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# Nature and Role of the Lytic Factor in Hemolytic Streptococcal Fibrinolysis.*

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The dissolution of human plasma-clot by cultures or culture-filtrates of Group A β-hemolytic streptococci was observed by Tillett and Garner.[1] The active agent, termed fibrinolysin, was also capable of lysing preparations of fibrinogen, as was indicated by the loss of clotting ability of the fibrinogen and by the alteration of its properties of solubility, heat coagulability,[2] and antigenicity.[3] It was not active on casein, gelatin, or peptone, and only slightly active on fibrins of animal origin.[4]

The action of fibrinolysin on human fibrin was accompanied by a slight increase in amino nitrogen. Since the end-product remained protein in nature, Garner and Tillett[2] concluded that the degradation of the fibrin molecule was slight. They stated that fibrinolysin was probably an enzyme, but that its action was specific for fibrinogen and fibrin of human origin.

Milstone[5] demonstrated that human fibrinogen, purified by repeated precipitation, was not affected by fibrinolysin, but that the process of streptococcal fibrinolysis required the presence of an accessory *lytic factor* associated with the euglobulin component of human serum. Lytic factor preparations *per se* generally showed no lytic activity on fibrinogen or fibrin.

In recent blood coagulation studies, Tagnon, Davidson, and Taylor[6] isolated from normal human plasma a euglobulin component, *globulin substance,* which, after treatment with chloroform, possessed both fibrinogenolytic and fibrinolytic properties. Subsequent studies[7] demonstrated that the chloroform-activated *globulin substance* was a true tryptic enzyme capable of digesting gelatin, casein, and hemoglobin.

Since the two substances, lytic factor and globulin substance, were associated with the euglobulin component of the serum proteins, since each was obtained from normal serum by a similar procedure of dilution and acidification, and since each was apparently a major participant in the fibrinolytic process, it seemed possible that the two preparations contained the same lytic agent. Moreover, though the relation of streptococcal fibrinolysin to the lytic factor remained obscure, it was similar in some respects to the interaction of chloroform and globulin substance.

The purpose of this work, therefore, is to report: (1) a study of the relationship of the lytic factor of Milstone to the serum tryptase, and (2) an investigation of the interaction of fibrinolysin and the lytic factor in an attempt to elucidate further the mechanism of hemolytic streptococcal fibrinolysis.

*Materials and Methods. Fibrinolysin Prep-*

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1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

2 Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 255.

3 Jablonowitz, J. M., *J. Immunol.*, 1939, **36**, 261.

4 Van Deventer, J. K., and Reich, T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 821.

5 Milstone, H., *J. Immunol.*, 1941, **42**, 109.

6 Tagnon, H. J., Davidson, C. S., and Taylor, F. H. L., *J. Clin. Invest.*, 1942, **21**, 525.

7 Kaplan, M. H., Tagnon, H. J., Davidson, C. S., and Taylor, F. H. L., *J. Clin. Invest.*, 1942, **21**, 533.

*aration.* The source of the fibrinolysin was a highly active fibrinolytic strain of Group A hemolytic streptococcus isolated from the blood stream of a patient with erysipelas. Fibrinolysin was obtained by the alcohol-precipitation method of Garner and Tillett[8] from a Seitz filtrate of an 18-hour culture in beef-heart infusion broth containing 0.05% added dextrose. The alcohol precipitate was redissolved in 1/100 M phosphate-buffered physiological saline, pH 7.4, and dried in 5 ml lots by the lyophile process. The dried product was highly stable, and upon reconstitution with distilled water, dissolved rapidly and completely.

*Fibrinogen.* Stable lyophilized preparations of human fibrinogen were obtained through the courtesy of Drs. E. J. Cohn and S. Howard Armstrong, Jr., of the Department of Physical Chemistry, Harvard Medical School.† The fibrinogen solution employed contained 0.15% fibrinogen in 1/100 M phosphate buffered saline, pH 7.4. Clots formed from the fibrinogen preparation were readily lysed by fibrinolysin, indicating that the preparation contained lytic factor.

*Thrombin.* A stable and highly active rabbit thrombin preparation ("Hemostatic Globulin," Lederle Laboratories, Inc.), was employed in a 1:10 dilution.

Lytic factor was prepared by the method of Milstone.[5]

Globulin substance was prepared by the acid precipitation method of Pohle and Taylor,[9] except that either defibrinated plasma or serum was used instead of citrated plasma.

For the determination of proteolytic activity the substrates used were fibrin, hemoglobin, and gelatin.

In the case of fibrin, lysis rate was determined on a standard fibrinogen-thrombin clot formed by the addition of 0.2 ml of a 1:10 dilution of rabbit thrombin to a mixture of 1.0 ml of the standard 0.15% fibrinogen solution and 1.0 ml of the test material. Lysis time was taken as the interval between the clotting time and complete dissolution of the fibrin clot. The clotting time was approximately 3 minutes.

