

Comparative Studies on Blood Coagulation Factor XIII<sup>1</sup> (40141)S. LOPACIUK,<sup>2</sup> R. P. MCDONAGH, AND JAN MCDONAGH*Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514*

Blood coagulation systems are found in all animal species that have a circulatory system. These range from the primitive cell agglutination mechanisms of the lower invertebrates to the complex cellular and plasma mammalian coagulation systems. From studies on comparative coagulation, it has become apparent that all systems have much in common with the human system. In the arthropods *Limulus polyphemus* and *Homarus americanus*, amebocytes in the hemolymph undergo aggregation, degradation, and release in a manner similar to mammalian platelets. The amebocytes release a clottable protein analogous to fibrin (1). In *Homarus* a transglutaminase enzyme crosslinks the clottable protein by a mechanism similar to the action of factor XIIIa on fibrin (2). The clotting system of the lamprey eel (*Petromyzon marinus*), one of the most primitive vertebrates, has been shown to have both fibrinogen and thrombin which are similar to the mammalian proteins but which also have distinct species differences (3).

The present study deals with the characterization and species specificity of factor XIII in twelve mammalian and three avian plasmas. Plasma levels of fibrinogen and factor XIII were determined for each species. Factor XIII in each species was characterized by gel filtration and by reactivity toward human and rabbit antibodies to factor XIII.

**Materials and methods.** Blood was carefully collected from live, healthy subjects into 0.1 vol of 3.8% sodium citrate. Anesthesia was not used except for guinea pigs and monkeys. Platelet or thrombocyte poor plasma was prepared and was tested immediately or was stored at -20°. Plasma was also obtained from two patients who had developed specific

antibodies to factor XIII. These were the Warsaw inhibitor and the Chapel Hill inhibitor (4). In some studies, an IgG immunoglobulin fraction from the Warsaw inhibitor was used. This was purified by Rivanol and ethanol precipitation (5).

Fibrinogen was assayed spectrophotometrically as clottable protein dissolved in alkaline urea (6), with human fibrinogen Fraction I-4 which was 98% clottable as the reference material. Factor XIII activity was assayed by the dansylcadaverine incorporation method (7), in which the fluorescent amine is enzymatically incorporated into casein by factor XIIIa. Dansylcadaverine was obtained from Sigma Chemical Company. Gel filtration of plasma samples was performed on columns of Biogel A-1.5m (2 cm<sup>2</sup> × 90 cm). Columns were eluted by upward flow with 0.05 M Tris -0.1 M NaCl, pH 7.4, and the effluent was monitored for protein absorbance at 280 nm and for factor XIII activity.

Reaction of plasma of each species to spontaneously occurring human antibodies to factor XIII and to rabbit antisera (Behring Diagnostics) to the *a* and *b* subunits of human plasma factor XIII was determined by Ouchterlony immunodiffusion. Neutralization of factor XIII in each species plasma by the antibodies was determined by modification of the dansylcadaverine assay. In order to obtain comparable antigen:antibody ratios, rabbit, guinea pig, canine, and bovine plasmas were diluted so that the factor XIII concentrations would be comparable to that in human plasma. In these studies, 0.1 ml plasma was incubated with 0.1 ml of antiserum or inhibitor IgG for 75 min at room temperature. This mixture was heated for 5 min at 56° and cooled. The total time elapsed before the addition of thrombin to activate factor XIII was 90 min.

**Results. Fibrinogen and factor XIII levels in the plasma of different species.** All mammalian and avian plasmas tested contained detectable levels of fibrinogen and factor XIII as

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measured by these assays (Table I). There was no correlation between fibrinogen concentration and factor XIII activity. Fibrinogen levels were found to be relatively constant within the 15 species, and the sample averages ranged from 2.16 to 4.24 mg/ml with a mean of  $3.25 \pm 0.73$ . Factor XIII activity was generated in all plasmas by activation with bovine thrombin. In all of the avian and eight of the mammalian plasmas, factor XIII activity ranged from 46 to 96% of that found in human plasma. Four mammalian plasmas had significantly higher levels. For guinea pig, rabbit, canine, and bovine plasmas the levels were 233%, 173%, 185%, and 480% of that in human plasmas.

