Transitory Variations in Serum Prothrombin Activity. (19502)

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When blood clots in the test tube, only a portion of the prothrombin is consumed. The amount of prothrombin activity remaining in the serum has recently been shown to have considerable clinical significance(1). Deficiencies in the anti-hemophilic globulin (thromboplastinogen) or the platelet-tissue thromboplastic enzyme result in less consumption of prothrombin and consequently more residual serum prothrombin activity. Quick (1) has demonstrated that serum prothrombin activity can be estimated by using deprothrombinized plasma as a source of fibrinogen. His data indicate that incubation of the whole clot may alter serum prothrombin activity quite differently from similar incubation of the separated serum. This fact is probably responsible for much of the difficulties and multiplicity of technics suggested for the estimation of prothrombin consumption(3). Quick suggests that prothrombin consumption be determined by incubating 3 aliquots of blood for various time intervals before separation of the serum and doing 7 estimations of prothrombin activity of these 3 sera after various periods of further incubation. This paper presents data describing serially the pattern of serum prothrombin activity following varying periods of incubation before and after separation of the serum from the clot. These data are sought in order to determine the conditions which will best reflect the prothrombin-consuming property of a specimen of blood. Knowledge of these conditions may make it possible to estimate prothrombin consumption reliably and reproducibly by a single determination on one aliquot of blood. Data are also presented on the use of fibrinogen in place of "prothrombin-free" rabbit plasma for determining serum prothrombin activity. A standardized, dry preparation containing fibrinogen, thromboplastin, $CaCl_2$, and NaCl in optimal amounts is employed.* The preparation is free from prothrombin, oxalate, and citrate(2) and is stable at 5°C for at least several months. This single reagent mixture is prepared for use by the addition of water, and permits the determination of serum prothrombin activity as simply as the usual plasma prothrombin time. The normal range of plasma prothrombin time with the thromboplastin employed is from 14 to 17 seconds.

A. *Effect of incubation before and after separation of serum from the clot. Method.* Three ml aliquots of blood were placed in a 37.5° water bath promptly after venipuncture. At various time intervals each aliquot was centrifuged at 3000 RPM for 5 minutes, the serum separated, and returned to the water bath. The serum prothrombin time of each aliquot of serum was estimated by adding 0.1 ml serum to 0.2 ml reagent mixture* at 37°. Estimations were made one minute after separation, and at intervals thereafter. *Data.* Fig. 1 illustrates the results obtained with normal blood. The beginning of each bar represents the time each aliquot of blood was incubated before centrifugation; the bar represents the period of centrifugation; the dot the serum prothrombin time one minute after separation of the serum; the curves the prothrombin time of each serum aliquot on subsequent incubation. There appear to be 3 phases of activity. 1) Serum separated as

* The reagent employed is a combination of thromboplastin and fibrinogen prepared as follows: Thromboplastin is first prepared as an aqueous extract of dried rabbit brain and lung tissue, and includes calcium chloride. Volumes are adjusted to give a preparation containing 0.0125 M calcium chloride and yielding a normal plasma prothrombin time of 15 seconds. Fibrinogen is precipitated from deprothrombinized oxalated beef plasma, and lyophilized to a dry state. This fibrinogen is placed in vials each containing 7 mg of clottable protein. Extract of rabbit brain and lung tissue is then added to each vial and the combination frozen and dried from the frozen state.

The authors wish to thank Dr. R. Kroc and E. White of Chilcott Laboratories, Morris Plains, N. J., for preparing this reagent.

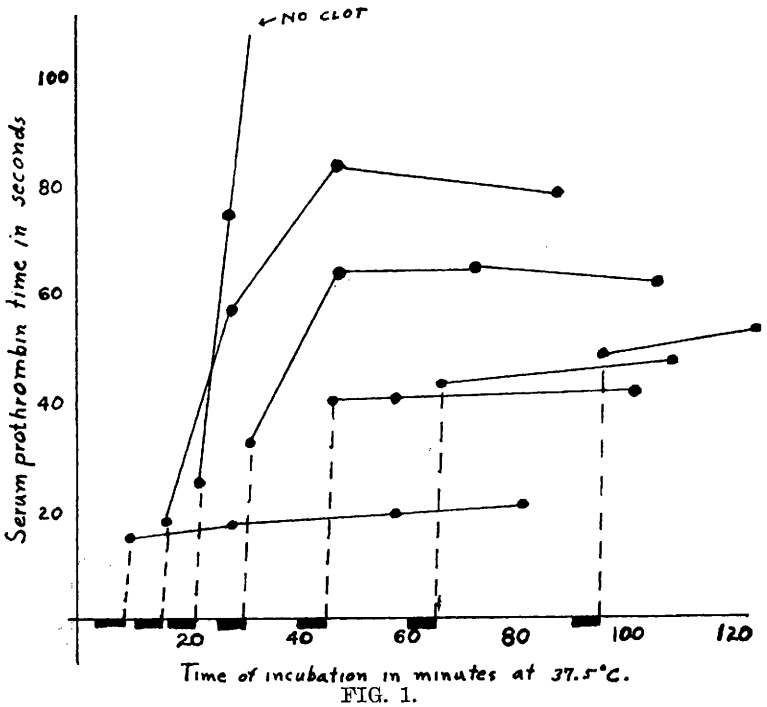

FIG. 1.

