13002

Quantitative Determination of Activity of Streptococcal Fibrinolysin.

L. R. CHRISTENSEN. (Introduced by L. R. Jones.)

From the Department of Bacteriology, St. Louis University School of Medicine, St. Louis, Mo.

A survey of the literature indicates that very few quantitative studies have been made on streptococcal fibrinolysin. The majority of attempts which have been made have involved serial dilutions of cultures or culture-filtrates which were incorporated in fibrin clots and in this way tested for lytic activity. Van Deventer[1] proposed as a unit of fibrinolysin that amount causing dissolution of a plasma fibrin clot at the end of 24 hours. Fraser and Madison[2] used essentially the same technic with the substitution of fibrin prepared from freshly-prepared fibrinogen and thrombin solutions. Later, Madison and Taranik[3] attempted a still further standardization of the test. In this procedure, the highest dilution causing lysis at the end of 2 hours contained one unit of fibrinolytic activity.

Massell, Mote and Jones[4] in an extensive study of antifibrinolysins devised a method for measuring the amount of antifibrinolysin present in a serum, based on their belief that fibrinolysin is quantitatively neutralized by antifibrinolysin. By appropriate modification of their procedure, it is possible to compare the lytic activity of 2 or more lysins, but absolute quantitative results are not obtained.

Lack of uniformity in the fibrin used as substrate, the effects of various media components, and the effects of other enzymes in the culture filtrates have seemed to render difficult the development of a satisfactory quantitative test. Further, in none of these reports has any data on retests been given, so it is impossible to evalute the reliability of the various procedures from the standpoint of reproducibility. Also, it has been noted in this laboratory that freshly-prepared fibrinogen samples vary in their susceptibility to lysis. It is believed that the chief factor in these differences in susceptibility is the variable protein content of such fibrinogen solutions.

---

1 Van Deventer, J. K., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 50.

2 Fraser, F. H., and Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 307.

3 Madison, R. R., and Taranik, J. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 1.

4 Massell, B. F., Mote, J. R., and Jones, T. D., *J. Immunol.*, 1939, **36**, 45.

With a standardized, stable substrate available through the use of dried fibrinogen and dried prothrombin,[5, 6] the possibility of the construction of a satisfactory quantitative fibrinolysin test was investigated.

The effects of fibrinogen concentration, temperature of incubation, and pH on fibrinolysis have been discussed in a previous paper,[6] together with the reasons for the use of 0.1% fibrinogen solution in the preparation of the standard fibrin clot. In the experiments to be reported, suitable culture-filtrates, serially diluted in M/10 phosphate buffer (pH 7.2-7.3) were added in 0.2 cc volume to 1.0 cc of the standard fibrinogen solution prepared from desiccated fibrinogen and the mixtures clotted by the addition of 0.1 cc of thrombin solution prepared from dried prothrombin. The fibrinlysin mixture had a pH of 7.2-7.3, and was incubated at 40°C in a water bath following clotting.

A typical lysis curve is presented in Fig. 1. Lysis time represents the time in minutes elapsing between clotting and complete dissolution of the fibrin clot. It can be seen that over the greater portion of its length the curve approximates a straight line. All lysis curves are of the same shape, although the slope of the curve varies inversely with the original strength of the fibrinolysin sample.

Determination of the Fibrinolytic Unit. Originally, it was decided to designate as one unit that dilution of lysin, obtained by in-

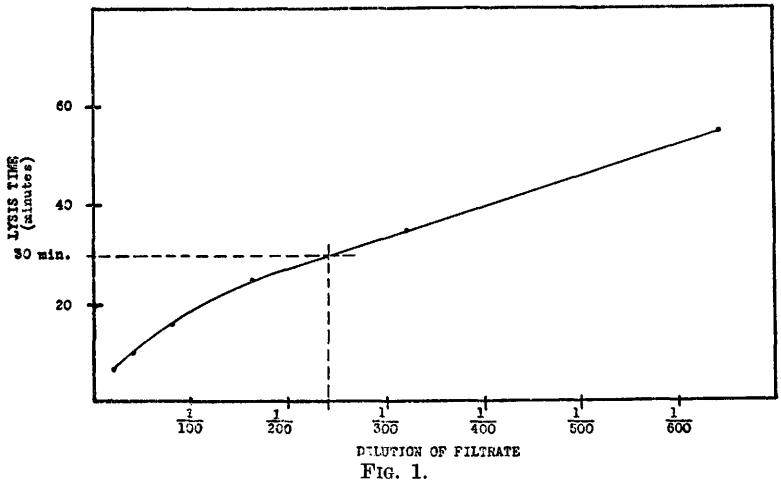

FIG. 1.
Quantitative Determination of Fibrinolysin.

