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Estimation of Trypsin by Fibrinolysis.

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It has been pointed out that the actions of crystalline trypsin on blood-clotting systems are: 1. thromboplastic,[1] 2. prothrombinolytic[2] and thrombinolytic,[3] 3. fibrinogenolytic and fibrinolytic.[4] Preliminary tests with varying amounts of crystalline trypsin (X.T. = preparation of Dr. T. E. Weichselbaum, Washington University, St. Louis) and commercial trypsin (F. T. = Fairchild Bros. and Foster) established the fact that fibrinogen and fibrin are more sensitive than thrombin to the lytic action of the protease. This is a serious handicap in attempts to assay trypsin by the thrombinolytic method previously suggested.[3] However, fibrinolysis offers an alternative and simpler technic for the enzyme assay, which may be made especially accurate if the fibrinolysis is followed by *relative turbidimetry* (*cf.*[4]) using the Evelyn photoelectric colorimeter.

Method. Essential reagents include a stable fibrinogen (F)[4] and a stable thrombin (*e.g.* T_G = 1:100 dilution of "rabbit clotting globulin," kindly supplied by Dr. I. A. Parfentjev,[5] Lederle Labs., N. Y.). In each of a series of enzyme dilutions, 1 cc trypsin is added to 5 cc F + 3 cc T_G + 1 cc saline (= control for experiments, other than those cited, in which it is desired to test effects of inhibitors, etc.), a few sec prior to the onset of clotting. Control of clotting conditions must include temperature and pH. Relative turbidity is measured at intervals by the galvanometer scale deflection of the Evelyn apparatus. Data are plotted in the form of a series of curves corresponding to the various dilutions of trypsin used. It is not the shape of the curves which is significant but their relative positions along the time axis. Hence, conversion of the galvanometer reading (G) into "photometric density" (L), by means of the formula L = 2—log G, is an unnecessary refinement of the method described.

Quantitation of tryptic fibrinolysis, timed with the photoelectric colorimeter. In order to determine the sensitivity of the "lytic-time" differences over a convenient range of observation times, dilutions varying by 10% were made from a stock trypsin solution (1:2000 F.T. = 100%). The data (G values) between 90% and 20% are illustrated in Fig. 1. The sharply rising end portions of the fibrinolytic curves are separated by marked time intervals.

An unknown solution (suitably diluted) of similarly-acting lytic agent could easily be assayed, well within 10%, from the position of its end-curve relative to the trypsin standards. Additional tests with crystalline trypsin indicate an absolute (practical) sensitivity of the test of the order of 1:100,000 X.T. (1:1,000,000 *final* conc.), depending, in part, on fibrinogen concentration, pH, and other factors which remain to be investigated. The method is suitable for serum tryptase, papain, and other proteases (*e.g.* venoms, bacterial fibrinolysins, etc.) which are active in a pH range (6.5 — 8.5) suitable for the thrombic-clotting of fibrinogen. It is an easy way to follow steps in the purification of such enzymes and their behavior with respect to small changes in pH, salt concentration, action of inhibitors, etc.

In the tests of Fig. 1 the following *clotting-times* were noted: (a) control (0) — 30 sec.; (b) other tubes — less than 1-2 min. Thus, neither thrombinolysis nor fibrinogenolysis were complicating factors of any practical

1 Ferguson, J. H., and Erickson, B. Nims., *Am. J. Physiol.*, 1939, **126**, 661.

2 Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 80.

3 Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.*, 1940, **24**, 169.

4 Ferguson, J. H., *J. Gen. Physiol.*, 1942, **25**, 607.

5 Parfentjev, I. A., *Am. J. Med. Sci.*, 1941, **202**, 578.

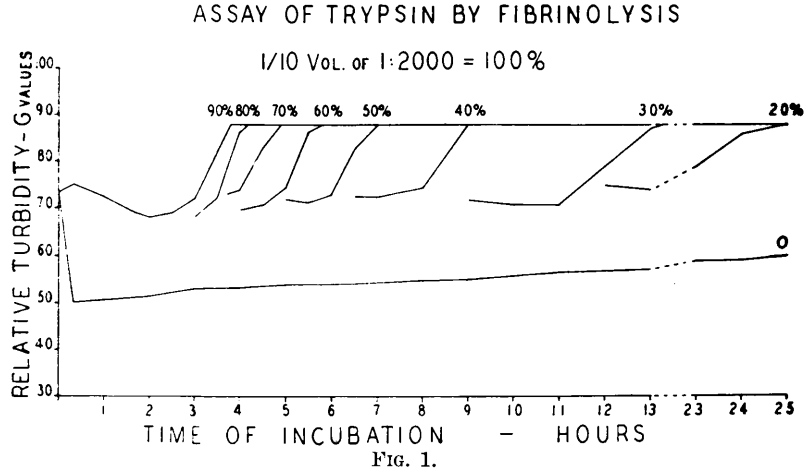

FIG. 1.

Temp. = 29°C; pH = 7.95. G values, with green filter (540): tube and saline (10 cc) = 100; initial reading (all tubes) = 73.0; final reading (all tubes, except control) = 88.0. The persistent turbidity is probably due to the faint opalescence attributable to lipids.

significance. Such fibrinogenolysis as occurs lessens the significance of the absolute turbidimetric values but, within limits, enhances the separation of the test curves. The method is valid, therefore, provided that a good clot is obtained within a minute or two of the control. The stock trypsin solution in the cited series could, with advantage, have been a little less dilute. With a 1-2 hr "lytic-time" for the 100% standard, the series should be completed, down to 20% or 10%, in 6-10 hr.

A good commercial trypsin affords a satisfactory standard of reference but it should be analyzed by some independent standard technic, such as the Anson and Mirsky[6] hemoglobin method. This was not done for the preparation cited, but four "end-points," *viz.* 1. incipient thrombinolysis in 1 hr, 2. complete thrombinolysis in 1-1½ hr, 3. fibrinogenolysis, in 15 sec; 4. fibrinolysis in 2-2½ hr, were obtained in parallel dilution series of (a) F.T. and (b) X.T. The 4 comparisons all placed the strength of F.T. at 0.4 × that of the crystalline trypsin, 1 mg of which assayed 4.24×10^{-3} [T.U.] Hb.

[6] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1933, **17**, 151, *et seq.*