soon as normal blood clots yields an apparent serum prothrombin time equal to or shorter than that of the original plasma, and loses that activity slowly on subsequent incubation. 2) If the separation is delayed for several minutes, there is considerable loss of prothrombin activity on subsequent incubation of the serum. 3) If a period of about one hour or more is allowed to elapse before the serum is separated, the prothrombin activity of the subsequently incubated serum remains relatively constant. Reproducible, consistent serum prothrombin estimations are obtained only after this relatively stable state is reached. In spite of incubation, blood from 5 known hemophilic subjects did not go beyond the first phase of high prothrombin activity. Because of these observations, the method described below has been adopted for routine estimations of serum prothrombin time (prothrombin consumption).

B. *Comparison of serum prothrombin time of normal and hemophilic blood. Method.* Two to 3 ml of venous blood is obtained with a minimum of trauma and is introduced gently into a 12 mm diameter glass tube. The specimen is placed in a water bath at 37.5°C for one hour. It is then centrifuged at 3000 RPM for 5 minutes. The serum is separated and returned to the water bath for several minutes. The serum prothrombin time is then estimated by adding 0.1 ml of serum to 0.2 ml of the thromboplastin-calcium-fibrinogen reagent,* and determining the time necessary for clot formation with the aid of a wire loop as described for routine plasma prothrombin time determinations. The end-point is sharp and is preceded by a fine flocculation.

Data. Twenty-five normal male subjects between the ages of 18 and 25 years were used for control studies. Blood was obtained by free flow into a test tube through a 19 gauge needle. The serum prothrombin time of 24 of these subjects averaged 41.1 seconds (range from 33 to 54 seconds) with a standard deviation of 2.1 seconds. One subject, not included in the average, had a serum prothrombin time of 89 seconds. Repeated estimations of the serum prothrombin times of 5 hemophilic subjects were never above 25 seconds, and usually between 14 and 20 seconds. The serum prothrombin time of one hemophilic

subject rose to 29 seconds several hours after a transfusion of 500 ml of normal whole blood. Two days later the serum prothrombin time had returned to 19 seconds. The serum prothrombin time of chronically ill hospitalized subjects averaged about 10 seconds higher than the young healthy control group. The significance of relatively high serum prothrombin time values found in some chronic disease states has not yet been determined.

Discussion. The 3 phases of serum prothrombin activity described in this report are suggestive of the presence of a substance which develops as clotting progresses, and interferes with prothrombin activity of serum if separated with the serum. This substance is apparently removed by the clot if the serum is not separated from the clot. Thus, during the first phase, only a small amount is formed and promptly neutralized. During the second phase some of the active substance is separated with the serum, and may destroy some of the protein moieties which contribute to prothrombin activity. If the serum remains with the clot, however, (third phase), all of this substance may be removed by the clot. This sequence is consistent with the concept that the substance is thrombin, since early in coagulation, thrombin is present only in traces (phase 1). Later it develops in appreciable amounts which may be separated with the serum (phase 2). This thrombin in serum, free from its normal adsorbents (fibrinogen, fibrin, and platelets), may exert a binding or proteolytic effect on other serum proteins, and may destroy some component of the prothrombin complex. However, if the serum is permitted to remain in contact with the clot (phase 3), the thrombin is adsorbed by the clot, leaving the residual prothrombin unaltered in the serum. This concept removes the necessity for postulating a new inhibitor substance to explain the observed facts. It is consistent with the data reported by others (3). Thus phase 1 represents only the initiation of prothrombin consumption; phase 2 represents a labile period of free thrombin activity; phase 3 represents a relatively stable period of residual prothrombin, reflecting the prothrombin consuming capacity of the blood.

The data demonstrate that prothrombin consumption can be estimated adequately by determining serum prothrombin time after one hour of clot incubation with the aid of a single, relatively stable, dry reagent preparation. The normal serum prothrombin time obtained with the preparation is about 40 seconds, as compared with about 25 seconds by the Quick technic. This difference is probably due to the difference in thromboplastin preparation plus the fact that only fibrinogen, free from extrinsic accessory clotting factors, is added. Much of the clinical significance of altered prothrombin consumption remains to be determined.

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Received January 14, 1952. P.S.E.B.M., 1952, v80.

Comparison of Parenteral and Oral Protein Feeding on Radiation Susceptibility in Protein-Depleted Rat. (19503)

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The damage that is apparent histologically in the intestinal epithelium following radiation has stimulated several investigations of intestinal absorption following radiation. Meed, Decker, and Bennett found no marked impairment of fat absorption from the intestine due

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