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Received October 8, 1956. P.S.E.B.M., 1957, v94.

Relation Between Degree of Fibrinogen Proteolysis by Plasmin and Fibrinolytic Paracoagulation.*† (22916)

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Fibrinolytic paracoagulation, or clotting of lysed fibrinogen or fibrin(1,2), may be obtained only during a period which depends on experimental conditions. Previous works suggested a relation between degree of fibrinogen proteolysis and fibrinolytic paracoagulation which is here studied.

Materials and methods. Bovin fibrinogen (Fraction I from Armour Laboratories) was purified according to Laki(3) yielding preparations with 90-96% of total protein clottable by thrombin; chloroform activated plasmin was prepared by the method described by Loomis(4,5); Parke Davis thrombin with 5 mg/ml (approximately 100 u/ml) and 2M sodium sulphate (reagent grade) were used to effect paracoagulation, and phosphates buffer pH 7.6 to control pH. Reaction mixture: 1.5 ml of buffer and 7.5 ml of plasmin were added to 30 ml of fibrinogen solution and the mixture incubated at 40°C. Standard conditions throughout were: fibrinogen 5.150 g/l, pH 7.6 and ionic strength 0.2. Varying plasmin concentrations were employed in different reaction mixtures to reach final values of 0.60, 0.40 and 0.20 mg/ml respectively. Measurements of clotting time, paracoagulation test and non-protein nitrogen were made on aliquots withdrawn at suitable intervals. Determination of clotting time was carried out as follows: 0.9 ml of reaction mixture were transferred to test tube and 0.1 ml of thrombin added. The tube was shaken lightly and placed in water bath. The stop watch was clicked as thrombin was blown in and the time required for formation of a clot was recorded. When the mixture became unclottable paracoagulation was obtained with 0.5 ml of sodium sulphate forcibly blown into test tube, 1 minute after addition of thrombin. Paracoagulation was repeated until a negative test was obtained (extinction) and the time required for extinction was measured from initial addition of plasmin. To determine acid soluble split products, 4 ml aliquots of reaction mixture were discharged into equal amount of 10% trichloroacetic acid. The mixture was thoroughly shaken, allowed to stand 20 minutes and then filtered. Micro Kjeldahl digestions were carried out on 5 ml of filtrate using the catalyst mixture recommended by Chibnal, Rees and Williams(6) and N estimations were made according to Ma and Zuazaga(7). Acid soluble material at zero time was calculated from micro Kjeldahl estimations made on fibrinogen and plasmin specimens prior to their mixture. At least 4 curves for each plasmin concentration were carried out. Results are summarized in Fig. 1.

Results. As expected, the time required for the mixture to become unclottable by

* Presented at meeting of Soc. Biol. Santiago de Chile, July 10, 1956.

† We are indebted to Dr. J. B. Lesh from Armour Laboratories for supplying bovin fibrinogen.

‡ Research fellows of University of Chile (1955-1957).

