Rate of Disappearance of Prothrombin from the Circulation[1]

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The rate at which prothrombin disappears from the circulation could give important clues as to the significance and utilization of this substance in the body. The problem, however, has not received adequate attention in the literature, chiefly because of the technical difficulties met with in prothrombin determination. Warren and Rhoads (*6*), while investigating the formation of prothrombin by the liver, found that the prothrombin level dropped rapidly to about 20% in 10 hr and 10% in 16 hr, after total hepatectomy in dogs. Their data, unfortunately, are subject to criticism because, when the liver is removed, labile factor and prothrombin are depleted simultaneously (*2*); the prothrombin time expressing, therefore, the combined deficiency of both factors. McGinty *et al.* (*1*), while studying the ability of purified beef prothrombin to restore the prothrombin level in dicumarolized dogs, noticed that the injected prothrombin would progressively decrease and disappear within 2 or 3 days. More analytical data were not given.

New techniques for the study of the speed of disappearance of prothrombin from the circulation have now become available with the introduction of methods for direct determination of the concentration of prothrombin (*5*), and for simple preparation of concentrates of prothrombin (*5*). The problem was, therefore, investigated in rabbits, in which prothrombin concentration had been drastically depressed with dicumarol treatment.

Five male rabbits of average size received daily 10 mg/kg body wt of dicumarol in gum acacia suspension,[3] introduced in the stomach by intubation. After 5 days of treatment the prothrombin level of the animals was usually lower than 5% of normal, while the concentration of the labile factor, determined with the method previously described by Quick and Stefanini (*4*), appeared normal or slightly decreased. At this point, we injected intravenously in each rabbit a freshly prepared concentrate of prothrombin, obtained from a volume of fresh oxalated rabbit plasma equal to that of the presumed plasma volume of the animal under investigation. For working purposes, this was considered equal to 5% of the body wt of the animal. No reaction or untoward effect was noticed. The prothrombin time was then determined in plasma obtained by centrifugation at 2000 rpm for 10 min of oxalated blood collected from the central artery of the ear at various intervals after the injection (30 min, and 1, 6, 12, 24, 36, 48, 60, and 72 hr). During this phase of the experiment the rabbits continued to receive 5 mg of dicumarol per kg body wt daily to prevent any formation of prothrombin by the liver. The concentrate of prothrombin was prepared by the method of Quick and Stefanini (*5*), the only modification being that the final product was treated with 1/10 vol of full-strength thrombin to remove the fibrinogen still present, stored at 4° C for 1 hr, and then incubated in a water bath at 37° C for 30 min in order to inactivate any remaining thrombin. The prothrombin time was determined with the one-stage method of Quick (*3*) and its values were checked by determining the concentration of prothrombin directly with the technique described by Quick and Stefanini (*5*). The results of the two procedures usually agreed within ± 5%.

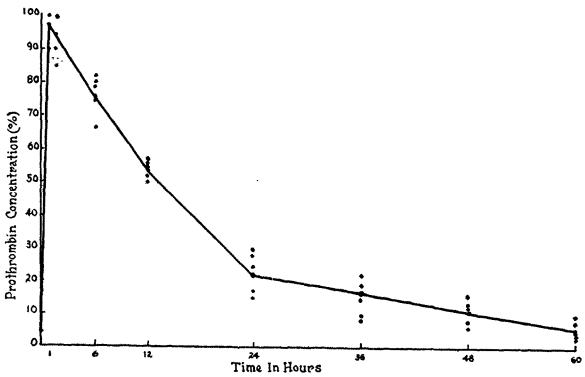

FIG. 1. The rate of disappearance of prothrombin from the circulation following intravenous injection of a concentrate of prothrombin in dicumarolized rabbits.

The average findings of the five experiments are presented in Fig. 1. Restoration of the prothrombin level to almost 100% of normal followed immediately the injection of the concentrate of prothrombin. About 50% of the prothrombin injected disappeared from the circulation within the first 12 hr, and about 80% in 24 hr. From this time on, a slow depletion continued until the original level was again reached, 48 hr—60 hr after the injection.

These findings show that prothrombin is promptly utilized or metabolized in the body, most of it disappearing from the circulation in the first 24 hr. The results also demonstrate that the concentrate of prothrombin prepared with the technique developed in our laboratory is capable of restoring to normal the prothrombin concentration of dicumarolized rabbits, in a definite quantitative relationship.

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[3] In a mortar, 250 mg of dicumarol and 5 g of finely pulverized acacia was added to 50 ml of distilled water and suspended by grinding carefully. One ml of the suspension contained, therefore, 5 mg of dicumarol. The suspension, well shaken before use, was kept in a refrigerator at 10° C.

References

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