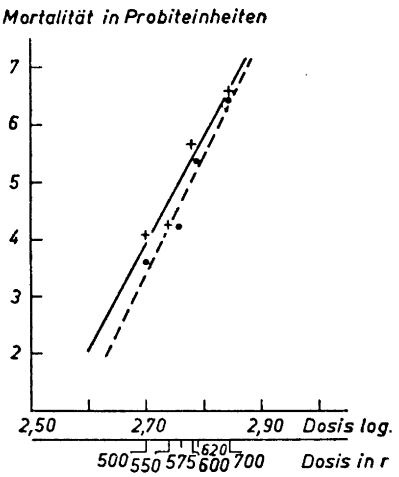


*Fig. 2.* Dosis-Wirkungskurven bei Ganzkörperbestrahlung.
•———•: Kontrollen.
+——+: 3-Mercaptomethylpyridin.
Abszisse: log. der in r gemessener Dosen, darunter die regelrechten r-Werte.
Ordinate: Prozent der gestorbenen Tiere in Probiteinheiten.

Zwischen den beiden DL 50-Werten besteht keine gesicherte Differenz (P = 0,8—0,9). Eine weitere Prüfung gibt die Gewähr, dass beide Regressionslinien parallel verlaufen (für Nicht-Parallelität: P = 0,9—0,8). Figur 2 zeigt die Verhältnisse graphisch. Die leichte Linksverschiebung der 3-Mercaptomethylpyridin-Kurve ist sehr wahrscheinlich auf eine gewisse Giftwirkung des Mercaptans zu beziehen und auf keinen Fall im Sinne einer Sensibilisierung zu deuten.

*Diskussion.* Die vorliegenden Ergebnisse bestätigen unsere ersten Beobachtungen, dass im Mercapto-Pyridoxin ein Schutzköper gegenüber energiereichen Strahlen vorliegt. Derselbe tritt sowohl nach parenteraler als auch peroraler Applikation auf und entspricht damit den Schutzstoffen der Cystein-Cysteaminreihe. Der perorale Schutz lässt sich mit dem nach Homocysteinthiolacton oder AET vergleichen. Mit diesem Befund lässt sich die Vorstellung einer spezifischen Strahlenschutzwirkung der Sulfhydrylkörper der Cystein-Cysteaminreihe nicht mehr aufrecht erhalten.

Entsprechend unseren Untersuchungen im Bereich der Cysteaminreihe prüfen wir auch beim Mercapto-Pyridoxin, welche Bedeutung den einzelenen Substituenten des Pyridinringes zukommt. Dabei zeigt sich, dass das einfache 3-Mercaptomethylpyridin vollkommen unwirksam ist.

Wie im Falle der Schutzwirkung der Cystein-Cysteaminkörper ist auch im vorliegenden eine Deutung des Schutzmechanismus nicht möglich.

Die Untersuchungen wurden mit Unterstützung der Deutschen Forschungsgemeinschaft ausgeführt.

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Eingegangen am 27. September, 1958.

## On the Significance of the Release of two Different Peptides from Fibrinogen during Clotting

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The first step in the clotting process when fibrinogen is incubated with thrombin is a release of two different peptides, A and B [1,2]. It has been shown that clotting also occurs after incubation with reptilase when only peptide A is released [3,4]. This raises the question of the biological significance of the release of peptide B.

The second step in the clotting process with thrombin has been shown to be a formation of an intermediate polymer with a varying length and a width corresponding to two parallel fibrinogen molecules [5]. Casassa [6] showed that it is possible by means of light-scattering to obtain the ratio of the molecular weight and molecular length (M/L) of this polymer. The second

