A. J., *J. Nutrition*, 1937, v14, 273.

6. Speirs, R. S., and Meyer, R. K., *Endocrinology*, 1949, v45, 403.

7. Halberg, F., and Visscher, M. B., *ibid.*, 1952, v51, 320.

8. Hurley, L. S., and Morgan, A. F., *J. Biol. Chem*, 1952, v195, 583.

9. Shukers, C. F., and Day, P. L., *J. Nutrition*, 1943, v25, 511.

10. Mulinos, M. G., and Pomerantz, L., *Endocrinology*, 1942, v31, 276.

11. Butler, L. C., and Morgan, A. F., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 655.

12. Thorn, G. W., Forsham, P. H., Recant, L., and Hills, G. A., *J. Clin. Endocrinol.*, 1948, v8, 589.

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Action of Fibrinolysin (Plasmin) on Proteins.* (20811)

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Previous workers(1,2) have found that the products of the action of fibrinolysin (plasmin) on fibrinogen and fibrin are of high molecular weight. In the method of Ungar and coworkers(3) for the assay of fibrinolysin, unchanged fibrinogen is precipitated as fibrin and the material remaining in the filtrate is measured. If the fibrinogen is precipitated with trichloroacetic acid (TCA), the reaction products are precipitated along with the unchanged fibrinogen and cannot be found in the filtrate. Similarly, the soluble material formed by the action of fibrinolysin on fibrin is largely precipitable by TCA. The action of rennin upon casein is similar in some respects to that of thrombin upon fibrinogen. After exposure to the action of fibrinolysin if unchanged casein is precipitated with rennin, there is more soluble material present than when the casein is precipitated with TCA. These observations confirm the conclusions of previous workers(1,2) that the product of the action of fibrinolysin consists of proteins or peptides of high molecular weight.

Methods. The buffer in all cases was that of Ungar and coworkers(3) consisting of 0.15 M phosphate (sodium salts) of pH 7.4 in 0.1 M NaCl. Solutions of 0.1% bovine fibrinolysin† and 0.2% bovine fibrinogen (Armour) in buffer were made up daily as needed. Casein (Pfanstiehl) was dissolved in sufficient buffer to give a 2% solution by heating 10 minutes at the temperature of boiling water, cooled, made up to the original volume with distilled water, and filtered. It was stored in the refrigerator as long as one week. Thrombin† was used as a solution in isotonic saline containing 300 units per ml. It was stored in the frozen state for periods up to 2 weeks. Rennin (Nutritional Biochemicals) was dissolved in distilled water as needed to give a 0.1% solution. Fibrinolysin was assayed for its activity for fibrinogen and fibrin by the method of Ungar *et al.*(3). Activity for casein was determined similarly using rennin as the coagulant. The substrate (1.0 ml of 2% solution) was acted on by various concentrations of fibrinolysin in a total volume of 4.5 ml of buffer. After one hour at 37°C sufficient 0.1 M citric acid (1.1 ml) to bring the pH to about 5.3 and 0.1 ml of rennin solution were added. After 10 minutes at 37°C, the pH was adjusted to about 4.8 (0.3 ml of citric acid). The pH's were chosen to approximate the optima found by Lundsteen(4) for enzymatic action of rennin on casein and for coagulation of the product. After 30 minutes at 37°C the precipitates were filtered, and the optical density of the filtrates was read at 280 mμ against blanks without casein. Readings were corrected for the reading on casein with fibrinolysin absent. Although the isoelectric point of casein was approached at pH 4.8 and its solutions became milky in

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TABLE I.

Substrate	Optical density change in filtrate/mg fibrinolysin/hr: Thrombin or rennin coagulation method	TCA precipitation method	Ratio
Fibrinogen	1.71	.013	131.5
Fibrin	.106	.022	4.8
Casein	.248	.076	3.3

appearance, no coagulation occurred when rennin was absent.

To determine activities using TCA as precipitant, substrate was incubated with enzyme for one hour at 37°C under the above conditions. Sufficient TCA was then added to give a final concentration of 5%. After 30 minutes the precipitates were filtered off and the filtrates read at 280 mμ against blanks without enzyme. Experiments on each substrate were run simultaneously using aliquots from the same solutions of enzyme and substrate and varying only the method of precipitation of protein. Activity appeared to be linear with enzyme concentration over the range investigated in each case. Differences in range of enzyme concentration and time of incubation depending on the substrate and the method of precipitation were necessitated by the limitations of the spectrophotometer. Fibrinogen was incubated with 0-0.4 mg of fibrinolysin for 20 minutes when it was precipitated with thrombin, whereas it was incubated one hour with 0-3.0 mg of enzyme when it was precipitated by TCA. The enzyme was assayed for activity on fibrin at levels of 0-2.0 mg, the incubation time being one hour. When unchanged casein was precipitated with rennin fibrinolysin was assayed at levels of 0-1.0 mg, compared to a range of 0-3.0 mg when TCA was used. When TCA was the protein precipitant the final volumes were 6.3 ml for fibrinogen and 6.6 ml for fibrin, rather than 6.0 ml, as in all other cases. Optical density differences were corrected to allow for the additional dilution.

