SPECIAL ARTICLES

PLASMA PROTHROMBIN CONCENTRATION IN DOGS GIVEN 3,3′-METHYLENEBIS(4-HYDROXYCOUMARIN) AND PURIFIED BEEF PROTHROMBIN

WHEN the prothrombin concentration of the blood becomes markedly reduced, hemorrhages of varying degree of severity are likely to occur. To avoid that difficulty the hypoprothrombinemia can in many instances be corrected. The practicability of administering prothrombin itself for that purpose has perhaps occurred to many, but heretofore it has not been possible to obtain a sufficient quantity of purified material for experimental purposes.

We have recently been able to obtain purified beef prothrombin in large amounts with the use of methods already described,[1] and have found that plasma prothrombin concentrations can be raised well above the danger zone for a significant period of time. For the experimental production of hypoprothrombinemia 6 dogs were given 3,3′-methylenebis(4-hydroxycoumarin). This compound, recently isolated from spoiled sweet clover hay by Campbell and Link,[2] was administered in two doses of 14 mg/kg each, 24 hours apart. Within 48 hours (Fig. 1) the plasma prothrombin concentration (measured by the two-stage method,[3] which in our hands had a standard error of 3.3 per cent. at various levels of activity) dropped below 20 per cent. of normal, and continued to drop until the fifth day, when slow spontaneous recovery occurred. One month later the experiment was repeated. When the level of prothrombin had again dropped below 5 per cent. the dogs were given a concentrated solution of purified beef prothrombin intravenously (17,000 units per cc in 0.9 per cent. NaCl + 0.09 per cent. potassium oxalate) in sufficient calculated dosage to raise the prothrombin level to 200 per cent. in 2 of the dogs and 100 per cent. in 4 of the dogs. The calculations were based on our observation that normal dog plasma contains 200 units of prothrombin per cc and the assumption that plasma volume equals 5 per cent. of the total body weight.

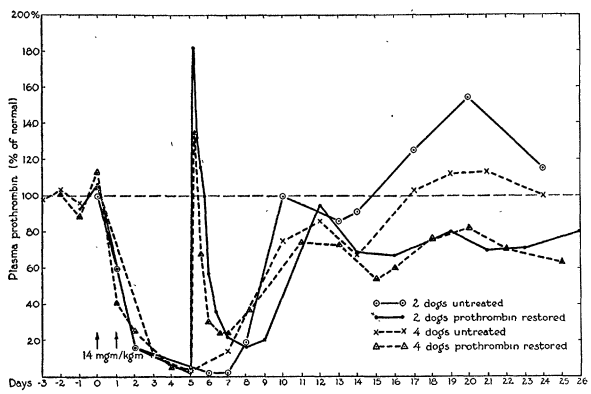

FIG. 1. After the control determinations, 2 doses of 14 mg/kg of 3,3′-methylenebis(4-hydroxycoumarin) were given orally 24 hours apart. Blood samples were taken at 1, 6, 12 and 24 hours after restoration of the prothrombin level with injected beef prothrombin.

The concentration of plasma prothrombin in the dogs given beef prothrombin solution rose promptly to approximately the expected level. The rate of disappearance of beef prothrombin, while initially rapid, noticeably paralleled the rate of disappearance of dog prothrombin resulting from the administration of 3,3′-methylenebis(4-hydroxycoumarin) to normal dogs. These curves suggest not only that beef prothrombin, on injection, can replace dog prothrombin in the physiological mechanism of blood clotting, but that the mechanism of destruction of the two may be the same.

Slow spontaneous increases of plasma prothrombin occurred in all animals during the recovery period. However, in dogs not receiving beef prothrombin there was evidence of an over-correction of the depleted plasma prothrombin. On the other hand, the plasma prothrombin levels in dogs given beef prothrombin did not attain their previous normal level during the 25-day experimental period. It is of interest that the animals tolerated the prothrombin preparations surprisingly well. They were kept under close observation, and reactions (retching, vomiting and defecation) of varying degree of severity were noted which were mainly dependent on the speed of injection. These can doubtless be ascribed to globulins still associated with the prothrombin itself, and with their removal the observed reactions may no longer occur.

SUMMARY

Purified beef prothrombin will antidote the hypoprothrombinemia induced in dogs by oral administration of 3,3′-methylenebis(4-hydroxycoumarin). If the hypothesis is proven that this chemical compound acts only by preventing the production of prothrombin in the liver then the life of injected beef prothrombin is probably of the same duration as that of the dog's own plasma prothrombin; namely, 2 to 3 days.

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