5 Christensen, L. R., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 569.

6 Christensen, L. R., *J. Inf. Dis.*, 1940, **66**, 278.

terpolation on the lysis curve, which lysed the standard clot in one hour. It was noted, however, that as the incubation period approached one hour, the tubes containing the higher dilutions of fibrinolysin showed marked shred formation in the clot due to denaturation of the fibrin. With experience, the true end-point of lysis could be approximated fairly accurately, but because of this difficulty, a shorter interval, 30 minutes, was adopted as the end-point of the titration. At this time-interval, lysis is more clear-cut, without the marked shredding of the fibrin seen on longer incubation, and by this time the lysis curves have straightened. One unit of fibrinolysin is defined, therefore, as that amount, in a volume of 0.2 cc, which lyses the standard fibrin clot in 30 minutes, this amount being determined by interpolation on the lysis curve. A simple calculation will then give the number of units of lysin in 1 cc of the original material.

The following example will illustrate the method: Serial dilutions of a sample of fibrinolysin obtained from strain 1212 were prepared and tested against standard fibrin clots, with the lysis times recorded in Table I.

When these data are plotted (Fig. 1), it is seen that the dilution lysing in 30 minutes, obtained by interpolation would be 1:240. This dilution contains 1 unit; therefore, 1 cc of the original, undiluted material contains 1200 units (240×5).

The unit herein described is admittedly arbitrary and artificial. It does, however, have definite advantages which make it practicable when large numbers of quantitative tests must be made. The test as described is simple to perform, results are available in a short time (30-45 minutes), and the short incubation period precludes growth of the microörganisms influencing the results when whole cultures are tested.

Evaluation of Method. To establish the reproducibility of the method, a batch of fibrinolysin was prepared and stored in the ice box. Experience has shown that if adjusted to the proper pH, such filtrates will show no significant variation in activity over several days. For test materials, batches of dried fibrinogen and of dried prothrombin were used, and successive titrations of the lysin were made over a period of 3 days. In Table II, the number of units was determined by the method outlined above. The figures re-

TABLE I.
Titration of Fibrinolytic Activity in Minutes.

Dilution	1:20	1:40	1:80	1:160	1:320	1:640
Lysis time, min	7	10	15½	25	35	54½

corded in the "fibrinogen" and "thrombin" columns refer to the solution prepared from the dried reagents. Thus, the first result in Table II indicates that the third solution of fibrinogen prepared from the dried material was clotted with the first solution of thrombin prepared from the dried prothrombin.

The first half of Table II represents those tests, selected from the entire series, in which the thrombin and fibrinogen solutions were not over 1 hour old at the time of testing. The remainder of the tests in the table illustrate the influence of the age of the test solutions (fibrinogen and thrombin) on the results. In these latter tests either one or both of the reagents was over one hour old at the time of testing.

From these data, it is quite evident that quantitative determinations are not reliable if either the thrombin or fibrinogen solutions are more than one hour old. However, when freshly-prepared solutions are used, agreement of successive determinations is excellent. It must be emphasized that fibrinogen and thrombin solutions will serve for qualitative tests of lysin activity for many hours, even several days if kept in the ice box. Evidently, however, the slight denaturation which occurs in even a short time exerts a marked influence on lysis time. The nature of the changes occurring in the reagents is not known, but it is of interest that in some cases lysis time is decreased and in others it is increased.

TABLE II.
Titration of Fibrinolytic Activity.

(Fresh reagents)			(Old reagents)		
Fibrinogen	Thrombin	Units	Fibrinogen	Thrombin	Units
3	1	1065	1	2	1185
4	3	1115	1	2	835
5	4	1245	2	1	685
5	4	1200	2	2	860
7	5	1120	3	2	810
9	7	1230	1	2	1435
10	8	1170	2	2	1355
10	8	1170	3	2	1310
11	9	1150	2	1	1575
11	9	1200	4	2	1280
11	9	1175	4	3	1315
12	10	1100	5	4	980
12	10	1075	5	4	930
			7	5	1425
			7	4	1600
			8	6	1600
1155	Mean		1198	Mean	
54.77	Standard Deviation		293.3	Standard Deviation	
4.74%	Coefficient of Variation		24.48%	Coefficient of Variation	