Lack of effect of heparin on factor Xla [letter]

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To the Editor:

A report by Beeler et al in the May 1986 issue of Blood claims to provide evidence that "plasma concentrations of antithrombin III completely saturated with heparin would become the dominant factor in the inhibition of factor Xa." This statement appears to differ from our summary also published in Blood in October 1982 that "heparin, at concentrations used in clinical practice, does not accelerate the inactivation of human factor Xa by antithrombin III." Therefore, we decided to analyze the possible source of the differences in the two reports.

First, it is important to consider the concentrations of heparin used in both studies. The experiment of Beeler et al, in a purified system, used a single concentration of heparin, 5.88 × 10⁻⁶ mol/L, which, based on their specific activity of 350 USP U/mg and a mol wt of 27,000 daltons, yields a concentration of 14.4 USP U/mL. This "saturating" concentration is 40-fold higher than the average pharmacologic concentration of 0.35 USP U/mL found during clinical anticoagulation and fivefold higher than that employed in cardiopulmonary bypass. In our study, we used a range of concentrations from 1 to 30 U/mL in a purified system using both amidolytic and coagulant assays (not estenolytic as stated by Beeler et al). We found no difference in the rate of inactivation of factor Xa by antithrombin III at 1 U/mL heparin in the purified systems. Unlike Beeler et al, we extended our study into plasma systems and were able to confirm the lack of effect of 1 U/mL heparin on the rate of inactivation of factor Xa.

Second, we also examined the suprapharmacologic concentration of heparin used in the study of Beeler et al. We documented a maximal 4-fold increase in the rate of inactivation. In our study, at 23 °C, which assumed the plasma ATIII concentration to be 4.7 μmol/L, the inactivation rate constant without heparin was 5.4 × 10⁵ mol/L⁻¹min⁻¹ and 2.7 × 10⁴ mol/L⁻¹min⁻¹ at 30 U/mL heparin. When these experiments were performed at 37 °C (unpublished results) using the plasma antithrombin concentration reported by Beeler et al of 2.5 μmol/L for plasma concentration, the second order rate constants were 3.1 × 10⁵ mol/L⁻¹min⁻¹ without heparin and 1.51 × 10⁵ mol/L⁻¹min⁻¹ with 30 U/mL heparin. Therefore, our data in the presence of heparin is in good agreement with that of Beeler et al, whose calculated second order rate constant is 1.59 × 10⁵ mol/L⁻¹min⁻¹. However, their second order rate constant in the absence of heparin of 4.7 × 10⁵ mol/L⁻¹min⁻¹ is only 15% of our value, explaining the apparent 40-fold increase.

Several problems in their experimental procedures may explain their lower value. No controls are presented in their paper for the loss of activity with factor Xa alone, or factor Xa with heparin. Indeed the loss of activity in the absence of heparin is stated to be approximately 50% of the rate constant making its accuracy suspect.

Moreover, contact proteins demonstrate biphasic loss of activity upon contact with plastic making a single time-point correction factor in error. In the experiments performed by Beeler et al, single reactions for each point were used which may lead to greater error in calculations of the actual concentrations of initial activity. When first order kinetics are performed by subsampling from a single reaction vessel, as in our study, one can extrapolate to time zero in order to determine the actual initial concentration of enzyme in the reaction mixture. Moreover, pseudo-first-order reaction rate constants are not accurately determined unless the reaction has undergone at least one half-life, which was not shown in the paper of Beeler et al. Further, our values for the inactivation rate of factor Xa by antithrombin III without heparin in purified systems agree closely with those observed in plasma. Thus, it appears that the differences observed by Beeler et al stem from an underestimation of the inactivation rate in the absence of heparin.

Our experiments in both purified and plasma systems at pharmacologic concentrations of heparin lead us to reassert our conclusion that alpha,-antitrypsin, not antithrombin III, is the major inhibitor of factor Xa in plasma. Our previous study, in collaboration with Dr Rosenberg, indicates that heparin had minimal effect in accelerating the inactivation of plasma kallikrein by antithrombin III in purified systems and no effect in plasma. A recent study in Blood from our laboratory indicates that heparin had little effect on factor Xa in purified systems and in plasma. Finally, in a recent study presented at the American Society of Hematology in December 1986 in collaboration with Dr Peter Harpel, we showed evidence for activation of the contact system during clinical cardiopulmonary bypass in the presence of 5 U/mL heparin. Thus, we find that heparin and antithrombin III, although critically important for thrombin, factor Xa, and possibly factor IXa, play little role in the regulation of the contact system of blood coagulation.

