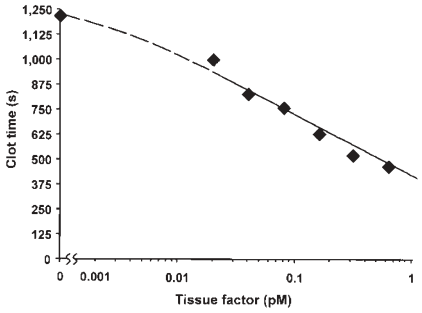

Figure 1 Titration of relipidated tissue factor in contact pathway–inhibited whole blood. Varying concentrations of tissue factor were added to CTI-blood, and clotting times were evaluated visually.

assess plasma clotting activity of blood tissue factor[8]. The concentration of a soluble form of blood tissue factor was almost five orders of magnitude higher than that reported by the same authors in plasma. But even under these conditions, the authors detected only limited activity, which was substantially lower than that observed for full-length tissue factor; this limited level of activity is similar to that reported for the extracellular domain of tissue factor[7]. Additionally, the limited decrease in the clotting time reported by Bogdanov *et al.*[8] (from 230 to 150 s) may be caused by uncontrolled contact pathway activity (that is, by the surface of the tube). Conventional assays with clotting times of >100 s without use of a contact pathway inhibitor are questionable. The hypothesis that soluble blood tissue factor is required to promote thrombus growth is more speculative than supported by experimental data. Although Bogdanov *et al.*[8] observed the accumulation of soluble tissue factor in the growing thrombus, no data suggesting the activity of this tissue factor were provided; potentially, soluble tissue factor may act as an inhibitor of coagulation by binding FVIIa into an inactive complex.

The addition of 5 pM active tissue factor to blood provides a clotting time of ~5 min (current study and previously published[5]), that is, similar to that observed in 'Simplate' bleeding assays[9]. The observation that active tissue factor in blood of healthy individuals does not exceed a concentration of a few femtomolar suggests that if clotting were to require blood-borne tissue factor, the concentration of this protein at the site of injury would have to increase by at least three orders of magnitude above that normally present. As a consequence, over the time required to accumulate enough blood tissue factor activity at the site of the injury, a life-threatening blood loss (>3 L) would occur even in the case of minor damage to the vasculature.

In conclusion, we believe that there are no reliable data suggesting the presence of significant quantities of functional tissue factor in the blood of healthy individuals. Blood donors were recruited according to a protocol approved by the University of Vermont Human Studies Committee, and their consent was obtained.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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The authors reply:

We do not question the validity of the measurements presented by Butenas and Mann, particularly because it had been previously shown that tissue factor antibodies did not prolong blood clotting times[1]. Nor do we dispute their statement that alternatively spliced tissue factor, which does circulate[2], has minimal activity, although the tested preparations were derived from *Escherichia coli*, perhaps thereby underestimating its activity. Moreover, this protein is found in all thrombi, thus supporting its further study.

Whole-blood clotting times are performed using nonflow conditions and therefore do not address the participation of blood-borne tissue factor in thrombogenesis, for which there is considerable evidence, apparently ignored by Butenas and Mann. Giesen *et al.*[3] showed that *ex vivo* deposition of fibrin-containing thrombi on a collagen surface was virtually eliminated by a monoclonal antibody to tissue factor. Inasmuch as these experiments used no tissues other than blood, one must conclude that blood contributed the tissue factor. An *in vivo* model developed by Himber *et al.*[4], in which thrombus growth within rabbit jugular veins or within a silastic jugular-jugular shunt was evaluated by radiolabeled fibrin accretion, rose linearly with time until a monoclonal antibody to rabbit tissue factor was perfused, after which there was essentially no thrombus growth. Further, a mouse transgenic model supported the role of circulating tissue factor in thrombogenesis[5].

A fundamental difference between the cited experiments and the current data of Butenas and Mann is that the latter measured *in vitro* blood coagulation under nonflow conditions whereas the former studied thrombus formation in flowing blood. It is abundantly clear that shear forces caused by laminar flow are involved in thrombogenesis[6]. A potentially critical observation emphasizing the role of shear force is that fresh platelets do not stain for tissue factor; however, following perfusion with tissue factor–positive cells under physiologically relevant flow conditions, platelet aggregates were markedly positive for tissue factor[7].

In summary, we do not dispute the results presented by Butenas and Mann; we do, however, seriously question the applicability of data derived from static assays to thrombogenesis, as well as the underlying rationale for this extrapolation.

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