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Note on Calcium Ion Requirements for Threone Activity.* (22555)

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The conversion of prothrombin to thrombin occurs in several ways. Two possibilities may be represented by the following equations:

$$\text{Prothrombin} \xrightarrow[\text{Ac-globulin}\ \text{Thromboplastin}]{Ca^{++}} \text{Thrombin}$$

$$\text{Prothrombin} \xrightarrow[\text{Ac-globulin}\ \text{Threone}]{Ca^{++}} \text{Thrombin}$$

In the first type of activation, thromboplastin of tissue origin is involved and it is known that a certain optimum calcium concentration best supports the reaction(s). This optimum is found to be about the same irrespective of whether whole blood or plasma is clotting (1,2) or whether purified prothrombin is being activated(3).

In the type of activation represented by the second equation, platelets are involved instead of thromboplastin; for, threone activity consists of the simultaneous presence, in suitable proportions, of platelet cofactor I from the plasma and platelet factor 3 from the platelets(4). This may be represented as follows: Platelet factor 3 + Platelet Cofactor I = Threone. In the present study we measured the calcium ion requirements for threone activity.

Methods. Purified prothrombin was prepared from bovine sources(5,6,7) and assayed by a modification of the 2-stage technic(8), and thrombin activity was measured as described by Seegers and Smith(9). Platelet suspensions were prepared as described previously(10); and in this study, it was found that they could also be replaced with purified platelet factor 3 obtained and assayed quantitatively for its activity as described(11). Platelet cofactor I concentrates were obtained from bovine sources and assayed according to procedures we hope to describe in detail in a later communication. Our preparations of platelet cofactor I were free of autoprothrombin II activity. The following reaction mixture was composed: (a) Purified prothrombin, 2400 u/ml; (b) platelet homogenates or purified platelet factor 3, 75 u/ml; (c) Platelet cofactor I concentrate, 65 u/ml; and, (d) $CaCl_2$ dissolved in imidazole buffer pH 7.25. The latter was the only variable. The reaction mixture was placed in a water bath at 28°C, and periodically samples were removed for the quantitative determination of thrombin activity. The results are presented on Fig. 1.

Results. In our study of the calcium ion requirements for the conversion of prothrombin to thrombin with threone, we found that there is no sharp optimum concentration. Once a certain minimum calcium ion concentration is supplied, the rate of prothrombin activation and the yield of thrombin is the same from 0.009 M to 0.04 M concentration (Fig. 1). With higher concentration, calcium is inhibitory. The wide range of calcium concentration most suitable for threone

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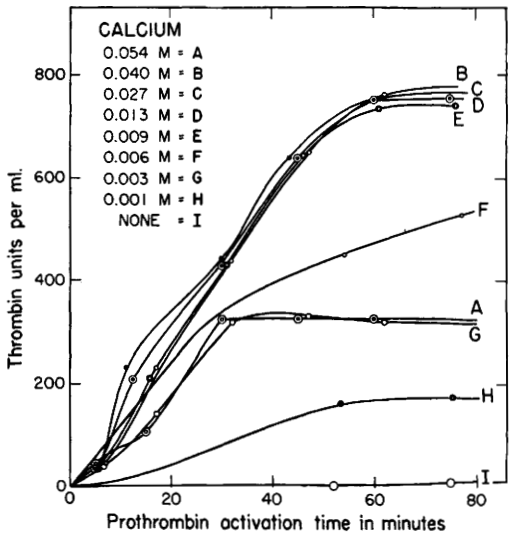

FIG. 1. Activation of purified prothrombin. Only calcium concentration was varied, as indicated for each curve. Molar concentration refers to concentration in reaction mixture described in the text.

activity is thus seen in sharp contrast with the narrow optimum range found with thromboplastin(3) as an activator of prothrombin.

Summary. When threone is involved in the conversion of purified prothrombin to thrombin, the optimum calcium concentration ranges from 0.009 M to 0.04 M. This is a much broader range than found when thromboplastin is used for the activation of prothrombin.

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Attempts to Identify Site of Production of Circulating 'Erythropoietin'. (22556)

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It is becoming rapidly established that humoral mechanisms underlie the erythropoietic effects of anoxia. Thus serum and plasma, or extracts derived therefrom, obtained from animals rendered anemic by bleedings(1-9) or by repeated injections of phenylhydrazine (9-13) exert considerable erythropoietic activity. Attempts to concentrate the active principle(s) from 'anemic' plasma and serum have led some to the conclusion that it is heat-stable and protein-free(9-15). In this connection, boiled filtrates of acidified plasma obtained from blood of phenylhydrazinized rabbits display marked erythropoietic actions in the rat (9-13). Relatively few investigators have attempted to locate the site of formation of the blood erythropoietic factor. Gordon *et al.*(5) detected slight activity in the livers of chronically bled rabbits and other experiments(16) appear to rule out those organs, presumably blood-forming in nature, which are injured by nitrogen mustard.

It seemed important to consider this problem in greater detail by subjecting a variety of organs to the chemical procedure employed