McNeely and Griffith demonstrated in the presence of absence of 0.5 U/mL heparin, the amounts of radiolabeled factor IX cleaved in plasma after exposure to glass (contact activation), phospholipid, and calcium were identical. They conclude, in agreement with us, that heparin has no significant effect on the reaction rate between antithrombin III and the enzymes preceding factor IX in the intrinsic pathway.

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To the Editor:

Colman and Scott have provided a detailed critique of our studies. We have previously pointed out numerous experimental problems in their recent investigations of the kinetics of factor XIa-antithrombin-heparin interactions and do not find it necessary to repeat them here. In any case, one of our pictures is more compelling than repeating a myraid of arcane kinetic arguments. Western blot analyses of our reaction mixtures graphically show the generation of large amounts of factor XIa-antithrombin complex in the presence of optimal levels of heparin but no visible interaction product in the absence of the mucopolysaccharide (Figure 2'). Unpublished data indicate that the factor Xla–antithrombin complex generated in the absence of heparin would have been detected if it were present at as little as 2.5% to 5.0% of the final concentration of factor XIa–antithrombin interaction product formed in the presence of mucopolysaccharide. Thus, heparin must be augmenting the antithrombin-dependent neutralization of factor XIa by 20- to 40-fold. We cannot even claim any great originality for this particular result since Kurachi and Davie' reported a similar finding employing SDS-PAGE and have reached similar conclusions about the magnitude of the mucopolysaccharide-induced augmentation of factor XIa–antithrombin interactions. Based upon the available evidence, we believe that our estimate of the acceleration of factor XIa–antithrombin interactions by heparin (40-fold) is more likely to be correct than that of Scott and Colman (two-to-fourfold). Indeed, the original studies of this phenomenon conducted by us more than a decade ago appear to be valid.

The effects of heparin therapy on factor XIa–antithrombin interactions were not considered within our publication. However, we did suggest that antithrombin fully complexed with the endogenous anticoagulantly active heparan sulfate of the endothelium could represent a critical biologic mechanism for neutralizing factor XIa within the circulation. We believe this to be the case because of the dramatic acceleration of factor XIa inhibition by antithrombin in the presence of saturating levels of mucopolysaccharide; the unusually strong dependence of the velocity of factor XIa neutralization on selective concentration of antithrombin by mucopolysaccharide on the surface of endothelial cells [see rate equation for factor XIa neutralization], and the known relatively slow actions of other protease inhibitors within the blood. This scenario might be quite different during heparin therapy when only a small fraction of antithrombin is complexed by the anticoagulant and when the protease inhibitor might not be concentrated within a small region of the vascular tree. We hope that our results lead other investigators to examine the intriguing possibility that the endogenous heparan sulfate–antithrombin system is critically involved in regulating the in vivo activity of the contact phase of the coagulation mechanism rather than generate endless disputes over the exact extent of inhibition of factor XIa by antithrombin in the presence of heparin.

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DISCRIMINANT ANALYSIS FOR PROBABILITY OF HEMOPHILIA A CARRIERSHIP

To the Editor:

Green et al recently reported a multilaboratory study on hemophilia A carrier testing. They concluded that a linear discriminant function of Factor VIII coagulant activity (F.VIII:C) and von Willebrand factor antigen (VWF:Ag) adjusted for age and ABO blood type provided the best discrimination and that the ratio F.VIII:C/VWF:Ag gave poor results. Our experience has been different. Using a similar analysis, we find the ratio method to be just as accurate and much easier for clinical use.

We have used both a bivariate linear discriminant function, prepared as described by Elston et al., and a univariate discriminator, the natural logarithm of the ratio F.VIII:C/VWF:Ag, as described elsewhere. The ratio has several advantages. The well-documented effects of age on F.VIII:C and VWF:Ag are approximately equal, and the ratio shows no significant regression on age in groups of 49 carriers, 81 normal women, and 100 blood donors (unpublished observation). Also, no significant difference in F.VIII:C/VWF:Ag between Type O and non-O women was reported by McCallum et al.3 This was confirmed in our blood donor sample. Therefore, neither age nor ABO blood type need be introduced into the discriminant if the F.VIII:C/VWF:Ag ratio is used. Green et al apparently used age-adjusted levels in calculating their ratios.

Table 1 shows our experience with discriminant analysis using the ratio method. Carrier and normal subjects are divided into those used in calculating the discriminant (Group I) and those tested later (Group II). The discrimination is slightly better than that reported by Green et al., although our sample size is considerably smaller. We found fewer misclassifications with the ratio (6.9%) than with the bivariate discriminant produced from the same data (9.2%, data not shown). Average odds favoring carriectors were 30:1 in the carriers