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Experimental Hypoprothrombinemia with Anti-Prothrombin Serum.

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A transitory reduction in the plasma prothrombin of rabbits may be produced by injecting into these animals an antiserum prepared in the guinea pig against fibrinogen-free rabbit prothrombin.

Fibrinogen-free prothrombin was isolated from the plasma of rabbits as follows:[1] About 100 cc of blood was collected from a large rabbit by cardiac puncture, in 5 cc of 5% sodium citrate; the plasma was separated off, passed through a Berkefeld V filter and the clear filtrate then heated carefully to 54-56°C for 2 minutes, the precipitated fibrinogen being filtered off and discarded. The plasma was then diluted 10 times with distilled water, and 1% acetic acid was added slowly with constant stirring until a pH of 6.0 was reached. The precipitate that formed was, after centrifugation, diluted in about 5 cc of salt solution, to which a few drops of 0.5% sodium bicarbonate were added. On standing at room temperature, most of the precipitate was dissolved. To prepare the antiserum the prothrombin solution from 100 cc of blood was injected intraperitoneally into a guinea pig weighing no less than 600 g, 5 times, at approximately 6-day intervals, the animal being being killed and bled out 10 days after the last injection. Rabbits weighing between 2.5 and 3.0 kg were used; they were fed a mixture of clover hay, timothy, oats, corn and dog chow. Blood was drawn from the heart or ear vein into a syringe containing 1.34% sodium oxalate solution (0.2 cc of the solution, 1.8 cc of blood). Prothrombin was measured according to the method of Quick.[2] With each determination in the experimental animals, control determinations were performed on plasma from normal rabbits.

Mixing increasing dilutions of the antiserum with a clear prothrombin solution brought about flocculation and precipitation of the prothrombin in dilutions up to 1-256. By incubating the undiluted antiserum with a given amount of oxalated rabbit plasma for 24 hours and following it with the addition of thromboplastin and calcium chloride it was possible to demonstrate a complete inacti-

1 Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.

2 Quick, A. J., *Am. J. Physiol.*, 1937, **118**, 260.

vation of the prothrombin in that plasma, and some reduction in prothrombin activity in dilutions of the antiserum up to 1-128.

Subcutaneous injections into 2 rabbits in doses of 0.2 to 0.4 cc per kg body weight of the animal produced no detectable external changes either locally or generally. In this respect the serum differs from antiplatelet serum which produces large local hemorrhages when injected subcutaneously.[3] In the doses employed, injections of the antiserum intravenously in 4 healthy, nonfasting animals produced only a very transitory reduction in the prothrombin, varying between 25 to 40% of normal, detected usually between 15 and 20 minutes after an injection. There was a quick recovery by the end of the first hour and no subsequent reduction was detected. Repetition of the injection the next day brought about a similar response, followed later by a rise of the prothrombin concentration above normal and failure of subsequent injections to bring about a drop.

In 4 animals that had been fasting for 3 to 4 days it became easier to produce and maintain a hypoprothrombinemia by this method. Chart I illustrates the effects of one or more intravenous injections in 2 of these rabbits deprived of food for 3 days before the antiserum was given and while the experiments were being carried out. Fasting for 3 days in itself produces only a very slight reduction in the plasma prothrombin. Two animals died during the experiments from hemorrhage induced by cardiac punctures. When size of the veins made it possible all specimens in one animal were collected from the ear vein. Selection of animals with large ear veins makes it possible to collect rapidly the required amount of blood.

The rapidity with which the prothrombin returned to normal after being reduced by the injection of antiprothrombin serum is another indication of the great lability of this substance. The body reserves of vitamin K and of whatever other substances are required for the production of prothrombin by the liver seem to make a preliminary period of fasting necessary to demonstrate a clear-cut diminution of the prothrombin after injection of the antiserum.

The fact that some of the prothrombin in the plasma may be associated with other protein fractions besides the globulin may partially explain the moderate and transitory nature of the reduction in the plasma prothrombin; the antiserum may only destroy that portion of the prothrombin linked with the globulin fraction. Heating of the plasma to precipitate the fibrinogen may have also precipitated or destroyed some of the prothrombin and affected the antigenic properties of this protein. In no instance was the plasma prothrombin

[3] Tocantins, L. M., *Arch. Path.*, 1936, **21**, 69.

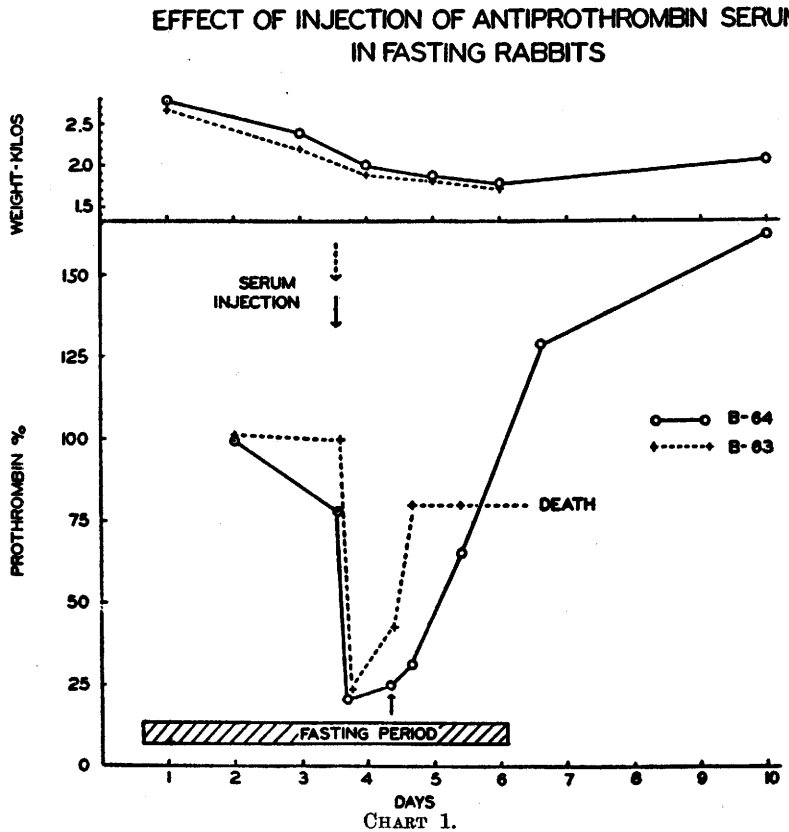

CHART 1.

Rabbit B-64 received 2 injections of the antiserum (solid arrow) and survived the experiment. Rabbit B-63 received 1 injection only (broken arrow) and died on the following day from a hemopericardium.

ever observed to go below 20% of normal; this may explain the relative scarcity of hemorrhagic manifestations encountered.

Summary. An antiprothrombin serum may be prepared in the guinea pig by injecting it with fibrinogen-free prothrombin isolated from rabbit plasma. Intravenous injections of this antiserum in fasting rabbits produce a hypoprothrombinemia of a transient and moderate character.