Results. Table I gives the average change in optical density of filtrate per hour per milligram of fibrinolysin. It can be seen that the products of the action of fibrinolysin on fibrinogen, which are non-coagulable by thrombin, are almost entirely precipitated in 5% TCA. They must therefore consist of peptides of high molecular weight. They could not be differentiated from fibrinogen by paper chromatography or paper ionophoresis under several conditions, and preliminary light scattering measurements indicate that their average molecular weight is of a comparable order of magnitude to that of fibrinogen. As the fibrinolysin preparation is not homogeneous, the very small amount of product which is soluble in TCA may result from proteolytic activity of a contaminating enzyme upon fibrinogen or the initial reaction products. Several authors(5-8) have found evidence for a multiplicity of enzymes with peptidase, proteolytic, or fibrinolytic activity in various blood sera.

The activity of the enzyme for casein and for fibrin is of a different magnitude than its activity for fibrinogen. Ungar and coworkers (9) have already demonstrated this difference in activity for fibrinogen and fibrin. This may be because of a difference in affinity of the enzyme for these substrates or because they are acted on by an enzyme present as a contaminant in low concentration. The insolubility of fibrin may also account for the relatively poor activity of the enzyme. The products from fibrin, as determined by lysis, and from casein, as determined by non-coagulability with rennin, are largely precipitated by TCA, as shown in Table I. However, about 20% of each product is soluble in 5% TCA, if the reaction rate can be assumed constant and linear with enzyme concentration. The portion of lower molecular weight may result either from simultaneous splitting of smaller fragments from the original protein by the principal enzyme or an impurity, or by further splitting of the primary products. Several proteolytic enzymes have been shown(10-13) to form primary products of high molecular weight, which are then broken down to smaller fragments. The action of fibrinolysin may well be similar in nature, except that the products of high molecular weight from fibrinogen do not readily undergo further reaction.

Summary. By the action of fibrinolysin fibrinogen is converted to products which are

not coagulated by thrombin, but which are precipitated by TCA. The enzyme is much less active for fibrin and casein. The former is lysed to give a product only about 20% of which is soluble in TCA. The product from casein, which is not coagulated by rennin, is also largely precipitated by TCA, although soluble to the extent of about 20%.

1. Holmberg, C. G., *Ark. Kemi, Mineral Geol.*, 1944, v17A, No. 28.

2. Seegers, W. H., Nieft, M. L., and Vandenvelt, J. M., *Arch. Biochem.*, 1945, v7, 15.

3. Ungar, G., Damgaard, E., and Hummel, F. P., *Endocrinology*, 1951, v49, 805.

4. Lundsteen, E., *Biochem. Z.*, 1938, v295, 191.

5. Holman, H. R., White, A., and Fruton, J. S., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 196.

6. Mole, R. H., *J. Path. Bact.*, 1948, v60, 413.

7. Cliffton, E. E., and Cannamela, D. A., *Blood*, 1953, v8, 554.

8. ———, *J. Applied Physiol.*, 1953, v6, 42.

9. Ungar, G., Damgaard, E., and Hummel, F. P., *J. Exp. Med.*, in press.

10. Petermann, M. L., *J. Biol. Chem.*, 1942, v144, 607.

11. ———, *J. Phys. Chem.*, 1942, v46, 183.

12. Moring-Claesson, I., *Biochim. et Biophys. Acta*, 1948, v2, 389.

13. Desnuelle, P., Rovery, M., and Bonjour, G., *Biochim. et Biophys. Acta*, 1950, v5, 116.

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The Depolarization of Nerve by Organic Compounds.* (20812)

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One of the perplexing aspects of the action of narcotic agents on nerve has been noted by Laget *et al.*(1), by Bishop(2), and by Brink(3). This is the fact that while the lower alcohols of the paraffin series depolarize nerve before a conduction block ensues, the higher alcohols and many other narcotic agents block conduction before there is any detectable depolarization. Indeed, there seems to be a slight increase in the membrane polarization associated with the action of the higher alcohols and with cocaine. In order to investigate this problem in more detail, we have measured the depolarization produced by a variety of organic reagents, and we have attempted to explain the results obtained in terms of a model.

Methods. In most experiments, bullfrog (*R. catesbiana*) sciatic nerves were used, although a few measurements were made on *R. pipiens* nerves. The general arrangement of the chamber for mounting nerves was similar to that used by Lorente(4). In most cases, the depolarization produced by 0.11 M KCl at one region along the nerve was compared with the depolarization produced by an experimental solution at another region along the nerve. Such measurements permitted the calculation of a depolarization rate relative to the rate of KCl depolarization as a standard. Measurements were made with a recording potentiometer so arranged as to record the potential between the KCl-Ringer segment and between the narcotic agent-Ringer segment at 16 sec. intervals. The sensitivity of this instrument with the nerve in the external circuit was about 0.1 mV, and the current drawn at balance, 10^{-9} amperes. The usual technic for measurement was to mount a nerve, previously dissected and kept in Ringer solution for 2 hours, in the chamber with Ringer in all electrodes. This was a check for any initial depolarization. Next, KCl, 0.11 M was added to electrode 1. Ringer to electrode 2, and test agent dissolved in Ringer to electrode 3. Recording was initiated, and continued usually for several hours after which all electrodes were changed to Ringer, and recording was continued to follow the course of repolarization. Throughout the course of the experiments, 95% O_2-

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