Hypercoagulability in Centenarians: The Paradox of Successful Aging

By D. Mari, P.M. Mannucci, R. Coppola, B. Bottasso, K.A. Bauer, and R.D. Rosenberg

With advancing age, an increasing number of healthy individuals have laboratory signs of heightened coagulation enzyme activity. Such biochemical hypercoagulability might be the basis of either the increased thrombotic tendency occurring with age or a harmless manifestation of this process. To see whether these alterations are also present in the very elderly who had aged successfully, 25 healthy centenarians were studied and results of coagulation and fibrinolysis measurements were compared with those obtained in two control groups of healthy adults, 25 ranging in age from 18 to 50 years and 25 from 51 to 69 years. Older controls had, in general, slightly higher values of several coagulation and fibrinolysis measurements than younger controls. Centenarians had striking signs of heightened coagulation enzyme activity, as assessed directly by measuring activated factor VII in plasma (P < .01, compared with either control group) or indirectly by measuring the plasma levels of the activation peptides of prothrombin, factor IX, factor X, and thrombin-antithrombin complexes (all P < .001). Heightened coagulation enzyme activity was accompanied by signs of enhanced formation of fibrin (high fibrinopeptide A, P < .001) and secondary hyperfibrinolysis (high D-dimer and plasmin-antiplasmin complex, P < .001). Plasma concentrations of fibrinogen and factor VIII were higher than in controls, whereas other coagulation factors were not elevated. In conclusion, this study shows the very elderly do not escape the state of hypercoagulability associated with aging, but that this phenomenon is compatible with health and longevity. Hence, high plasma levels of the coagulation activation markers in older populations do not necessarily mirror a high risk of arterial or venous thrombosis.

THE PROCESS OF aging in humans is accompanied by modifications of the blood coagulation system. It is well established that the plasma concentrations of some coagulation factors (such as factor VII, factor VIII, and fibrinogen) increase progressively with age.1-3 However, these procoagulant proteins are normally present in plasma in such a large excess that the pathophysiologic significance of the relatively modest increases that occur with aging is uncertain. Using sensitive immunoassays for markers of coagulation enzyme activation, Bauer et al4-6 showed that with age advancing from 42 to 80 years, an increasing number of healthy men developed a laboratory picture of enhanced activity of coagulation enzymes, expressed indirectly by high plasma levels of the activation peptides that are cleaved from prothrombin, factor IX, and factor X when these zymogens are converted into the corresponding active enzymes.4-6

Because thrombotic disease increases with age, biochemical hypercoagulability in the elderly might be the basis for their increased thrombotic tendency. On the other hand, the phenomenon may simply be a harmless manifestation of the aging process. We attempted to evaluate these possibilities by studying coagulation and fibrinolysis in a group of healthy centenarians. Centenarians are an important natural model of successful aging and may help to identify crucial mechanisms for the maintenance of homeostasis in body systems.

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Blood sampling and processing. Venous blood was obtained between 8:00 and 10:00 AM from the antecubital veins by two-syringe techniques using 19-gauge needles. Blood samples for factor IX and X peptide assays were drawn into plastic syringes preloaded with the following anticoagulant: 38 mmol/L citric acid, 75 mmol/L sodium citrate, 136 mmol/L dextrose, 6 mmol/L EDTA, 6 mmol/L adenosine, and 25 U/mL heparin. The ratio of anticoagulant to blood was 0.2:1.0 (vol/vol). For the prothrombin fragment assay, an anticoagulant containing a synthetic thrombin inhibitor, EDTA and aprotinin was purchased from Byk-Sangtec (Dietzenbach, Germany) and the ratio of anticoagulant to blood was 0.1:0.9 (vol/vol). For fibrinopeptide A assay, an anticoagulant provided by the manufacturer of the assay kit (see below) and containing unspecified amounts of heparin and aprotinin was used (anticoagulant/blood ratio: 0.2:1.0). All of the remaining coagulation and fibrinolysis assays were carried out on blood collected in sodium citrate at a final concentration of 3.8% (wt/vol). All plasma specimens were centrifuged at 4°C for 20 minutes at 2,000g and stored at -80°C until assayed within 2 to 3 months. Assays of the factor IX and X activation peptides and the prothrombin fragment 1+2 were carried out in Boston, MA. All other assays were done in Milan, Italy.

Assays of coagulation activation markers. Activated factor VII (factor VIIa), the enzymatic form of factor VII, was measured by a one-stage, prothrombin time-based assay using a truncated soluble form of recombinant tissue factor (kindly supplied by Dr Y. Nemerson, Mount Sinai Hospital Medical School, New York, NY) that, upon repidification, reacts with factor VIIa but not with one-chain factor VII. The factor X activation peptide is cleaved from factor X when this zymogen is activated by factor VIIa-tissue factor complex or by activated factor IX-activated factor VIII-activated surface complex; the factor IX activation peptide is cleaved from factor IX when this zymogen is activated by factor VIIa-tissue factor or activated factor XI. Both peptides were then measured by double-antibody radioimmunoassay (RIA) in plasma extracted before assay to eliminate nonspecific interference from other plasma proteins. Prothrombin fragment 1+2 is a measure of the cleavage of prothrombin by activated factor X (factor Xa) being released from the aminoterminal portion of the molecule during its conversion to thrombin. It was measured in plasma by a previously described double-antibody RIA. Thrombin-antithrombin complex, a measure of thrombin generation and neutralization by antithrombin, was assayed by enzyme-linked immunosorbent assay (ELISA) using a kit from Behringwerke (Marburg, Germany). Fibrinopeptide A is a measure of the formation of fibrin being released from the α-chain of fibrinogen when thrombin converts fibrinogen to fibrin. It was measured by ELISA in plasma extracted twice with bentonite to remove fibrinogen, using a kit from Boehinger Biochemica (Mannheim, Germany).

