Chemistry. — *On the spreading of fibrinogen.* By E. GORTER, L. MAASKANT and G. J. VAN LOOKEREN CAMPAGNE. (From the Laboratory of the Children's Hospital of the University of Leiden, Holland.) (Communicated by Prof. J. VAN DER HOEVE.)

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a. The influence of trypsin on the spreading of fibrinogen.

Up till now we had studied the spreading of fibrinogen only superficially. In a previous communication the suggestion was made, that fibrinogen probably did not spread because the particles of the sol had too little solubility. The reason of this retarded investigation was owing to the experimental difficulties of the preparation of pure fibrinogen.

GORTER and VAN ORMONDT [1]) showed that spreading of well-purified myosin is very difficult to obtain.

If, however, small quantities of trypsin are added to the myosin solution spreading is facilitated and it seems reasonable to think that an enzyme can by inducing a protein to increase its surface prepare it for hydrolysis into smaller peptides and amino-acids.

It seemed to us, that the same circumstances, examined by GORTER and VAN ORMONDT with regard to myosin, influence the spreading of fibrinogen.

Spreading of freshly prepared and well-purified fibrinogen even on a strongly acid solution p_H 1.0 can be scarcely observed. Now it is possible to induce fibrinogen solutions to spread by adding to the solution a trace of a proteolytic enzyme, by which the fibrinogen is transformed into a well-spreading substance.

This process is dependent on the temperature of the solution and on the amount of the trypsin added and it takes some time before the final effect of the action of the trypsin on the fibrinogen is obtained, when the concentration of trypsin is rather low.

Fig. 1 shows the results of measurements of spreading, when different concentrations of trypsin are added to a fibrinogen solution (5 mg./m.l.) after various periods of time at the same temperature, while the temperature is kept constant (38°). It appears, that before degradation into small particles, which have no longer spreading properties the spreading remains maximum rather a long time, even when the trypsinconcentration is high ($^1/_{50}$).

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[1]) E. GORTER and H. v. ORMONDT, Bioch. J., Vol. XXIX, No. 1, pag. 48 (1935).

We have also studied the influence of temperature on the first part of the enzymatic process and for this purpose we constructed a curve

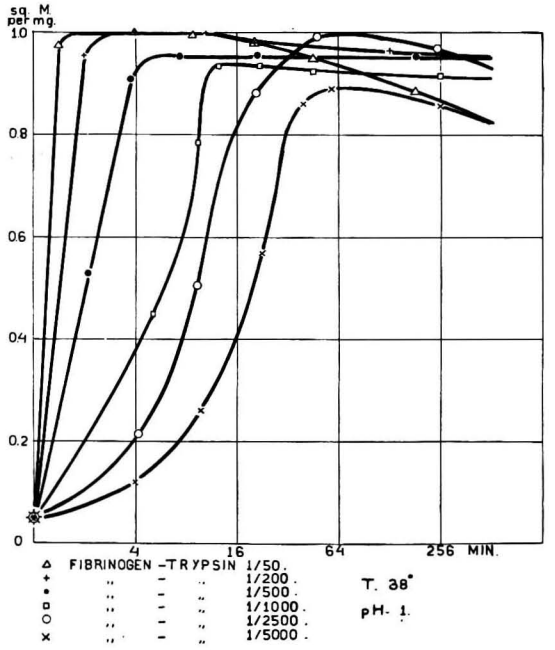

Fig. 1.

(fig. 2), which shows that the same area for the spreading of fibrinogen after exposure for a given time to the action of trypsin can be obtained by

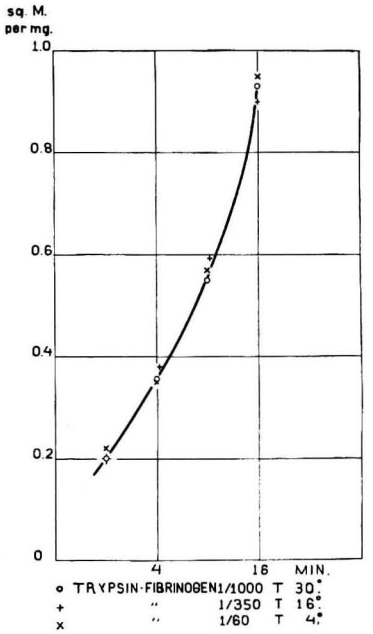

Fig. 2.

