occurs within 2 hours following the removal of the tourniquet in fatal shock induced by leg tourniquets in albino rats. 2. There is no decrease in the percent of lymphocytes in blood smears made below the tourniquet at the end of a 5-hour period. 3. Dibenamine hydrochloride increases the onset of death in tourniquet-shock and increases the lymphocyte decline.

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17227. Protecting Effect of Heparin on the Inactivation of Thrombin by Heat.*

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Thrombin and thrombokinase, unlike prothrombin, are oxidizable and become inactive in their oxidized form, as reported previously.[1] Further investigations proved that γ-quantities of heparin inhibit completely the inactivation of thrombin by oxidation. This property of heparin, unknown till now, showed itself to be specific; it can be concluded, therefore, that heparin possesses not only an antagonistic action on thrombin, but, under certain circumstances, has also qualities protecting thrombin.[2] The inactivation of the coagulation factors by means of oxidation supports our previous theory on the role of oxidation in blood coagulation. Our experiments show that increased oxidation diminishes coagulability of blood, whereas oxygen deficiency accelerates coagulation. Therefore breathing itself must be the essential regulator in blood coagulation *in vivo* as has been shown.[3] The thrombin-protecting effect of heparin, which contrasts with its inhibitory effect on coagulation, is not clarified in our earlier work. It is supposed that this question could be answered by clearing up the nature of the plasma factor (co-factor) of heparin. This supposition is supported by the fact that the unknown plasma factor can modify the heparin effect, as may be seen by the different reactions of heparin against plasma and purified fibrinogen. Heparin has no coagulation-inhibitory effect on purified fibrinogen.[4]

In the present work we investigated whether heparin has an inhibiting effect on heat inactivation of thrombin, similar to its protecting effect toward oxidation. We used for our investigations thrombin and heparin (Liquemin, Hoffmann-La Roche). The activity of thrombin was measured with a solution of fibrinogen, containing 15 mg/ml of dry substance; the time of coagulation was noted in seconds. The solutions used for heat inactivation contained thrombin mixed with varied quantities of heparin. In each experiment the mixtures of thrombin and heparin were kept exactly 10 minutes in the water bath. A control series was similarly treated without heparin. The activity of thrombin was measured 1 hour after the samples were removed from the water bath.

It can be seen from Table I that the inactivation of thrombin by heat is inhibited most efficiently by an equal quantity of heparin. Smaller amounts of heparin inhibit less; however, a larger amount does not essentially increase the inhibitory effect. The heat inactivation of thrombin in the presence of an equal amount of heparin is demonstrated in a curve which is essentially similar to the inactivation curve of thrombin following oxidation.

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1 Palos, L. A., *Nature*, in print.

2 Palos, L. A., *Experientia*, 1949, **5**, 207.

3 Palos, L. A., *Acta Medica Scandinavica*, in print.

4 Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918-19, **47**, 328.

TABLE I.
Activity of Thrombin after Keeping 10 Min. at Different Temperatures in Water Bath with and without Heparin.

Temp. °C	Thrombin: 1 mg Heparin: 1.5 mg pro ml dest water	1 mg 1 mg	1 mg 0.5 mg	1 mg 0 mg
20	15″	15″	16″	16″
30	16″	16″	20″	20″
40	17″	18″	22″	29″
50	23″	24″	34″	50″
60	25″	26″	51″	500″
70	28″	29″	66″	1200″
80	34″	36″	88″	∞
90	44″	45″	90″	∞
100	51″	52″	260″	∞

Without heparin inactivation of thrombin is strongly accelerated at 50°C; between 70 and 80°C it loses its activity, *i.e.*, the fibrinogen cannot be further coagulated. In contrast, a solution of thrombin containing a corresponding amount of heparin, after even 10 min. boiling (100°C), causes a strong coagulation of fibrinogen; the activity diminishes only to one-third of the initial value.

The protecting effect of heparin shows differences concerning oxidation of thrombin and inactivation by heat. In the first case γ-quantities of heparin were enough to stop oxidation completely. For protecting the inactivation by heat there are required at least equal quantities of heparin to produce a marked effect. Total inhibition of heat inactivation could not be obtained. If the treatment with heat is prolonged, the thrombin loses its activity despite the protecting effect of heparin, but much more slowly than without heparin.

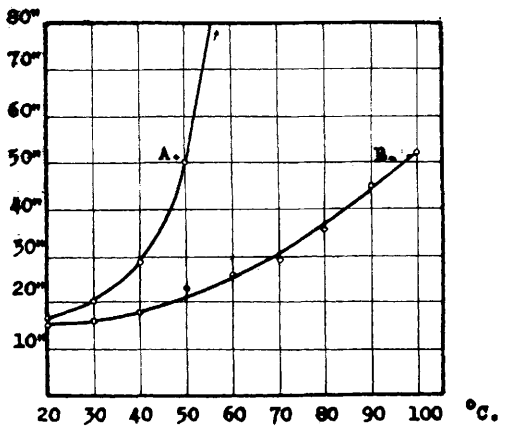

FIG. 1.
Activity of thrombin after keeping 10 minutes at different temperatures in water bath without (A) and with (B) heparin.

A similar effect to that of heparin could not be produced with other, not esterified di- and polysaccharides, neither in the oxidation experiments nor in the investigation on heat inactivation.

Synthetic polysaccharidesulfonates, which correspond to heparin in inhibition of coagulation, were not investigated.

These new facts, the total inhibition of oxidation and the high grade of inhibition of inactivation of the thrombin by heparin may lead to a new conception of the relationship between thrombin and heparin.

Summary. The inactivation of thrombin by heat is sharply inhibited, if it is mixed with equal parts of heparin. Heating a mixture of thrombin-heparin (1:1) for 10 minutes to 100°C causes only a slight decrease of activity.

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