

Figure 1. Mucocutaneous Manifestations of Necrolytic Migratory Erythema.

Bullous, erosive oral mucositis with areas having lichenoid features are visible on the tongue and the upper and lower lips (Panel A) and inside the right cheek (Panel B). The patient's entire body, including the back, is covered with purplish, confluent erythematopapulous scaling and crusting lesions with a keratotic surface (Panel C). Histologic analysis of the skin (Panel D, left) shows hyperparakeratosis and spongiosis, accompanied by the presence of necrotic keratinocytes, and vascular proliferation. Histologic analysis of the oral mucosa (Panel D, right) shows a prominent neutrophilic, eosinophilic, and plasmacellular infiltrate (hematoxylin and eosin).

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Prothrombin Time for Detection of Contaminated Heparins

TO THE EDITOR: The recent "heparin scandal" resulted from the use of contaminated heparin that caused serious adverse events including death.¹ The contaminant was identified as synthetically oversulfated chondroitin sulfate (OSCS).² Despite the missing final proof of a cause-and-effect re-

lationship, OSCS was shown to have pharmacologic effects that may contribute to the observed allergic-type reactions.³ Furthermore, OSCS is suspected to be responsible for an observed increased incidence of heparin-induced thrombocytopenia type 2.⁴ Revised monographs about heparin in

U.S. and European pharmacopeias now include two mandatory tests to identify OSCS — nuclear magnetic resonance spectroscopy and capillary electrophoresis. However, these methods are technically challenging, not widely established, and not easily applicable in clinical practice.

We report on the use of prothrombin time to detect OSCS contamination in both unfractionated heparin and low-molecular-weight heparins. As expected, only plasma concentrations of 5 μg per milliliter (about 1 IU per milliliter) or greater of unfractionated heparin and 10 μg per milliliter of unfractionated heparin contaminated with 17.4% OSCS (contaminated unfractionated heparin) slightly prolonged the prothrombin time (by 9% and 12%, respectively) (Fig. 1A). At concentrations of 5 μg per milliliter or less, contaminated unfractionated heparin, but not unfractionated heparin, shortened the prothrombin time. However, it is essential to use a prothrombin-time reagent that does not contain a heparin-neutralizing compound such as polybrene, since this compound also antagonizes the effects of OSCS.

To verify these observations, we synthesized OSCS by sulfating chondroitin 4-sulfate.⁵ Whereas chondroitin 4-sulfate did not modify the coagulation time, OSCS showed weak anticoagulant activity at concentrations of more than 10 μg per milliliter, but it shortened the prothrombin time more than contaminated unfractionated heparin. Unfractionated heparin supplemented with 17.4% OSCS produced effects that were identical to those of contaminated unfractionated heparin. The reduction of the coagulation time by unfractionated heparin containing OSCS increased with the increasing content of OSCS (Fig. 1B). Contaminated low-molecular-weight heparins such as enoxaparin were also detectable (Fig. 1B).

Highly sulfated polysaccharides such as OSCS are known to act as anticoagulants, but they also induce contact activation promoting *in vitro* coagulation.³ However, with activated partial-thromboplastin time assays (also used for the assay of heparin in the U.S. and European pharmacopeias) in which the coagulation is initiated by contact activators, such effects cannot be recognized. In

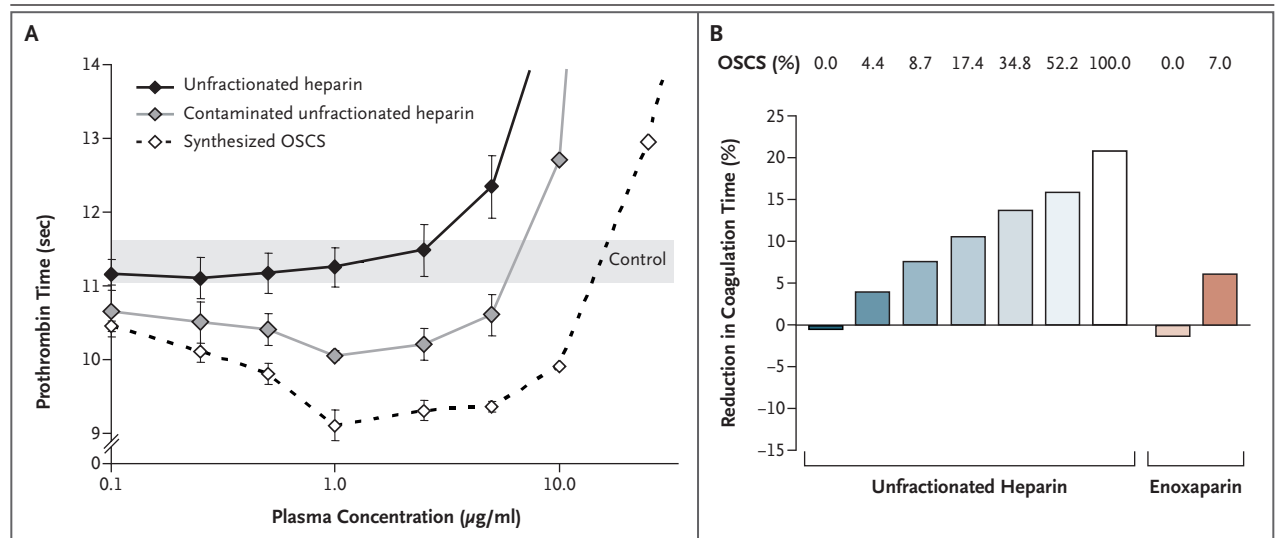


Figure 1. Reduction of Coagulation Time in the Prothrombin-Time Assay by Heparins Containing Oversulfated Chondroitin Sulfate.

Pooled human plasma samples were treated with unfractionated heparin (Heparin Na, lot no. 73508019, Novartis), unfractionated heparin contaminated with 17.4% oversulfated chondroitin sulfate (OSCS) (Rotexmedica), or synthesized OSCS by mixing 10 μl of solution containing a concentration 10 times higher than the plasma concentration in 0.9% sodium chloride with 90 μl of plasma. The control was plasma supplemented with sodium chloride. Panel A shows the concentration-dependent effect of the samples on the prothrombin time. The reagent used was Thromborel S (Dade-Behring), and the instrument was a KC10 Coagulometer (Amelung). Similar results were obtained by turbidimetry (Behring Coagulation System, Dade-Behring). The standard deviations of the coagulation times were generally 2.5% or less. Maximum shortening of the prothrombin time was observed at a plasma concentration of 1 μg per milliliter. I bars denote standard deviations. Panel B shows the percentage reduction of the prothrombin time by unfractionated heparin, unfractionated heparin containing 4.4 to 52.2% OSCS, OSCS, enoxaparin, and enoxaparin containing 7.0% OSCS, each at a plasma concentration of 1 μg per milliliter. The percentage reduction was calculated with the formula $100 - ((\text{mean sample coagulation time} \div \text{mean control coagulation time}) \times 100)$.

contrast, the prothrombin time is suitable for determining such procoagulant effects for two reasons. First, the coagulation is induced by thromboplastin. Second, the prothrombin time is quite insensitive to heparins, so that any procoagulant effect of OSCS is not obscured.

In conclusion, the prothrombin time could be used in a validated form as a sensitive screening test for the quality control of heparins. In clinical practice it could serve as a simple and fast assay to check the applied heparin when heparin-induced thrombocytopenia type 2 or allergic-type reactions develop in a patient who is receiving heparin.⁴

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