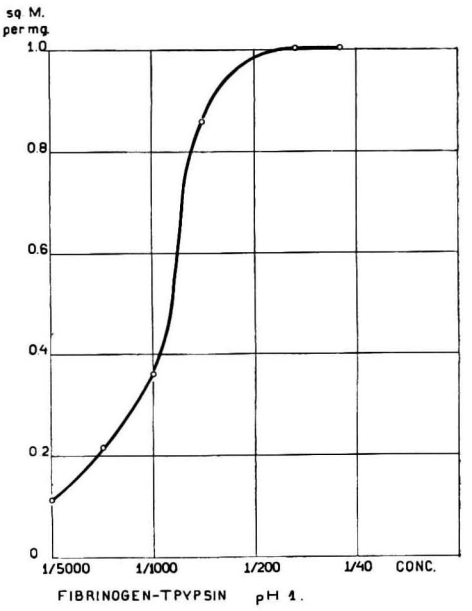

Fig. 3.

varying both temperature and concentration of trypsin. It is logical, that if the temperature is increased, the concentration must be lowered to get the same effect.

Fig. 3 showed the effect of varying concentration of trypsin on the spreading of fibrinogen, whereas the time of action of trypsin is 4 minutes and the temperature is 38°.

We then investigated the influence of the hydrogen concentration of the liquid in the trough on the spreading of fibrinogen. This fibrinogen was at first induced to spread by adding a trace of trypsin ($^1/_{1000}$). It appeared from these spreading measurements, that there is a pronounced minimum at the acid side of the iso-electric point ($p_H = 4.7$). See fig. 4.

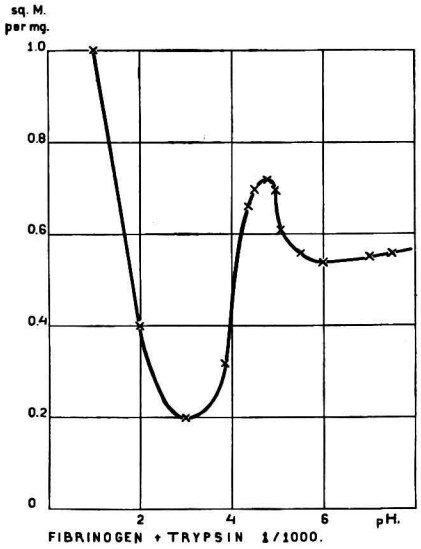

Fig. 4.

b. The influence of prothrombase on the spreading of fibrinogen.

We succeeded in preparing a very active prothrombase-preparation. When converted into thrombase 0.01 mg. prothrombase clotted 1 cc. of a fibrinogen solution (5 mg./m.l.) within half a minute at 38°.

Fig. 5 shows the influence of the hydrogen concentration on the spreading of this prothrombase. On the acid side of the iso-electric point ($p_H = 4.8$) the spreading of prothrombase is less diminished, whereas on the alkaline side of the iso-electric point, there is a minimum.

It appeared, that a similar p_H area curve was shown by euglobulin [1]), prepared in our laboratory from bloodserum and it is interesting to mention, that the prothrombase is described in the literature as having a euglobulin character.

Now the curves in fig. 6 show the results of measurements of spreading,

[1]) Not yet published.

when two different concentrations of prothrombase are added to fibrinogen after various periods of time at the same temperature.

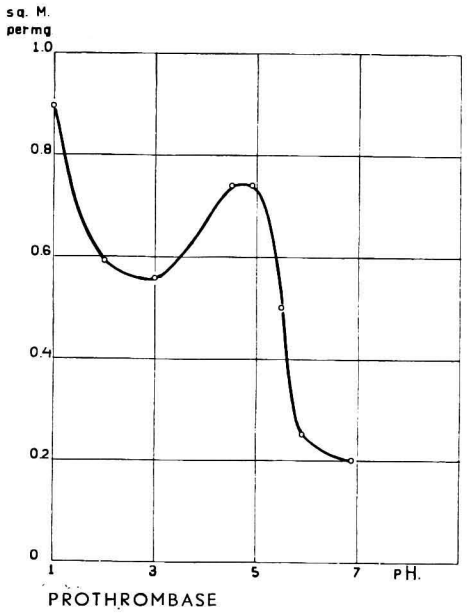

Fig. 5.