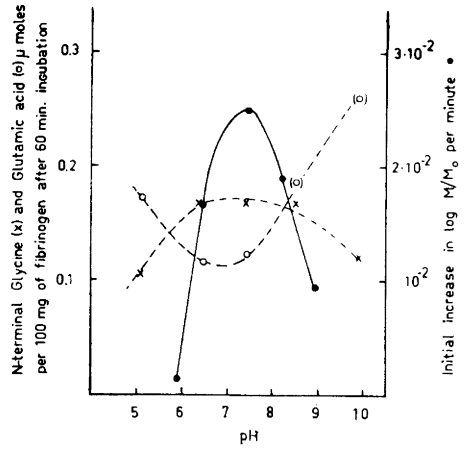


*Fig. 1.* N-Terminal analysis on glycine and glutamic acid after incubation of 0.17 % fibrinogen with 0.024 NIH-units/ml of reptilase for 60 min. The initial polymerization rate of the protein has been plotted for comparison. M is the weight-average molecular weight of the polymer and $M_0$ is the molecular weight of fibrinogen.

step of the clotting can conveniently be studied in urea solutions, where step three, the visible clotting, is inhibited [7].

The aim of the present investigation, of which this is a preliminary report, was to see whether an intermediate polymer could be formed even by reptilase as the coagulating agent.

The first experiment was planned to determine the pH-optimum of the action of reptilase as well as to assure that only peptide A was split off within the working range of the pH. The experimental conditions were described earlier [4]. N-Terminal glycine, glutamic acid and tyrosine were determined after incubation of fibrinogen with reptilase for 1 h and 17.5 h at room temperature. The protein concentration was 0.17 %, the reptilase concentration 0.024 NIH-units/ml and the medium was tris-imidazole buffer and sodium chloride, ionic strength 0.2. The values for glycine and glutamic acid were corrected with respect to the tyrosine values [8]. The initial polymerization rate was followed by light-scattering [4]. Fig. 1 shows that the decrease of glutamic acid, the increase of glycine and the initial polymerization rate all had pH-optima at pH 6—8. Within the error of the method, the disappearance of one N-terminal glutamic acid corresponded to the appearance of one N-terminal glycine. After 17.5 h incubation the ratio of N-terminal glycine to N-terminal tyrosine (Table 1) was, within the experimental errors, 1:1, indicating that most of the fibrinogen had been transformed to fibrin at all pH's studied and that only one peptide, A, had been split off.

*Table 1.* Ratio of N-terminal glycine and tyrosine after incubation of fibrinogen with reptilase for 17.5 h. The experimental conditions are described in the text.

| pH | 5.1 | 6.35 | 7.4 | 8.5 | 9.9 |
|---|---|---|---|---|---|
| Glycine / Tyrosine | 1.12 | 0.81 | 0.97 | 0.97 | 0.95 |

In the second experiment, thrombin and reptilase were added in a concentration of 0.03 NIH-units/ml to different solutions of 1 % fibrinogen containing 0.02 M tris-imidazole buffer pH 6.35 and 0.38 M sodium chloride. The solutions were incubated in dialysis bags for 9 h at 37°C. The clots formed were then dissolved by dialysis for 6 h against 2 M urea containing 0.01 M tris-imidazole buffer pH 7.45 and 0.22 M

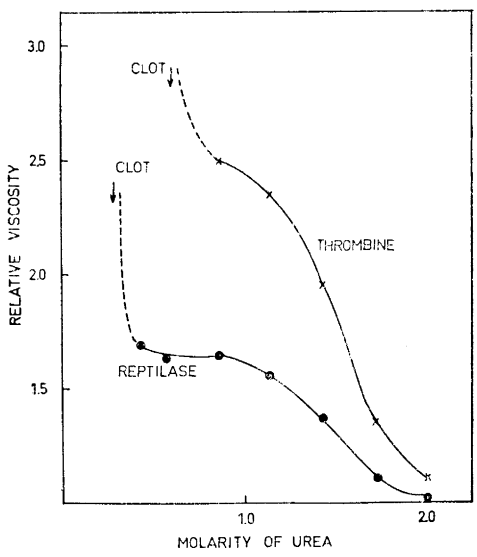


*Fig. 2.* Relative viscosity at different urea concentrations of fibrin formed by the action of thrombin and reptilase. Protein concentration 0.14 %.

sodium chloride. The solutions were diluted, the protein concentration being 0.14 %, keeping the ionic concentration constant, at varying urea concentrations. The viscosities of the solutions were measured in an Ostwald viscometer at 20°C, 18—24 h after dilution. No correction for non-Newtonian flow was applied. The result is shown in Fig. 2. The increase in the range of 0.4—2 M urea of the viscosity of fibrin formed by reptilase and in the range of 0.7—2 M urea of the viscosity of fibrin formed by thrombin is most probably an indication of the formation of urea soluble fibrin polymers.

