vine thrombin. This inhibitor appears to be different from the antithrombic activities described so far(8). It should be noted that the considerably lower concentrations of thrombin derivatives prepared from bovine thrombin were sufficient to inhibit the latter, than were required to inhibit thrombin. This species-specific inhibitory effect may be related to differences between bovine and human thrombin and to the ease with which the respective enzymes can be inhibited by the same inhibitor. On the other hand, the possibility may also be considered that the modification of an enzyme may result in a derivative which will inhibit preferentially the specific enzyme from which it has been prepared.

The heated thrombin preparations and the redissolved alcohol precipitates have been administered intravenously to rabbits and guinea pigs without toxic or thromboembolic sequelae.

*Summary.* An alcohol precipitate obtained from heated bovine thrombin preparations promoted the early phases of the coagulation mechanism, but did not clot fibrinogen in the manner of the unheated preparation. These materials inhibited bovine thrombin at lower concentrations that human thrombin in the clotting of fibrinogen.

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## Antifibrinolytic Activity of Derivatives of Fibrinolysin.‡ ( 22781)

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Fibrinolysin and its inhibition and activation have been intensively investigated in recent years in relation to coagulation and hemostasis. The clinical investigation of bleeding patients(1) as well as the fractionation of lyophilized human platelet material suggested the presence of an inhibitor of fibrinolysin in these materials. Antifibrinolysin, moreover, has been demonstrated previously in bovine platelets(2).

In the search for specific inhibitors of fibrinolysin, an attempt was made to produce an inactive derivative which would specifically inhibit the enzyme.

*Materials and methods.* Bovine fibrinolysin* was suspended in water, saline or buffer (imidazole pH 7.2), and heated at 90°C for periods varying from 5 to 30 min. Coagula formed during the heating were removed by centrifugation at 3,000 g for 10 min. The supernatant (M.F.) was precipitated with 3 volumes ethyl-alcohol to yield a white, water-soluble material (MFP). The amount of MFP obtained was approximately 4.5% of the weight of the starting material. The fibrin-plate method previously described (3) was used for the determination of fibrinolytic activity. The method was modified, however, in regard to times and temperatures of incubation. Human fibrinolysin activity was obtained by activating normal human serum with Streptokinase (Varidase).*

*Results.* The soluble material remaining after heating bovine fibrinolysin at 90°C was

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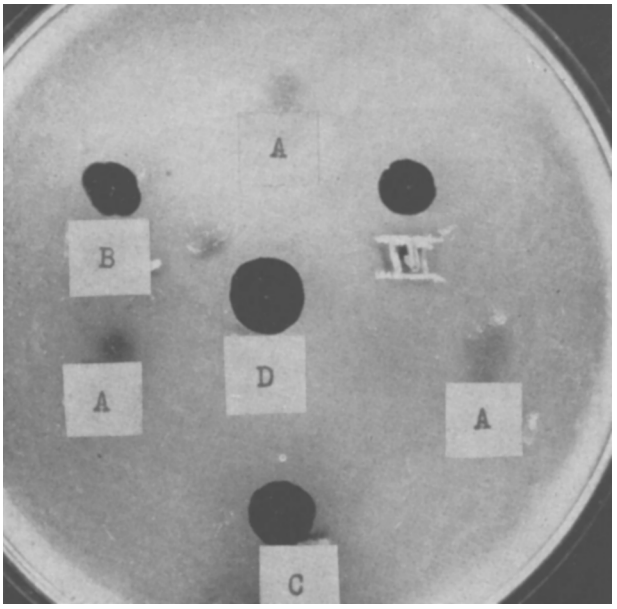


FIG. 1. A. 10 mg/cc active fibrinolysin solution mixed 1:1 with MF derived from 10 mg/cc of fibrinolysin. B. 10 mg/cc active fibrinolysin diluted 1:1 with saline. C. 10 mg/cc active fibrinolysin (undiluted). D. 20 mg/cc active fibrinolysin (undiluted).

insoluble in alcohol and gave a strongly positive orcinol reaction, indicating the presence of carbohydrate.

Both the supernatant (M.F.) as well as the material precipitated from it by alcohol (MFP), inhibited the activity of fresh fibrinolysin, as illustrated in Fig. 1.

In another experiment with MFP† the fibrin plates were incubated for 48 hours. Lysis of fibrin started at the point of application of fibrinolysin and spread to other areas of the plate. The area on which MFP had been applied (Fig. 2 and Fig. 3) was protected from lysis, while despite some lysis at the center of the site of application of a mixture of active and modified fibrinolysin, the surrounding fibrin was not lysed. Qualitatively similar results were also obtained with the supernatant (MF) obtained from heated fibrinolysin.

