At present he is using this arrested growth as a base line, adding nutrients one by one and determining which will renew bone growth.

We have not as yet tried the single-food-choice technique on larger animals, such as the dog, cat, or monkey. Some nutritional observations made in 1816 by Magendie (*2*) suggest that it may work quite as well on dogs and within much the same time limits. Magendie found that dogs kept on an exclusive diet of butter or sugar or gelatin lived 30–36 days, which is about the same length of time that rats live on these foodstuffs alone.

In summary, a method of prolonged partial starvation has been described which provides ideal preparation for dissection of spinal, peripheral, and autonomic nerves of the rat; also for the differentiation between glandular and fatty tissue.

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## Fibrinolytic Activity of Purified Thrombin[1]


**M. Mason Guest and Arnold G. Ware[2]**

***Department of Physiology and Pharmacology, Wayne University College of Medicine, Detroit, Michigan***


Highly purified thrombin at a concentration of 9,000 units per ml will completely dissolve a 0.5% fibrin clot at 37.5° C within a 90-min period. Evidence that thrombin is responsible for dissolution of the clots is based upon the degree of purification of thrombin preparations, failure of prothrombin from which the thrombin was derived to lyse fibrin clots, equal ability of thrombin prepared by two different methods of activation to lyse fibrin, relative lack of inhibition of fibrinolysis in the presence of antifibrinolysin (antiplasmin) or soybean antitrypsin, and heat inactivation of thrombin which parallels the decrement in lytic activity.

Reagents used in fibrinolytic tests consisted of fibrinogen substrate, prothrombin or thrombin buffered with imidazole, and in some experiments antifibrinolysin or soybean antitrypsin. Fibrinogen, prepared from bovine plasma by the freeze–thaw technique (*21*) was free of demonstrable fibrinolysin (plasmin) and profibrinolysin (plasminogen). Prothrombin was prepared from bovine plasma by techniques which have been described (*20, 22*). These products had specific prothrombin activity values ranging from 1,200 to 1,400 units[3] per mg of dry weight. Thrombin was prepared from prothrombin by activation with citrate or thromboplastin (*18, 20, 22*). Reactants in the lytic tests were buffered at pH 7.2 with 1% imidazole. At this concentration imidazole has been found to be strongly bacteriostatic (*6*).

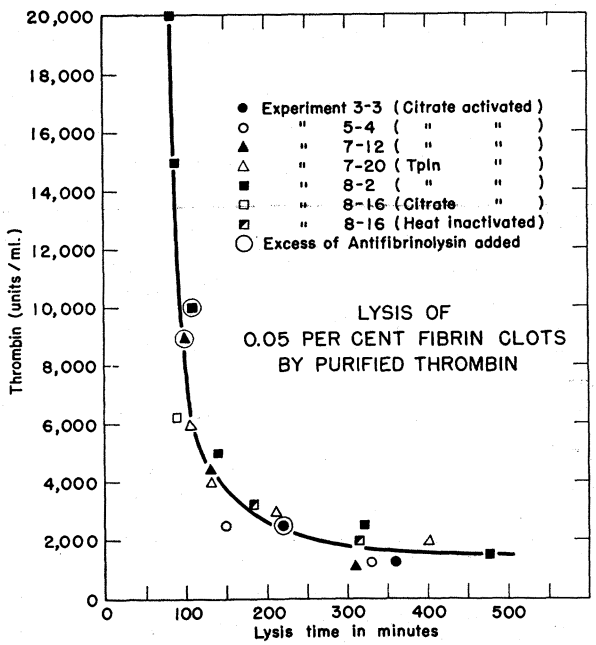


FIG. 1.

All tests for fibrinolytic activity were carried out at 37.5° C. Fibrin clots were formed in test tubes approximately 7 mm inside diam and 75 mm in length. To 0.2 ml of a 0.1% fibrinogen solution was added an equal volume of the substance to be tested. Lysis was considered to be complete when material in the tube flowed freely as the tube was tipped with the open end at an angle of 10° below the horizontal. At the point of complete lysis the solutions became clear and nonviscous. After the lysed solutions had stood for varying periods of time a flocculent precipitate appeared. The nature of this precipitate has not been investigated.

The plot on the graph shown in Fig. 1 results in deviation from the smoothed curve within the limits of error of the analytical methods used. Each experiment, indicated by a distinctive symbol, represents a single thrombin preparation which was used in its most concentrated form and in one or more dilutions.

All prothrombin preparations were checked for fibrinogenolytic and fibrinolytic activity prior to conversion to thrombin. Fibrinogen solutions containing prothrombin were clotted solidly by addition of thrombin after 72 hr of incubation. Similar solutions, to which a trace of thrombin was added at the start of incubation, clotted but did not lyse. These results indicate that prothrombin does not have a measurable lytic action on either fibrinogen or fibrin.