Assays of coagulation factors. The coagulant activities of factors II, VII, VIII, IX, and X were measured by standard one-stage functional assays using congenitally deficient plasmas containing less than one percent coagulant activities. Values were expressed in percent of pooled normal plasma. Plasma fibrinogen was measured by a fibrin polymerization assay (Boehringer Biochemica). The plasma levels of the factor X and factor IX activation peptides were very elevated in centenarians (P < .001) (Table 1), with little overlap with the values measured in both younger and older controls (Fig 1). The plasma levels of the factor X and factor IX activation peptides were significantly higher in centenarians than in the control groups (Fig 1). The prothrombin fragment 1+2, thrombin-antithrombin complex and fibrinopeptide A were also very elevated in centenarians (P < .001) (Table 1 and Fig 2).

There were positive correlations between the levels of the factor IX and factor X activation peptides and between either of them and factor VIIa levels (Table 2). The levels of factor VIIa and of the factor X activation peptide were correlated with those of the prothrombin fragment 1+2 (Table 2). There were no significant correlations between the plasma concentrations of activation markers and creatinine, blood urea, and results of liver function tests, such as albumin and aminotransferases (not shown).

Coagulation factors. Plasma levels of fibrinogen, factor VII, and factor VII tended to be higher in older controls, but the differences from younger controls were statistically significant only for factor VIII (Table 1). Fibrinogen and factor VIII were significantly higher in centenarians than in either control group (Table 1). The concentrations of factor II (prothrombin), factor IX, and factor X were not significantly different or were lower than those found in the control groups (Table 1).

Anticoagulant proteins. In centenarians, the two components of the activated protein C anticoagulant pathway that are measurable in plasma (protein C and protein S) did not differ from either control group (Table 1). The tissue factor pathway inhibitor, the main regulator of the tissue factor
factor VIIa pathway, and antithrombin, the main inhibitor of thrombin, were also not different (Table 1).

**Fibrinolysis measurements.** Plasminogen, the key zymogen of the fibrinolytic system, and the two principal inhibitors of the system (plasminogen activator inhibitor type I and α2-antiplasmin) were not elevated in centenarians (Table 1). However, centenarians had clear signs of hyperfibrinolysis, expressed by very high levels of D-dimer (an index of plasmin activity on cross-linked fibrin) and plasmin-antiplasmin complex (an index of plasmin formation and neutralization) (both *P < .001* compared with either control group) (Table 1 and Fig 3). The latter two measurements were positively correlated with each other and with the indexes of thrombin generation and activity (Table 2).

**DISCUSSION**

A large proportion of centenarians had laboratory signs of coagulation activation, expressed by high levels of enzymes, activation peptides, and enzyme-inhibitor complexes. Procoagulant proteins, such as fibrinogen and factor VIII, that epidemiologic studies have shown to be useful predictors of cardiovascular disease in middle-aged individuals, were also elevated in centenarians. Yet, these individuals were healthy and their striking biochemical abnormalities were not associated with a disease state, even though the presence of asymptomatic atherosclerosis cannot be ruled out.

Elevated plasma concentrations of markers of coagulation activation might be caused by increased production or by decreased clearance. In their study of age-related changes in activation markers, Bauer et al previously measured the half-life of the prothrombin activation fragment after its infusion into healthy and their striking biochemical abnormalities were also elevated in centenarians. Yet, these individuals were healthy and their striking biochemical abnormalities were not associated with a disease state, even though the presence of asymptomatic atherosclerosis cannot be ruled out.

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HYPERCOAGULABILITY IN CENTENARIANS

Fig 1. Plasma concentrations of activated factor VII, factor X activation peptide, and factor IX activation peptide in 25 centenarians (cen, ○), 25 controls aged 51 to 69 years (con 2, □), and 25 controls aged 18 to 50 years (con 1, ●). The solid horizontal lines denote the geometric mean values of each parameter in each group.