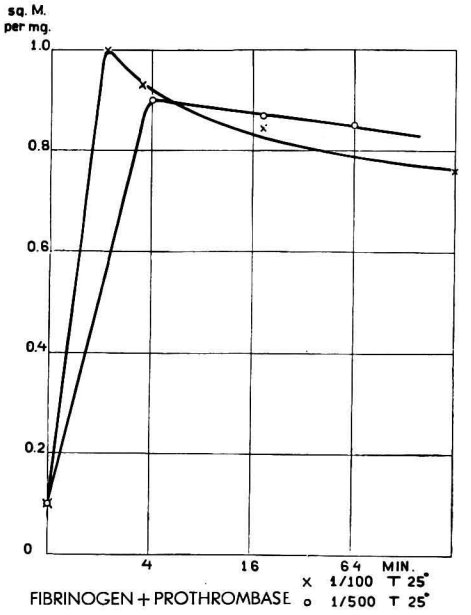

Fig. 6.

So it is possible to induce a fibrinogen solution to spread by adding a small amount of prothrombase.

Preparation of fibrinogen according to MELLANBY [1]).

200 cc. plasma, obtained from horse blood (0.2 % kaliumoxalate) were diluted to 2 L. with distilled water. By adding 6 cc. n. acetic-acid the p_H of the solution was reduced to 6.8.

After standing some hours at roomtemperature the clear solution was poured from the precipitate. This precipitate was then washed several times with distilled water, whereafter it was dissolved in 100 cc. of a physiological salt solution.

To this solution were added dropwise and with stirring 43 cc. of a saturated $(NH_4)_2SO_4$ solution. The precipitate was collected by centrifuging and washed two times with a $^1/_3$ saturated $(NH_4)_2SO_4$ solution, again centrifuged and dissolved in 200 cc. of a physiological salt solution.

The fibrinogen was reprecipitated from this solution by adding 80 cc. of a saturated $(NH_4)_2SO_4$ solution, centrifuged, washed with a $^1/_3$ saturated $(NH_4)_2SO_4$ solution and dissolved in a physiological salt solution in the manner described above.

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1) J. MELLANBY, J. of Physiol. **38**, 24 (1909).
See also C. OPPENHEIMER, Die Fermente **3**, 1425 (1929).

We were able only a few times to repeat this manipulation three times without too much loss of fibrinogen and so that the fibrinogen formed a clear solution after the last recrystallization.

The solution was then dialyzed in the ice-box against a physiological salt solution. The concentration of the pure solution was determined by means of micro-Kjeldahl determinations.

Preparation of prothrombase.

The prothrombase was prepared from the plasma of cow blood (containing 0.2 % K-oxalate) as described by J. MELLANBY [1]).

200 cc. plasma were diluted ten times with distilled water and the precipitation of prothrombase was obtained by the addition of 1 % acetic-acid, until the reaction was approximately $p_H = 5.3$.

After standing some hours the fluid was poured from the precipitate and the diluted suspension of globulins was centrifuged. The precipitate was then suspended in 100 cc of distilled water. This solution was treated with 100 cc. of a diluted calciumbicarbonate solution, which was prepared by diluting ten times a saturated solution of calciumoxyd at room-temperature, whereafter the p_H was reduced to 7 by blowing carefully CO_2 into the solution.

This calciumbicarbonate solution and the proteinsuspension were mixed with gentle shaking for 10 minutes. Then the solution was centrifuged immediately.

The prothrombase was precipitated from the waterclear solution by adding dropwise 1 % acetic-acid until the reaction of the solution was about at $p_H = 5$.

The prothrombase was centrifuged very rapidly and this precipitate was dried at once by means of acetone. For our experiments the stable prothrombase in a dry state was dissolved in a 1 % $NaHCO_3$ solution.

Discussion.

It is rather difficult to give a biological interpretation concerning the above mentioned investigations. These spreading experiments show, that fibrinogen is broken down somewhat under the influence of prothrombase and trypsin and they indicate, that fibrinogen is made more suitable especially under the influence of prothrombase of being converted into fibrin.

Summary.

Spreading of well-purified fibrinogen is very difficult to obtain, whereas trypsin and prothrombase are both spreading-promoting substances. The conditions, governing this phenomenon have been studied.

[1]) Proc. Roy. Soc. Serie B., **107**, 271 (1930).