Gelatin liquefaction was measured viscosimetrically by Ostwald viscosity tubes in a constant temperature bath at 37.5°C. The gelatin was a 3% solution in 1/20 M phosphate buffer, pH 7.4; it contained thymol as a bacteriostatic agent. Proteolytic activity was determined as follows: 4.0 ml of gelatin were mixed with 2.0 ml of the test preparation, and 5.0 ml of this mixture were then transferred to a viscosity tube. The temperature of the reagents was adjusted to 37.5°C before mixing. Viscosity efflux times at 37.5°C were determined at 20-minute intervals. Rate of proteolysis was measured by the percent decrease in efflux time of the substrate-enzyme mixture. The initial efflux time was determined by extrapolation to 0 time.

The hydrolysis of hemoglobin was measured by the liberation of tyrosine, as determined colorimetrically by the method of Anson.[10] Merthiolate to a final concentration of 1:10,000 was used as the bacteriostatic agent in the hemoglobin substrate preparations.

Bacteriological cultures on all enzyme-substrate mixtures were negative in both plate and broth culture.

All determinations of pH were made by the glass electrode.

*Results.* A. *Relation of Lytic Factor to Serum Tryptase.* Globulin substance treated with chloroform possesses proteolytic properties. When lytic factor preparation was incubated with 1/10 its volume of chloroform for 48 hours at 25°C, and the chloroform then removed by centrifugation and aeration, a proteolytic material was elaborated as was indicated by its action on gelatin, hemoglobin, and fibrin (Table I). Globulin substance and lytic factor preparation not treated with

[8] Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239.

† The products of plasma fractionation employed in this work were prepared by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, from blood collected by the American Red Cross, under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[9] Pohle, F. J., and Taylor, F. H. L., *J. Clin. Invest.*, 1937, **16**, 741.

[10] Anson, M. L., *J. Gen. Physiol.*, 1938, **22**, 79.

TABLE I.
Proteolytic Activity of Lytic Factor and Globulin Substance Preparations Activated by Chloroform, Alcohol, and Acetone.

| Exp. No. | Preparation | Incubated 2 days with 1/10 vol. | Gelatin Change in relative viscosity in 2 hr % | Hemoglobin Increase in tyrosine (equivalents/ml) in 24 hr | Fibrin Lysis time |
|---|---|---|---|---|---|
| 1 | Lytic Factor | Chloroform | 12.7 | 36.6 | 17 min |
| 2 | Globulin Substance | " | 5.4 | 27.6 | 26 " |
| 3 | Lytic Factor | — | 0 | 0 | >24 hr |
| 4 | Globulin Substance | — | 1.1 | 0 | >24 " |
| 5 | Lytic Factor | Alcohol | 6.3 | 11.1 | 80 min |
| 6 | Globulin Substance | " | 5.2 | 15.0 | 10 " |
| 7 | Lytic Factor | Acetone | 4.1 | 8.7 | 90 " |
| 8 | Globulin Substance | " | 9.1 | 16.2 | 10 " |

TABLE II.
Proteolytic Activity of the Lytic Factor Activated by Fibrinolysin.

| Exp. No. | Preparation | Fibrinolysin, ml | Saline, ml | Gelatin Change in relative viscosity in 2 hr % | Hemoglobin Increase in tyrosine (equivalents/ml) in 24 hr |
|---|---|---|---|---|---|
| 1 | 1.0 ml Lytic Factor | 0 | 1.0 | 1.1 | 0 |
| 2 | 0 " " " | 1.0 | 1.0 | 0 | 0 |
| 3 | 1.0 " " " | 1.0 | 0 | 23.9 | 16.8 |
| 4 | 1.0 Globulin Substance | 0 | 1.0 | 1.0 | 0 |
| 5 | 1.0 " " | 1.0 | 0 | 26.6 | 13.2 |

chloroform demonstrated little or no activity (Exp. 3 and 4, Table I).

When the two preparations were made from equal volumes of defibrinated plasma, the amount of activity on hemoglobin in both cases was fairly comparable at the end of 24 hours, as indicated by the tyrosine levels at this point.

Liberation of the serum tryptase by other organic solvents such as alcohol and acetone has been reported.[11] The incubation of lytic factor and globulin substance with 1/10 volumes of alcohol and acetone also resulted in the elicitation of proteolytic activity. (Exps. 5, 6, 7, 8, Table I).

The lytic factor preparation of Milstone may then be activated by chloroform and other organic solvents to yield a true proteolytic enzyme. The liberated tryptase is comparable in its activity on the substrates studied to the enzyme obtained from globulin substance. Both lytic factor and globulin substance preparations are comparable in their quantitative content of the proteinase.

Lytic factor was originally obtained by Milstone from diluted serum at an acid reaction corresponding at pH 5.0-5.2; globulin substance was obtained by Pohle and Taylor from diluted plasma at pH 6.0.

The results obtained above have indicated that the iso-electric precipitates at the two close pH values probably contain the same lytic constituent.