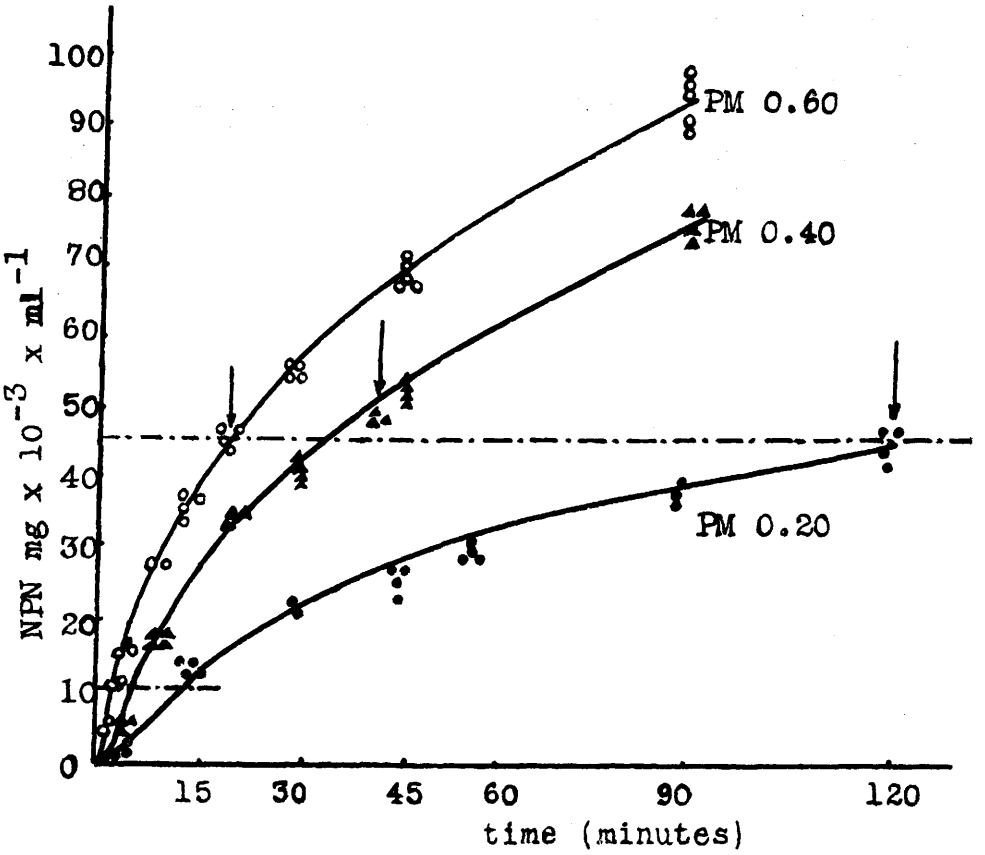

FIG. 1. NPN liberation and paracoagulation. Plasmin (PM) 0.60, 0.40 and 0.20 mg/ml of reaction mixture. Times for mixture to become unclottable by thrombin, 2½, 5 and 13 min., and for extinction of paracoagulation 20, 40 and 120 min. respectively. Arrows show experimental values when extinction was reached. An additional curve was obtained with saline instead of plasmin, but no increase in NPN was recorded.

thrombin and for extinction of paracoagulation differed for the 3 plasmin concentrations employed. Nevertheless, increase in NPN was the same for all curves at the time of extinction and amounted 45.7 ± 0.54 x 10^{-3} mg/ml of reaction mixture. NPN was not determined at time the mixture became unclottable by thrombin, but from interpolation data it was calculated to be nearly the same for the 3 series and amounted to approximately 10 x 10^{-3} mg/ml of reaction mixture. Though NPN liberation was mentioned by previous authors(8-10) who studied fibrinogen residues after lysis, it was neglected, and attention was drawn to the large residues. Holmberg(9) suggested that under the action of plasmin the fibrinogen molecule is split off into some few components whose molecular weights from sedimentation and diffusion constants were calculated to be about 100,000. A small loss of N was attributed to possible attack on products of lysis by TCA acid. Kaplan(10) showed that the products of the action of plasmin on fibrinogen, which are non-coagulable by thrombin, could not be differentiated from fibrinogen and he suggested that the very small amounts of acid-soluble products which appear might result from a proteolytic activity of contaminant enzyme on fibrinogen.

The close relationship shown in our curves between levels of liberated NPN and behavior of lysed fibrinogen, when tested against thrombin and the paracoagulant substances, indicates that NPN liberated comes from plasmin-fibrinogen reaction, and that inten-

sity of paracoagulation is closely related to degree of fibrinogen proteolysis by plasmin. In this way, the small amount of acid-soluble material liberated is comparably important to the liberation of fibrinopeptides from fibrinogen by thrombin (3-4% of total N)(11), which gives rise to important changes in the original molecule. It is a matter of further study whether plasmin-liberated peptides play an active role as inhibitor or dissolution agents on the clotting of fibrinogen and on fibrin clot respectively.

Summary. Fibrinogen proteolysis by plasmin and the clotting of lysed fibrinogen were studied. Close relationship was found between increase in NPN and behavior of lysed fibrinogen.

We wish to express our thanks to Prof. R. Honorato for his guidance and encouragement.

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Received October 15, 1956. P.S.E.B.M., 1957, v94.

Comparative Metabolism of Ca^{45} and Ra^{226}.* (22917)

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In experimental biology, the double tracer technic is a simple but powerful method. It is especially useful for comparative studies of metabolically similar elements, since biological variability is greatly reduced. In the studies described below, the radioactive isotopes Ca^{45} and Ra^{226} were used since the skeleton was of primary interest. The metabolism of radium, while qualitatively the same as that of calcium, quantitatively is unique, and the difference is of value in the study of skeletal metabolism. In addition, knowledge of the metabolism of radium relative to that of calcium is important in the problem of radium toxicity.

Methods. A 5-month-old beagle dog from a colony of several hundred(1) served as the subject. The pup was ½ to ¾ expected maximum size, and its skeleton was still growing rather rapidly. 71.8 μc Ra^{226} and 1.60 mc Ca^{45} in 8.2 ml isotonic saline, pH ca. 2 (HCl), were administered. These amounts, which would not cause detectable radiation damage in a short term study, permitted the measurement of very small samples. The injection standard was measured with the same syringe subsequently used to inject the dog. A conventional proportional counter† was used to measure both Ca^{45} and Ra^{226}. Aliquots of ashed samples were plated on stainless steel discs, which were flamed to evolve radon and its daughters, then allowed to stand 1 to 2 hours to permit decay of any remaining radon daughters. Immediately afterward, alphas (from Ra^{226}) were measured, then both alphas and betas (from Ca^{45}) were counted. The error in determining Ca^{45} by difference was minimized by injecting 22.5 μc Ca^{45} per μc Ra^{226}. Measurement of both isotopes in the same sample eliminates the effect of preparation errors in

* Supported by the U.S. Atomic Energy Commission.

† Nuclear Measurements Co., Model PC-1.