*Characterization of animal factor XIII by gel filtration.* When each of the animal plasmas was chromatographed on Biogel A-1.5m, no significant differences in the protein profiles were observed. For each species the partition coefficient,  $K_{av}$ , for factor XIII was determined. For rat, guinea pig, and rabbit factor XIII  $K_{av} = 0.39 \pm 0.01$ . For all other species  $K_{av} = 0.32 \pm 0.01$ .

*Neutralization of mammalian and avian plasmas by antibodies to factor XIII.* Factor XIII of all plasmas was neutralized to varying degrees by two human antibodies to factor XIII (Fig. 1A and B). For all mammalian

plasmas the maximum neutralization obtained was approximately 80% or more with both antibodies. Avian factor XIII was less reactive toward these antibodies. The maximum factor XIII inhibition by the Chapel Hill inhibitor for the three avian plasmas was 60%; for the Warsaw inhibitor it ranged from 50 to 75%. Rabbit antiserum to the *a* subunit of plasma factor XIII did not have the broad specificity of the spontaneously occurring human antibodies (Fig. 1C). The neutralization of human and monkey factor XIII by rabbit anti-*a* subunit was similar to their neutralization by the human antibodies. All other animal plasmas were less reactive toward rabbit anti-*a* subunit, although 80% maximum neutralization was obtained with canine, bovine, sheep, and goat plasmas. Factor XIII in the three avian plasmas was not neutralized by rabbit anti-*a* subunit. As expected, rabbit factor XIII was also not neutralized by this antiserum.

*Immunodiffusion studies.* Although rabbit antiserum to *a* subunit neutralized factor XIII in all the mammalian plasmas, as determined by assay of residual factor XIII activity, on Ouchterlony immunodiffusion precipitin lines formed against this antiserum only with human, bovine, canine, feline, sheep, and goat plasmas. No precipitin lines were observed with porcine, guinea pig, or rat plasmas, and monkey plasma was not tested. In contrast, a precipitin line was formed between rabbit antiserum to *b* subunit (also referred to as subunit S) and all other mammalian plasmas (Fig. 2). The three avian plasmas did not precipitate with antiserum to *b* subunit or *a* subunit.

*Discussion.* In this study plasma levels of fibrinogen and factor XIII were determined for 15 species. Plasma fibrinogen concentrations were relatively constant and ranged from 86% to 170% of the value for human fibrinogen (Table I). The mean concentration for all species tested was 3.25 mg/ml. These results can be compared to those of Glazunova (8), who found a mean of 2.23 mg/ml for six of the species tested in this study, and to those of Didisheim et al. (9), who found that plasma fibrinogen varied from 45% to 245% in seven of the species studied here, as compared to human fibrinogen.

In the comparative study of Schwartz *et al.*

TABLE I. PLASMA LEVELS OF FIBRINOGEN AND FACTOR XIII IN VARIOUS SPECIES.

Species	No. of subjects	Fibrinogen mg/ml	Factor XIII <sup>a</sup> $\mu$ mole DC incorp./30 min/0.2 ml
Man	7	$2.50 \pm 0.32^b$	$5.5 \pm 0.7^b$
Monkey (Macaca mulatta)	3	$3.63 \pm 0.22$	$4.7 \pm 0.5$
Rabbit	4	$2.50 \pm 0.91$	$9.5 \pm 3.6$
Rat	5	$2.35 \pm 1.30$	$5.2 \pm 0.7$
Guinea pig	4	$2.47 \pm 0.56$	$12.8 \pm 5.8$
Dog	3	$3.09 \pm 0.45$	$10.2 \pm 2.3$
Cat	3	$3.80 \pm 0.56$	$5.0 \pm 1.4$
Horse	4	$4.24 \pm 0.71$	$3.1 \pm 0.5$
Pig	3	$3.58 \pm 0.32$	$3.7 \pm 0.5$
Ox	4	$4.22 \pm 0.39$	$26.4 \pm 4.2$
Goat	4	$4.14 \pm 0.42$	$5.1 \pm 2.8$
Sheep	4	$3.61 \pm 1.10$	$3.9 \pm 1.6$
Goose	4	$2.16 \pm 0.34$	$4.4 \pm 1.3$
Chicken	4	$2.87 \pm 0.62$	$5.2 \pm 1.7$
Turkey	4	$3.58 \pm 0.90$	$5.3 \pm 0.9$