B. *Fibrinolysin-Lytic Factor Reaction.* Milstone reported that the addition of lytic factor preparation to purified fibrin was without effect, but on the admixture of fibrinolysin, clot dissolution occurred readily. To determine whether this was a case of true proteolytic action, each of the following—fibrinolysin, lytic factor preparation, and a lytic factor-fibrinolysin mixture—was incubated with gelatin, hemoglobin, and fibrin. Lytic factor alone and fibrinolysin alone were without effect; but in the presence of both sub-

[11] Stephan, R., and Wohl, E., *Z. exp. Med.*, 1921, **24**, 391.

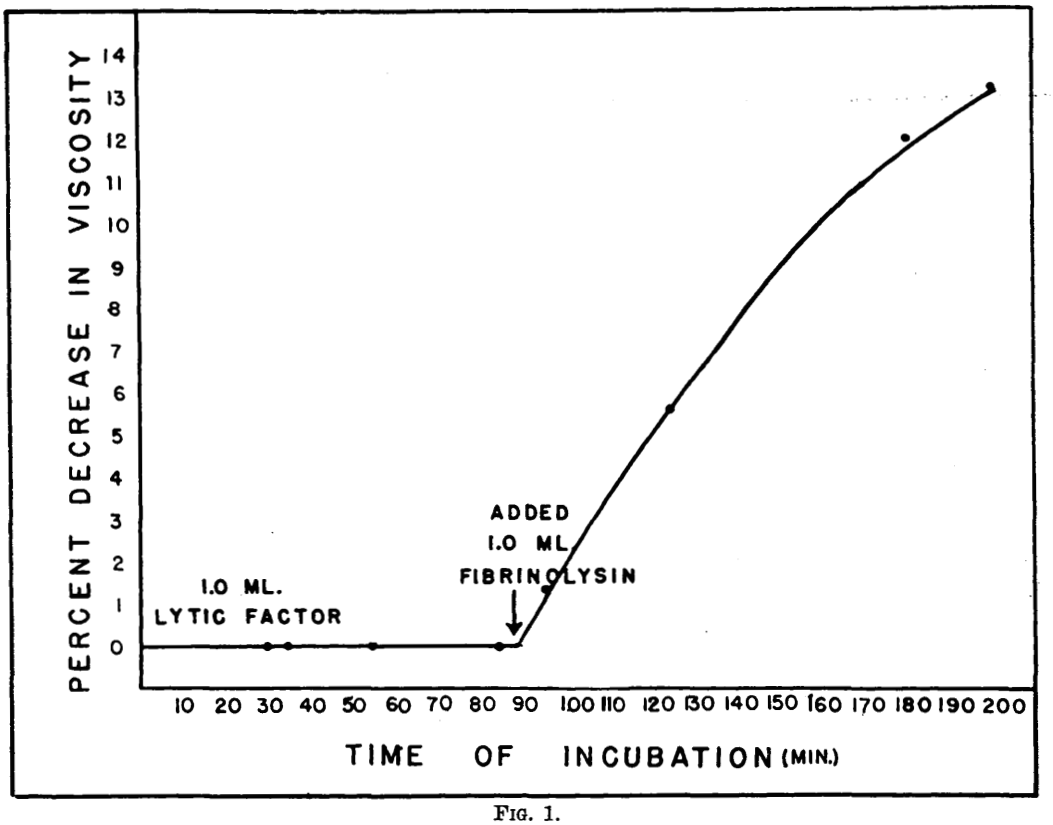


FIG. 1.
Activation of the lytic factor by fibrinolysin with gelatin as substrate.

stances, hydrolysis of gelatin and hemoglobin was marked (Table II) (Fig. 1).

Identical results were obtained when globulin substance was used instead of lytic factor (Exps. 4, 5, Table II).

In no case was fibrinolysin alone active on gelatin or hemoglobin substrates; proteolysis occurred only upon the concomitant action of fibrinolysin and lytic factor. However, since lytic factor itself could be converted by chloroform, alcohol, or acetone to an active proteinase, it must be concluded that the function of fibrinolysin then involves the activation of the serum tryptase from an inert precursor. Christensen,[12] in a recent abstract, has independently reported activation of the lytic factor by fibrinolysin.

The chemical basis for the activation reaction is obscure. In general, activation by the organic solvents proceeds at an exceedingly slow rate, while activation by fibrinolysin is comparatively rapid and suggestive of a monomolecular catalytic reaction. Studies of the chemical nature and the kinetics of the lytic factor-fibrinolysin reaction are being continued.

*Summary.* The lytic factor of Milstone and the globulin substance may both be activated by certain organic solvents to yield a true proteolytic enzyme. Both lytic factor and globulin substance are comparable in their quantitative content of the proteinase.

Fibrinolysin was found to activate the serum tryptase from an inert precursor.

[12] Christensen, L. R., *J. Bact.*, 1944, **47**, 65.