Fig 2. Plasma concentrations of prothrombin fragment 1 + 2, fibrinopeptide A, and thrombin-antithrombin complex. See Fig 1 for symbols.

cate excessive generation of factor Xa under in vivo conditions. The levels of factor X activation peptide were as high as those found in patients with disseminated intravascular coagulation. In normal individuals, when there is no pathologic trigger of blood coagulation and no need for enhanced hemostasis, generation of factor Xa occurs predominantly through the enzymatic action of the factor VIIa-tissue factor complex, which activates factor X. The factor VIIa-tissue factor pathway is also largely responsible for the activation of factor IX. In centenarians, the plasma levels of the factor IX activation peptide were very high, even higher than those found by Bauer et al., using the same assay method, in elderly individuals aged 71 to 80 years (mean values: 391...
Table 2. Correlation Coefficients (r values) Between Markers of Coagulation and Fibrinolysis Enzyme Activity in Centenarians

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<th></th>
<th>FXP</th>
<th>FIXP</th>
<th>F1+2</th>
<th>FPA</th>
<th>TAT</th>
<th>D-D</th>
<th>PAP</th>
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<tr>
<td>Factor VIIa (FVIIa)</td>
<td>0.55*</td>
<td>0.45†</td>
<td>0.60*</td>
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<td>Factor X activation peptide (FXP)</td>
<td>0.62†</td>
<td>0.66†</td>
<td>0.44†</td>
<td>0.52*</td>
<td>0.38</td>
<td>0.45†</td>
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<tr>
<td>Factor IX activation peptide (FIXP)</td>
<td>0.34</td>
<td>0.41†</td>
<td>0.35</td>
<td>0.29</td>
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<tr>
<td>Prothrombin fragment 1+2 (F1+2)</td>
<td></td>
<td>0.47†</td>
<td>0.51*</td>
<td>0.51*</td>
<td>0.46†</td>
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<td>Fibrinopeptide A (FPA)</td>
<td></td>
<td></td>
<td>0.58*</td>
<td>0.47†</td>
<td>0.46†</td>
<td>0.44†</td>
<td>0.54*</td>
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<td>Thrombin-antithrombin complex (TAT)</td>
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<td>D-dimer (D-D)</td>
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<td>Plasmin-antiplasmin complex (PAP)</td>
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* P < .01.
† P < .05.
‡ P < .001.

pmol/L in centenarians v 274 in 71- to 80-year-old individuals). In centenarians, there was a positive correlation between the elevated concentrations of factor X activation peptide and those of the factor IX activation peptide, suggesting that they are associated phenomena. Therefore, it is likely that both are the results of the heightened enzymatic activity of factor VIIa-tissue factor complex, as in physiologic conditions. However, the elevations of factor VIIa concentrations were less marked than those of the two activation peptides and the correlations weaker. Because factor VIIa has a high affinity for its cofactor tissue factor expressed on cell surfaces,15 the measurement of the levels of this enzyme in plasma may reflect only a small portion of the enhanced activity of the factor VIIa-tissue factor complex exerted mainly at the level of the cell surfaces.

Consistent with the findings of heightened factor X activation, the late reactions of blood coagulation were also activated in centenarians. Prothrombin fragment 1+2, the product of prothrombin cleavage by factor Xa, was markedly elevated. It is impossible to compare the high values of this fragment in centenarians with those found by Bauer et al14 in younger individuals aged 71 to 80 years because the current assay uses a different antibody that gives lower values in healthy individuals. There were also signs of enhanced thrombin generation (high thrombin-antithrombin complexes), fibrin formation (high fibrinopeptide A), and hyperfibrinolysis (high D-dimer and plasmin-antiplasmin levels), which were probably the expression of excessive fibrin formation and dissolution with values similar to those usually found in patients with disseminated intravascular coagulation.

In an attempt to explain mechanistically the dramatic activation of the coagulation system found in centenarians, we surmise that in these individuals there is an enhanced expression and availability of tissue factor, which would, in turn, augment factor VII activation. Tissue factor is usually not expressed in the intact vasculature,16 but perhaps changes associated with aging can modify the physiologic situation, induce the expression of tissue factor, and thereby accelerate the factor VIIa/tissue factor pathway. The natural regulatory mechanisms may thus be overwhelmed, leading to a state of marked coagulation system activation. The nature of these changes can only be speculative at the moment. Tissue factor expression can be induced by a variety of agonists, such as endotoxins, inflammatory cytokines, activated lymphocytes, growth factors, and oxidized low-density lipoproteins.16 It has been shown that in centenarians there is a marked increase in the number of circulating activated T lymphocytes (CD3 and HLA-DR positive),17 which may interact with macrophages-monocytes and/or endothelial cells and upregulate the expression of tissue factor in these cells.18 This mechanism for enhanced tissue factor expression has been
documented to cause the hypercoagulable state associated with unstable angina.19

Despite high coagulation enzyme activity leading to discrete fibrin formation and secondary hyperfibrinolysis, centenarians were healthy and had no current or past thrombotic episodes. In general, the clinical significance and predictive value of biochemical markers of hypercoagulability remain to be established and are currently being evaluated in prospective studies carried out in middle-aged individuals. Because this investigation shows that marked alterations of these markers are compatible with health and longevity, plasma levels of coagulation activation markers in a study population must always be interpreted in concert with an appropriate control group, and high levels do not necessarily mirror a high risk of arterial or venous thrombosis. The same reasoning can be applied to procoagulant proteins, such as factor VIII and fibrinogen, which are predictors of cardiovascular disease in middle-aged individuals and yet are elevated in healthy centenarians.

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