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THE IMPROVED ONE-STAGS ASSAYS FOR FACTORS V AND X ACTIVITIES.

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ABSTRACT

Factors II,V, and X participate in both the intrinsic and extrinsic coagulation systems and their activities can be determined by either the activated partial thromboplastin time (APTT), prothrombin time (PT) or Russell's viper venom (RVV) techniques. Conventionally, the activity of factor X is determined by the RVV technique and that of factors II and V are determined by the PT technique. The relative sensitivity of the onestage assays for these coagulation factors employing the APTT, PT and RVV techniques were re-evaluated. With the utilization of the same specific substrates and normal control plasma sample, the sensitivity of each test system was judged by the slope of its standard reference curve. It was found that the APTT technique offerred no particular advantage over the other techniques and its clotting times were inconveniently long. The slope of the RVV reference curve for factor V activity was about twice that of the PT reference curve. Vice versa, the slope of the PT reference curve for factor X activity was better than that of the conventional RVV reference curve.

The results obtained indicated that, for factor V assay, the conventional PT technique should be replaced by a more sensitive RVV technique. For factor X assay, the PT technique is more sensitive than the conventional RVV technique but should be used only when the congenital factor X deficiency plasma is employed as substrate.

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INTRODUCTION

At the present time, the activities of most of the coagulation factors are conveniently determined by one-stage assay technique utilizing specific coagulation factor-deficient plasma as substrate. Generally, the activity of factor X is determined by the Russell's viper venom (RVV) technique [1] and that of factors II and V are determined by prothrombin time (PT) technique (2,3). Since factors II, V and X participate in both the intrinsic and extrinsic coagulation systems, their activities can be determined by either the activated partial thromboplastin time (APTT), PT or RVV techniques. The relative sensitivity of the one-stage assays for determination of their activities employing the APTT, PT and RVV techniques were re-evaluated.

MATERIALS AND METHODS.

The normal control plasma (Verify), APTT (Platelin plus activator) and PT (Simplastin) reagents were obtained from General Diagnostics (Morris Plains, N.J.). The specific congenital factor-deficient plasmas were obtained from DADE (American Hospital Supply, Miami, Fla.) and the RVV reagent was supplied by Diagnostic Reagents Ltd. (Thame, Oxon, England). Dilutions of normal control plasma were made in Imidazole buffered saline citrate (IBSC) solution. The reference curves of factors V and X activities were made by the APTT, PT and RVV techniques [1,3-5].

RESULTS.

For factor V assay, the slopes of the APTT and RVV reference curves were almost twice that of the PT reference curve. The clotting times of the APTT test were too long. For factor X assay, the slope of PT reference curve was apparently higher than that of the RVV curve.

COMMENTS.

The sensitivity of the assay method is reflected by the slope of the reference curve [6]. While simple in theory and procedure, the one-stage assay may often be inaccurate by lack of sensitivity, as evident by a flat reference curve. As judged by the slope of the refernce curves, the APTT technique offered no particular advantage over the other two techniques. In addition, its prolonged clotting times makes it inconvenient and inaccurate when the activity of the coagulation factor to be measured is low. The RVV technique appears to be the most sensitive assay for factor V activity and should be considered as the choice method in place of the conventional PT technique. Although the PT technique is apparently more sensitive than the RVV technique for

