COLD-PROMOTED ACTIVATION OF FACTOR VII AND SHORTENING OF THE PROTHROMBIN TIME

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INTRODUCTION

The previous speakers have summarized our knowledge concerning the biochemistry, function, synthesis and molecular biology of the coagulation factors II, VII, IX, X, and protein C and S. In particular, they have highlighted the role of the gamma carboxyglutamic acid, the biosynthetic rates and the specific steps in the activation of these proteins. This has led to a clear understanding of the biologic effect of the vitamin K antagonists. The studies of tissue factor have elucidated various aspects of this apoprotein and its function. Yet with this great body of knowledge, there is still a great deal of consternation about the test most commonly utilized to clinically monitor the effects of vitamin K antagonists, the prothrombin time.

As you have just heard, the calibration of therapeutic range by the International Normalized Ratio has been proposed so that the intensity of the anticoagulation can be better standardized throughout the world. Recent difficulties have been recognized in interpreting the blood test which monitors the potency of the anticoagulation. A variety of the important aspects which affect the prothrombin time must be considered in the decision-making process of the clinician in administering this drug to patients and maintaining a safe level of anticoagulation.

The prothrombin time is the most common coagulation procedure performed in the hemostasis laboratory. It is used as a measure of the extrinsic coagulation system to monitor warfarin anticoagulant therapy, as an indicator of hepatic disease, to detect inhibitors of blood coagulation and as a general screening test for blood coagulation. It is important in all blood coagulation assays that the sample be obtained without inducing cellular activation, coagulation factor activation or activation of other systems which may modify hemostasis, i.e., fibrinolysis, prekallikrein-bradykinin, etc. It is hoped that the sample collected for blood coagulation analysis is a reflection of the blood circulating in the patient, i.e., neither in-vitro activation nor other artifacts have occurred in the sample. In an attempt to standardize the pretest variables, the National Committee on Clinical Laboratory Standards (NCCLS) has provided a guideline for the collection, transport and preparation of blood specimens for coagulation testing and performance of coagulation assays,

1, and a second document on the proposed guideline for the one-stage prothrombin time.2 These two guidelines are intended to increase the uniformity in the collection, storage and preparation

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of blood or plasma before and during coagulation testing. Adherence to these guidelines should reduce the number of potential artifacts in the pretest and testing period.

Some of the major areas of concern which have been examined are the concentration of sodium citrate anticoagulant most efficacious for performing the prothrombin time, the type of collection system most suited for reducing or eliminating in vitro activation of blood, and the processing and storage of plasma or whole blood to maintain the blood as near as possible to its in-vivo state.

Studies of the sodium citrate anticoagulant at 109 and 129 mM (3.2% and 3.8%, respectively) have indicated no major differences in the prothrombin time in normal individuals or in those individuals who were receiving oral anticoagulants.3 Four collection systems for analyzing the prothrombin time, a borosilicate evacuated system, a siliconized borosilicate evacuated system, a new generation of siliconized borosilicate evacuated tubes and the use of polypropylene or polystyrene tubes were examined. The prothrombin time of blood collected in the borosilicate or the siliconized borosilicate tubes had a progressive shortening of the prothrombin time.3-5 In general, by 2 hours the prothrombin time of normal or anticoagulated blood had shortened by 12-15% and by 4 hours was shortened by 22-28% (Figure 1). The blood collected in the new generation of siliconized borosilicate tubes showed a marked improvement in the in-vitro shortening of the prothrombin time.

Normal blood showed insignificant amounts of shortening over a 4- to 6-hour incubation period, and the blood from the majority of anticoagulated patients (60%) showed no significant activation when held at 40°C for 4 hours.6 At 4 hours 40% of the patients had a 10-27% shortening of the prothrombin time (Figure 2). At 6 hours this decrease in the prothrombin time varied between 12-42%. When blood was collected in polypropylene tubes and the prothrombin time analyzed, less than 10% shortening of the prothrombin time was seen in patients receiving oral anticoagulants and in normal individuals. All samples are stable for at least 6 hours either as plasma separated from cells or whole blood in polypropylene tubes.

The Dutch group and the group from Manchester, England, have shown similar results when the blood of normal individuals or patients receiving oral anticoagulant therapy had been analyzed in an evacuated tube system.4,7 van den Besselaar and Loeliger found that the prothrombin time was shortened when the blood was collected and maintained at ambient temperatures.4 Their results are very similar to our results in that polystyrene tubes showed minimal activation while either glass or a variety of siliconized borosilicate evacuated tubes showed significant shortening of the prothrombin time. They showed that acid treatment of siliconized glass markedly reduced the in-vitro shortening of the prothrombin time. The glass tubes had to be acid treated for 12 days.

van den Besselaar et al. have stated that the new siliconized process of the evacuated tube system is suitable for long-term storage of blood for the determination of the prothrombin time.8 These results seem to be in disagreement with those mentioned above,8 and this may be due to the fact that pooled plasma was used in their studies to determine the stability of plasma. It is not stated how many individual patients were studied over a time course, and the paper describes only 2 patients studied in this manner. These data, then, may not be much different from our observations since approximately 1 out of 3 patients will have a marked shortening of their prothrombin time in blood collected in these evacuated tubes.

Thomson, who has similar data concerning borosilicate or siliconized borosilicate tubes, has indicated that there is not sufficient evidence on the reliability of the evacuated tube system. She has suggested that it might be worthwhile, if possible, to employ the system most commonly used in Europe and the United Kingdom, i.e., a syringe and a specially prepared tube for coagulation studies.7