<sup>a</sup> Factor XIII activity was determined as  $\mu$ mole dansylcadaverine (DC) incorporation per 30 min in 0.2 ml plasma.

<sup>b</sup> Mean  $\pm$  SD.

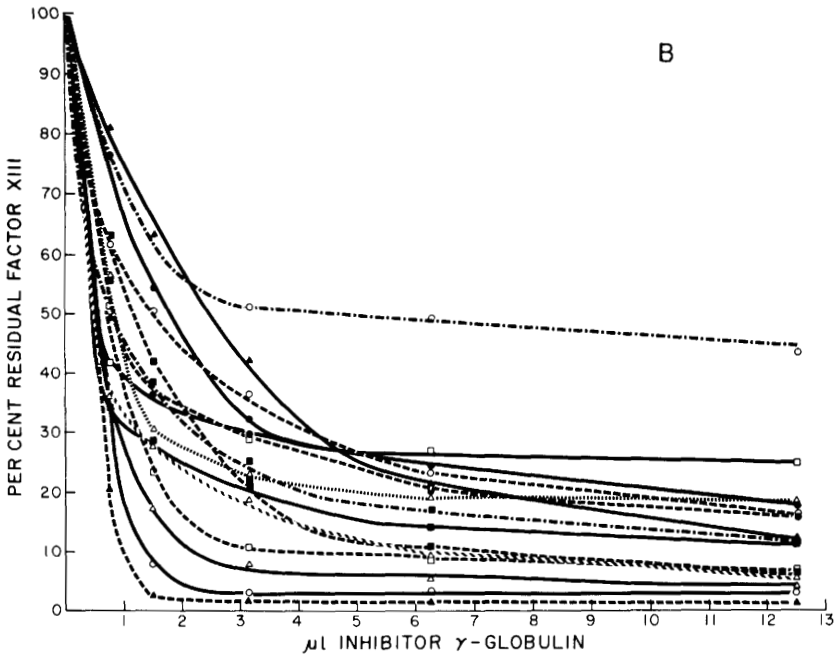
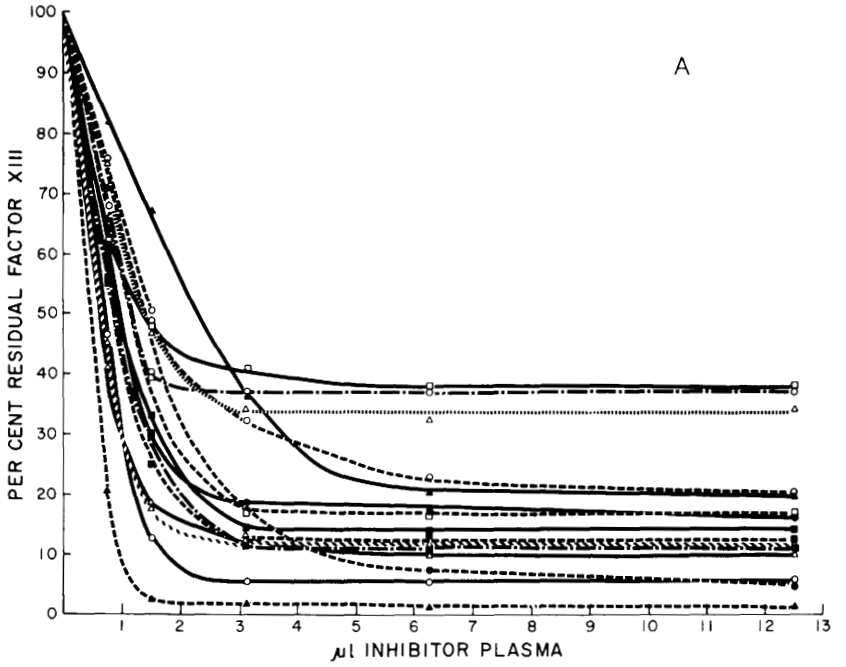