In order to compare the two intermediate polymers by light-scattering a third experiment was performed identical with the second except that the enzyme concentration was now kept at 0.06 NIH-units/ml in the original incubation mixture and the incubation time was 18 h. The N-terminal analysis in the first experiment indicates that during this incubation time, all

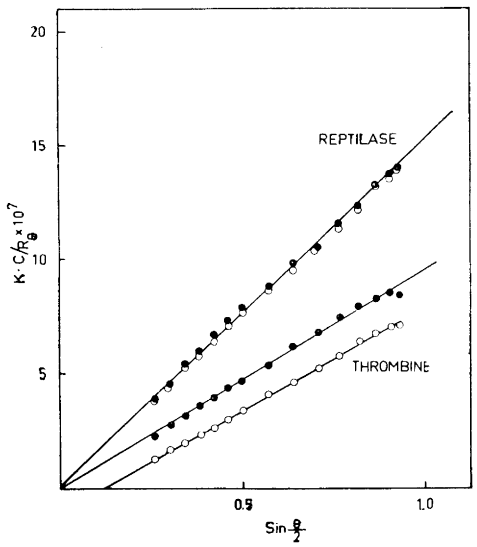


*Fig. 3.* Evaluation of light-scattering data according to Casassa. ($K = 2\pi^2 n^2 (dn/dc)^2 / N\lambda^4$, where $n$ is the refractive index of the solvent; $dn/dc$ the refractive increment, 0.197 ml/g; $N$, Avogadros number and $\lambda$ the wavelength of the light. $c$ is the concentration of protein in g/ml and $R_\Theta$ is the reduced angular intensity measured at the angle $\Theta$.) ● = protein concentration 0.07 %; ○ = protein concentration 0.14 %.

fibrinogen is converted to fibrin. The intermediate polymers formed were studied in 1 M urea at protein concentrations of 0.07 and 0.14 % 18—24 h after the dilutions were made. The angular distribution of the scattered light at a wavelength of 4 360 Å was measured at 35°—135° in a Brice-Phoenix light-scattering photometer using a cylindrical glasscell [8]. The solutions had been cleaned from dust in the usual way [4]. The results were evaluated according to Casassa [5] (Fig. 3). The slope of the line $K \cdot c/R_\Theta$ *vs.* sin $\Theta/2$ is related to the weight-average of M/L. For reptilase formed fibrin at 0.14 % concentration, the slope in four experiments was $1.50 - 1.78 \times 10^{-6}$ and for thrombin formed fibrin $0.87 - 1.16 \times 10^{-6}$. The averages correspond to M/L values of $0.73 \times 10^{11}$ and $1.16 \times 10^{11}$, respectively. If calculated according to Casassa, this should mean polymers 1.1 and 1.8 times wider than fibrinogen. At l ast at the present conditions, it seems as if reptilase formed fibrin was polymerized mainly end-to-end while thrombin formed fibrin had a tendency for lateral aggregation.

*Note added in proof.* The reptilase was obtained from AB Pentapharm, Basel. A new batch of this enzyme, obtained from the same firm, after this note was submitted for publication, had the same activity as thrombin, *i.e.* it splitted off both peptide A and B. Light-scattering data of fibrin obtained with this enzyme were identical with data of thrombin formed fibrin. This is in agreement with the observation that a lateral aggregation occurs when peptide B is split off. The reason for the different activities of the two reptilase batches is unknown.

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Received September 23, 1958.