The effect of heating for various periods of time was investigated. Optimal inhibition was obtained with materials heated for 5 to 10 minutes, while heating for more than 20 minutes tended to decrease the inhibitory activity.

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When modified bovine fibrinolysin was tested against human fibrinolysin (*i.e.*, serum activated with Streptokinase), fibrinolytic activity was not antagonized (Table I).

*Discussion.* Products obtained by heating bovine fibrinolysin preparations were found to inhibit the activity of the unmodified enzyme. The same material did not appear to affect the fibrinolytic activity of human serum treated with streptokinase. It should be noted that the successful formation of inhibitors by heat denaturation varied with the nature of the starting material and the procedures employed, in a manner not understood at the present time.

Analogous inhibitory phenomena have been

TABLE I. Effect of MFP on Human Serum Fibrinolysin.

| | Avg diameter of lysed area (mm) |
|---|---|
| Activated human serum 1:1 with 20 mg MFP*/cc | 16.5 |
| *Idem*, with 10 mg MFP/cc | 16.0 |
| " with saline | 16.2 |

* MFP refers to the alcohol precipitate, *i.e.*, 20 mg of dried precipitate/cc of physiological saline.

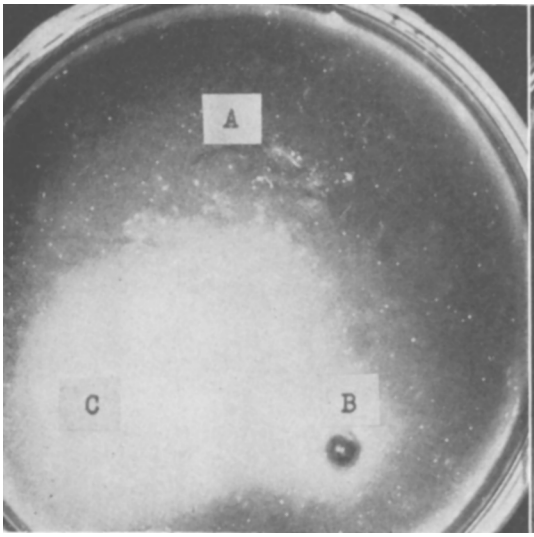


Fig. 2

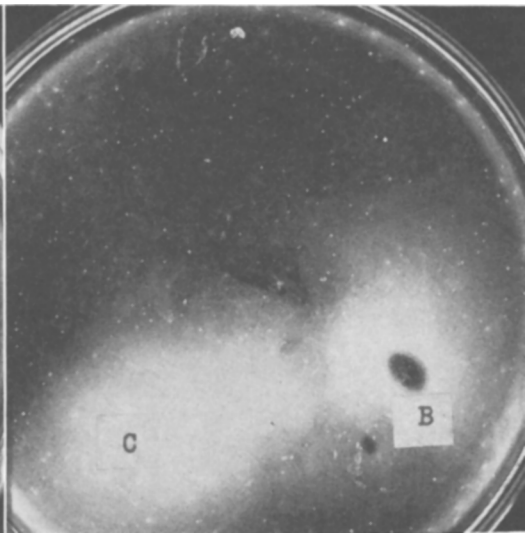


Fig. 3

FIG. 2. A. Fibrinolysin, 20 mg/cc, diluted 1:1 with saline. B. Fibrinolysin, 20 mg/cc, diluted 1:1 with MFP,* 20 mg/cc. C. MFP, 20 mg/cc.

FIG. 3. A. Fibrinolysin, 20 mg/cc, diluted 1:1 with saline. B. Fibrinolysin, 20 mg/cc, diluted 1.1 with MF† obtained from 20 mg fibrinolysin/cc. C. MF, 20 mg/cc.

* 20 mg of dried alcohol precipitate were dissolved in 1 cc of physiological saline.

† MF refers to supernatant obtained from heating a solution of 20 mg of fibrinolysin/cc.

observed in parallel studies of thrombin and in preliminary studies with a number of other enzymes which are not related to the coagulation mechanism.

The failure of an inhibitor derived from a bovine fibrinolysin preparation to affect human fibrinolysin activity (Table I) is of interest. Studies are in progress to determine the species-specificity of this inhibitory effect, particularly in view of the known differences between functionally similar enzymes derived from different tissues and different species.

*Summary.* Derivatives of bovine fibrinolysin have been prepared which inhibited the enzyme from which they are derived, but did not inhibit human fibrinolytic activity.

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