FIG. 1. Inhibitory effect of human and rabbit antibodies on the factor XIII activity in plasma of different species. A: antibody from the Chapel Hill inhibitor patient; species tested in increasing order of inhibition are chicken, turkey, goose, pig, cat, horse, dog, sheep, rat, goat, ox, rabbit, man, guinea pig, monkey. B: antibody from the Warsaw inhibitor patient; species tested are turkey, chicken, goose, dog, pig, guinea pig, cat, ox, sheep, horse, rat, goat, rabbit, man, monkey. C: rabbit antibody to human subunit  $\alpha$ ; species tested are chicken and rabbit together (no inhibition), turkey, goose, guinea pig, rat, pig, cat, horse, sheep, dog, ox, goat, man, monkey.

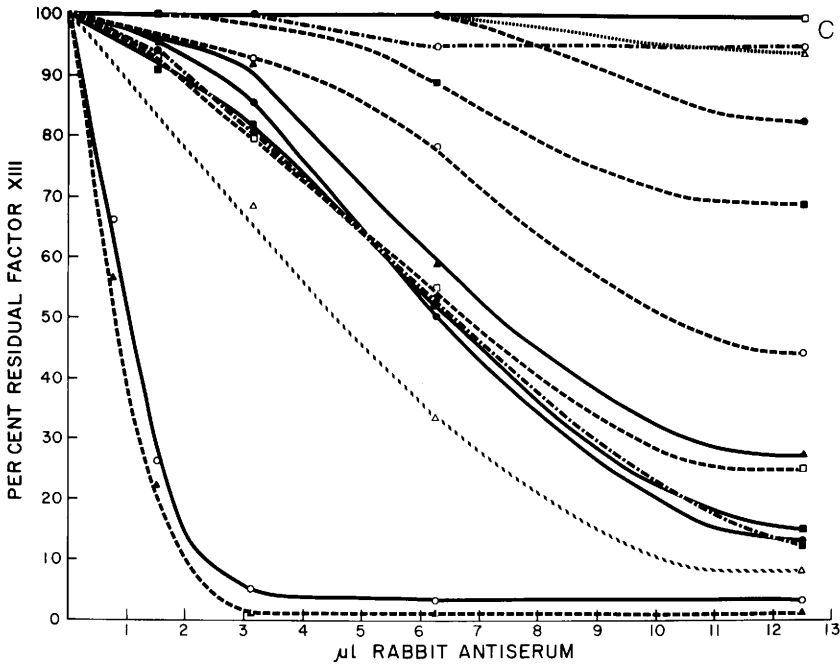


FIG. 1C.

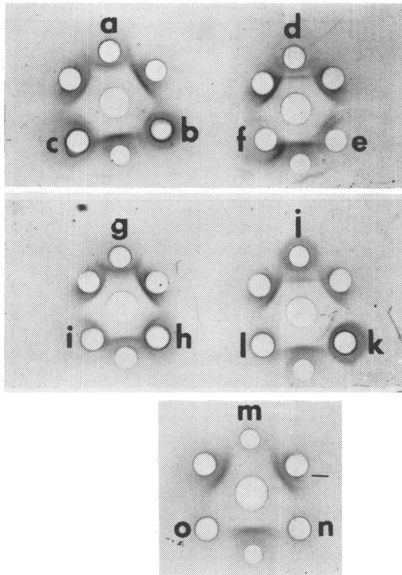


FIG. 2. Immunodiffusion of rabbit antiserum to human factor XIII *b* subunit against plasma from various species. The unmarked wells in each pattern contained human plasma. Other plasmas tested were (a) monkey, (b) ox, (c) pig, (d) dog, (e) cat, (f) rabbit, (g) sheep, (h) goat, (i) horse, (j) guinea pig, (k) rat, (l) chicken, (m) turkey, (n) goose, (o) goose.

