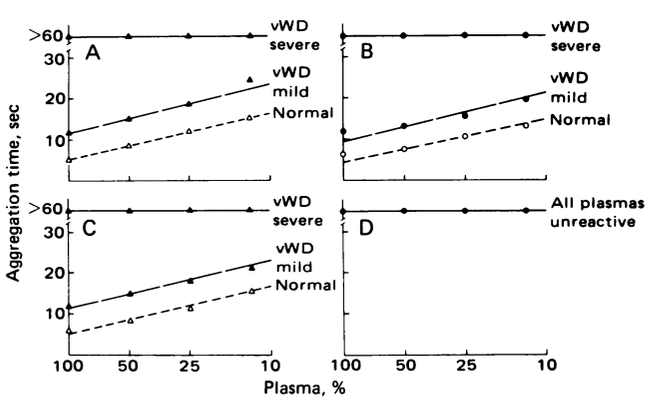

FIG. 3. Bioassay of PAF/vWF in human plasma with venom coagglutinin and either fixed human (*A*) or canine (*C*) platelets or with ristocetin and either fixed human (*B*) or canine platelets (*D*).

Table 5. Comparative bioassays of PAF/vWF in heterozygous and homozygous human, porcine, and canine vWD plasmas with venom coagglutinin or ristocetin and with human or dog platelets

vWD plasmas	Genotype	PAF/vWF, %: Venom coagglutinin, Dog platelets	Venom coagglutinin, Human platelets	Ristocetin, Human platelets
Human	Heterozygous	29	27	28
	Homozygous	<5	<5	<5
Porcine	Heterozygous	41	42*	42*
	Homozygous	<5	<5*	<5*
Canine	Heterozygous	44	48	Neg.
	Homozygous	<5	—	Neg.

* With PAF test only.

vWF in which both human and canine platelets were used are shown in Fig. 3 *A* and *C*. The close correspondence of the values obtained for the same plasmas with the assays with venom and with ristocetin (Fig. 3*B*) is remarkable. The use of the coagglutinin procedure for bioassay of PAF/vWF in vWD plasmas of three different species is shown in Table 5. Here too the closeness of the PAF/vWF values obtained with the different assay systems with coagglutinin is striking. The assays in ristocetin-refractory systems, such as human or porcine plasmas with dog platelets, gave the same values as in the ristocetin-sensitive systems with human platelets. The canine vWD plasma was obtained from a recently discovered strain of dogs with this disease (Table 5). The results obtained with the coagglutinin assay make it obvious that these dogs are deficient in PAF/vWF.

The venom thromboagglutinating factor may be a universal platelet aggregating agent with PAF/vWF in mammalian plasmas. Not only was the coagglutinin active with all species combinations of plasma and platelets reported here, but preliminary studies with other species combinations of plasma and platelets previously found to be ristocetin-resistant (4) showed all to be active with venom. The thromboagglutinin is thus more than a ristocetin substitute. The mechanism of action of the venom coagglutinin is unknown. The same is true of ristocetin, in spite of many studies exploring the basic mechanisms involved in its interaction with PAF/vWF and platelets. The broad-spectrum nature of coagglutinin, which overcomes the species specificity barrier, should provide a new probe for resolving the enigma of the platelet–plasma PAF/vWF reaction.

We acknowledge helpful discussions with Dr. Robert H. Wagner on the chromatography studies. The research was supported in part by Grants HL-01648 and HL-06350 from the National Heart, Lung and Blood Institute and by the Kurt Orban Foundation.

1. Cooper, H. A., Mason, R. G. & Brinkhous, K. M. (1976) *Annu. Rev. Physiol.* **38**, 501–555.
2. Griggs, T. R., Webster, W. P., Cooper, H. A., Wagner, R. H. & Brinkhous, K. M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2087–2090.
3. Allain, J. P., Cooper, H. A., Wagner, R. H. & Brinkhous, K. M. (1975) *J. Lab. Clin. Med.* **85**, 318–328.
4. Brinkhous, K. M., Thomas, B. D., Ibrahim, S. A. & Read, M. S. (1977) *Thromb. Res.* **11**, 345–355.
5. Blatt, P. M., Brinkhous, K. M., Culp, H. R., Krauss, J. A. & Roberts, H. R. (1976) *J. Am. Med. Assoc.* **238**, 2770–2772.
6. Brinkhous, K. M., Shermer, R. W. & Read, M. S. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 673 (abstr.).
7. Brinkhous, K. M., Graham, J. E., Cooper, H. A., Allain, J. P. & Wagner, R. H. (1975) *Thromb. Res.* **6**, 267–272.
8. Sarji, K. E., Stratton, R. D., Wagner, R. H. & Brinkhous, K. M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2937–2941.
9. Cooper, H. A., Wilkins, K. W., Johnson, P. R. & Wagner, R. H. (1977) *J. Lab. Clin. Med.* **90**, 512–521.
10. Stratton, R. D., Wagner, R. H., Webster, W. P. & Brinkhous, K. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4167–4171.
11. Weiss, H. J., Hoyer, L. W., Rickles, F. R., Varma, A. & Rogers, J. (1973) *J. Clin. Invest.* **52**, 2708–2716.
12. Stibbe, J. & Kirby, E. P. (1976) *Thromb. Res.* **8**, 151–165.