8891 P

Coagulation of Blood by Proteolytic Enzymes (Trypsin, Papain).

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Crude or crystalline trypsin in proper concentration coagulates human, dog, rabbit, guinea pig and horse plasma or whole blood. It does not clot fibrinogen directly, but reacts with prothrombin to form thrombin. This activation is independent of the presence of either calcium or platelets. It follows that neither of these is necessarily an intrinsic part of thrombin. Trypsin also coagulates blood *in vivo,* which suggests that circulating blood contains free and reactive prothrombin, rather than an inactive hypothetical complex from which prothrombin is liberated only after blood is shed.

An excess of trypsin digests fibrinogen, prothrombin and thrombin; and too little trypsin has no effect. The activation of prothrombin therefore takes place within a relatively narrow zone of enzyme concentration, which varies directly with the amount of protein present. At this optimum zone, there is a dynamic equilibrium between the activation of prothrombin to thrombin and the destruction of both reagents by the enzyme.

Since trypsin, in activating prothrombin, has qualitatively the same effect as the physiological system calcium plus platelets (or calcium plus tissue extracts), it is tentatively suggested that these systems contain a proteolytic enzyme similar to trypsin which reacts with prothrombin to form thrombin.

It has been further found that the proteolytic enzyme papain, freed of calcium, nevertheless coagulates whole blood, plasma, or fibrinogen. In this case, the enzyme does not activate prothrombin, but acts directly on fibrinogen to form a soft fibrillar gel resembling fibrin, with a marked tendency to resolution. If one admits this clot to be fibrin, this constitutes strong evidence that the physiological coagulant thrombin is also a proteolytic enzyme with a specific reactivity for fibrinogen.

The working hypothesis here suggested as to the mechanism of physiological coagulation is, therefore, that the calcium and platelet together contain a proteolytic enzyme which, like trypsin, transforms prothrombin to thrombin; and that the product of this reaction, thrombin, is itself a proteolytic enzyme which, like papain, converts fibrinogen to fibrin. A complete analogy for this hypoth-

esis is found in the recently reported activation of chymotrypsinogen by trypsin to form a new proteolytic enzyme, chymotrypsin (Kunitz and Northrop).[1]

8892 C

Effect of Cinchophen on the Liver and Other Tissues of the Dog.*

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During a study of peptic ulcers produced by the administration of cinchophen we had the opportunity of studying the effects of large amounts of cinchophen on the liver and other organs of the dog. In view of the divergence of opinions regarding the effects of cinchophen on the livers of experimental animals, it seems advisable to record our observations on this subject.

Specimens of liver were obtained from 131 dogs which were normal in every case before the experiment was begun. They were given the routine kennel care and were fed a balanced diet of a calculated weighed amount sufficient for their caloric requirements. Cinchophen was for the most part administered orally; in a few cases it was given rectally, parenterally, or through intestinal fistulas.

Thirty-one dogs, whose average weight was 17 kg., were daily given 2 gm. of cinchophen well mixed with their food for a period varying from 3 to 60 days. The average length of time over which administration of the drug to this group was continued was 27 days; the average total dose of cinchophen was 50 gm.

Fifty-four dogs were given 2 gm. of cinchophen daily, with an occasional rest day, for an average of 30 days but in a few cases over a period of time as long as 114 days. To this group the amount of cinchophen administered varied from 36 to 228 gm. during the course of treatment.

Thirteen dogs, whose average weight was 13 kg., were given varying doses of cinchophen by routes other than by mouth, the usual dose exceeding 1 gm. daily. To members of this group an average of 21 gm. of the drug was given in an average of 13 days.

[1] Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1935, **18**, 433.

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