(10), polyacrylamide gel electrophoresis in sodium dodecyl sulfate was used to examine fibrin crosslinking in a number of species. These investigators found  $\gamma$ -dimers and  $\alpha$ -polymers in their mammalian crosslinked fibrin clots and primarily  $\gamma$ -dimers in their avian fibrin clots, which indicated the presence of factor XIII activity in those plasmas. In the present study, factor XIII activity was quantitated and was found to vary widely. The values ranged from 56% to 480% of the mean human value. There was no species correlation between fibrinogen concentration and factor XIII level ( $r^2 = 0.02$ ). Factor XIII activity was also not correlated with evolutionary development; in this study, the highest value was found in bovine plasma and the lowest in equine plasma. The three avian plasmas had approximately the same level of factor XIII activity as human plasma.

Further species characterization of factor XIII was performed by gel filtration of plasma samples on an agarose column which was calibrated for molecular weight. Factor XIII from all plasmas, except rat, guinea pig, and rabbit, eluted from the column with a partition coefficient ( $K_{av}$ ) of 0.32 and an

apparent molecular weight of 340,000. For the rodent and rabbit plasmas,  $K_{av}$  for factor XIII was 0.39 and the apparent molecular weight was 175,000. Human plasma factor XIII is composed of two polypeptide subunits, *a* and *b*, with molecular weights of 75,000 and 88,000. It appears that in human plasma, factor XIII circulates as a tetramer with the structural formula,  $a_2b_2$ , and a molecular weight of 340,000 (11). On the basis of gel filtration data, the same subunit composition seems likely for most of the animal plasmas tested. The three rodent and rabbit exceptions could have *a* and *b* subunits in a dimer complex, with the formula, *ab*. It is unlikely that plasma factor XIII of these species is analogous to platelet factor XIII (which has only *a* subunit), since the rodent plasma also reacted with monospecific rabbit antiserum to *b* subunit (see below).

Even though the mammalian and avian plasmas tested all have factor XIII activity, distinct species differences can be observed (Figs. 1 and 2). Most mammalian factor XIII molecules appear to be immunochemically similar to the human molecule, with monkey factor XIII resembling human factor XIII the most closely. Rodent and rabbit factor XIII molecules have both *a* and *b* subunits, but they appear to be smaller and are less reactive to rabbit antibody to *a* subunit. Avian factor XIII molecules react poorly to human anti-factor XIII and do not react at all to rabbit antibodies to human *a* and *b* subunits. It is interesting that avian plasmas, which are also deficient in factor XII (12) and factor VIII (13), and which have unusual fibrinogen molecules (14), also have factor XIII molecules which are particularly unique.

Although significant species differences exist, the similarities of the various animal factor XIII molecules to human factor XIII are striking and indicate that the fibrin crosslinking system is an important part of the coagulation mechanism, which has been conserved during evolutionary development. The specific variations found in avian factor XIII probably are indicative of the different line of development in those species.

**Summary.** Factor XIII activity and fibrinogen concentrations were determined in multiple, fresh, platelet-poor plasma samples from 15 species of vertebrates. Factor XIII and fibrinogen levels varied independently. In monkey, rat, cat, horse, pig, sheep, goat, goose, chicken, and turkey plasmas, factor XIII activity ranged from 56 to 96% of that found in human plasma. For guinea pig, rabbit, canine, and bovine plasmas the values ranged from 173 to 480%. Fibrinogen concentration in all species ranged from 2.16 to 4.24 mg/ml. Factor XIII of all species was neutralized to varying degrees by two human antibodies to plasma factor XIII and, except for rabbit and avian plasmas, by rabbit antiserum to human *a* subunit. Rabbit antiserum to human *b* subunit formed a precipitin line with plasma of all species except rabbit and avian.

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