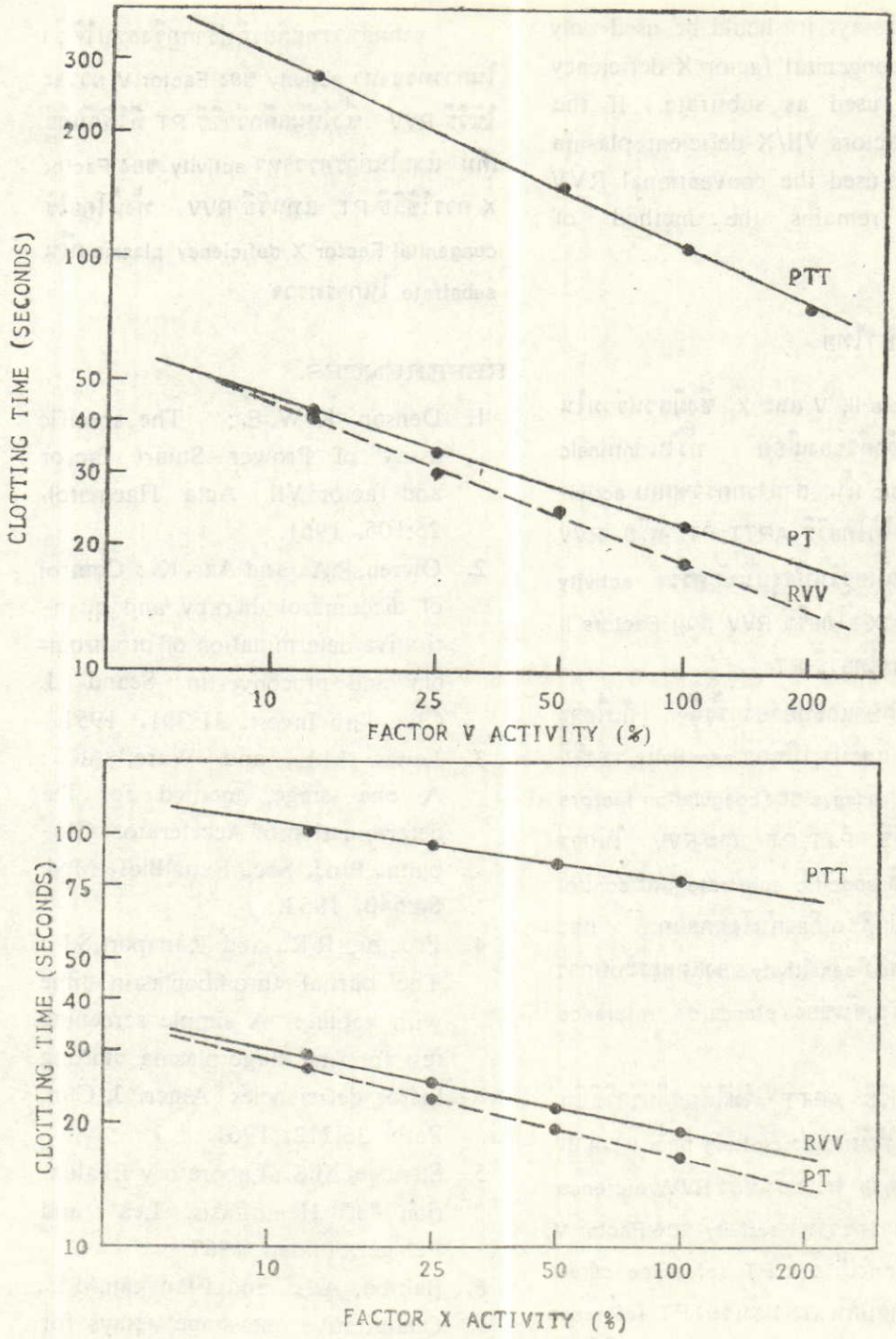

FIGURE I: Standard reference curves for factors V and X assays by APTT, PT and RVV techniques.

factor X assay, it should be used only when the congenital factor X deficiency plasma is used as substrate. If the artificial factors VII/X-deficient plasma substrate is used the conventional RVV technique remains the method of choice.

ย่อเรื่องภาษาไทย

Factors II, V และ X ซึ่งมีส่วนร่วมในระบบการแข็งตัวของเลือด ทั้งใน intrinsic และ extrinsic นั้น สามารถตรวจสอบ activities ของมันได้โดยวิธี APTT, PT หรือ RVV เท่าที่ปฏิบัติกันอยู่ในปัจจุบันมักตรวจ activity ของ Factor X โดยวิธี RVV ส่วน Factors II และ V ตรวจโดยวิธี PT

การศึกษาและทดลองครั้งนี้ ก็เพื่อจะตรวจสอบ และประเมินค่า senstivity ของวิธี one—stage assays ของ coagulation factors เหล่านี้โดยวิธี APTT, PT และ RVV ในการทดลองได้ใช้ specific substrate และ control plasma ค่าปกติชุดเดียวกันโดยตลอด และประเมินผลของ sensitivity ของแต่ละระบบการตรวจจากความชันของ standard reference curve

พบว่าวิธี APTT ไม่ให้ผลดีกว่าวิธีอื่น นอกจากนี้ยังให้ผลของ clotting time ยาวนานกว่าปกติอีกด้วย ความชันของ RVV reference curve ในการตรวจหา activity ของ Factor V นั้นมากเป็นสองเท่าของ PT reference curve และในทางกลับกัน ความชันของ PT reference ในการตรวจหา activity ของ Factor X ก็ดีกว่าของ RVV reference curve

จากผลการทดลองที่ปรากฏจึงสรุปได้ว่าในการตรวจหา activity ของ Factor V ควรจะใช้วิธี RVV ซึ่งให้ผลดีกว่าวิธี PT ที่ใช้กันอยู่เดิม และในการตรวจหา activity ของ Factor X ควรใช้วิธี PT แทนวิธี RVV ทั้งนี้โดยใช้ congenital Factor X deficiency plasma เป็น substrate ในการตรวจ

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