Since prothrombin preparations from which thrombins were derived had no fibrinolytic activity, it is evident that the lytic property appeared as a result of prothrombin activation. Two methods of activation were used.


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[2] Present address: Department of Biochemistry, University of Southern California Medical School, Los Angeles.

[3] The unit of prothrombin and the unit of thrombin used in this work are those described by Ware and Seegers (*24*).

Maximum conversion of prothrombin to thrombin with citrate occurred within 24 hr at room temperature in the presence of a high concentration of sodium citrate. Thrombin was then precipitated with ammonium sulfate, and the precipitate was dialyzed against deionized water at pH 7. Thrombin was also prepared by the addition to prothrombin of calcium and resedimented thromboplastin, free of fibrinolytic contaminants. As indicated on the graph, the lytic activity of thrombin prepared by either method was essentially the same, which suggests that the lytic property is independent of the method and reagents used in the conversion process.

In three different thrombin preparations lysis times were determined in the presence of approximately 240 units of bovine antifibrinolysin (*12*).[4] This concentration of antifibrinolysin will inactivate 24 units of fibrinolysin within about 15 min at 28° C, and 1 unit of fibrinolysin will lyse 1 ml of a 0.1% fibrin clot at 28° C in 120 sec in an isotonic saline solution buffered with imidazole (*4*). No difference in lysis times could be detected, in the presence of equal thrombin concentrations, between the tubes containing antifibrinolysin and those not containing antifibrinolysin. The failure of antifibrinolysin to inhibit lysis of the clot furnishes evidence that the lysis is not caused by contaminating fibrinolysin.

In one experiment soybean antitrypsin,[4] in sufficient concentration to completely inactivate 10 mg of crystalline trypsin (*11*), was dissolved in the fibrinogen solution before adding thrombin. Thrombin activity in this test was 9,000 units per ml. Since thrombin activity is roughly 1,000 units per mg of the dry solid, antitrypsin added was in excess of the amount required to inactivate all of the thrombin if it were assumed to be crystalline trypsin. The clot containing antitrypsin completely lysed in 375 min, while the clot containing the same amount of thrombin but free of antitrypsin lysed in 100 min. The partial inhibition of lysis which occurred was probably not specific or lysis would have been completely prevented. The partial inhibition is more likely associated with some of the other effects which soybean antitrypsin appears to have on organic substrates in addition to its inactivation of trypsin (*3, 5*).

Purified thrombin in saline solution is inactivated at a measurable rate by heating at 50° C (*17*). One thrombin preparation was tested for lytic activity prior to heating and after heating to 50° C for 15 and for 30 min. The unheated material contained 12,500 thrombin units per ml. After heating for 15 min, 6,560 units remained; while after heating for 30 min, 3,900 units remained. The plot of these points on the graph follows the dilution curve, indicating that the decrement in thrombin activity is equivalent to the decrement in lytic activity as measured by the method of dilution. Since it is improbable that a contaminating lytic principle and thrombin both have the same rate of heat inactivation, the experiment indicates that fibrinolytic activity resides in thrombin itself.

Our data indicate that thrombin will produce complete lysis of a clot within 1 to 4 hr only if its concentration is roughly equal to that of the fibrin. Earlier workers (*1, 7, 9, 14, 15*), who have reported that thrombin has the ability to redissolve the blood clot, did not have available sufficiently potent thrombin to lyse fibrin clots. Since purified thrombin has been available only recently, it is probable that thrombin preparations of the workers cited contained fibrinolytic contaminants which caused the described lysis. Seegers (*17*) and Hudemann (*8*), in reporting that thrombin does not have fibrinolytic activity, had preparations which were not contaminated but the thrombin was not sufficiently concentrated to cause dissolution of the clots.

In addition to its action on fibrinogen and on fibrin, there is evidence that thrombin also affects other substrates. Thrombin can either inactivate prothrombin (*13*) or activate it to thrombin (*19*), depending upon time and concentration factors. Traces of thrombin can apparently change the plasma type Ac-globulin to the active serum type and in higher concentrations it can destroy Ac-globulin (*23*). It reacts with antithrombin (*25*), and it has been claimed that thrombin has an effect on the stability of organic and inorganic colloids (*10*). Quick suggested that thrombin labilizes platelets (*16*). Recent data also indicate that it may have hypertensinase activity (*2*). It is conceivable that all of these actions are mediated through one specific portion of the thrombin molecule. On the other hand, it may be that different parts of the molecule are responsible for the permutations.

[4] Kindly supplied by E. C. Loomis of Parke, Davis and Company.

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