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The Extinction Coefficient of Canine Fibrin in Alkaline Urea at 282 mμ* (33934)

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The measurement of the concentration of fibrinogen in plasma by an isotope dilution method (1) requires the determination of the specific radioactivity (counts per minute per milligram of protein) of fibrin in alkaline urea. The concentration of fibrin (mg/ml) is obtained from the measurement of the optical density of an alkaline urea solution of this protein and the use of an extinction coefficient. This extinction coefficient has been measured for fibrins in man and the cow (2) and in rabbit (1) and the results show close agreement among these species.

In a study of the metabolism and distribution of fibrinogen in beagle dogs (3) all fibrinogen concentrations were determined by the isotope dilution method. Since no data exist, to the knowledge of the authors, on the extinction coefficient of canine fibrin dissolved in alkaline urea, it was considered desirable to determine this value rather than rely on a value obtained from other species.

Methods and Results. Fibrinogen from the plasma of 4 mongrel dogs was isolated, purified, and the coagulability was determined according to the method of Atencio *et al.* (1). A thrombin (Parke, Davis & Co., Detroit, Mich.) solution containing 8–10 NIH units of thrombin was added to the fibrinogen solution and the fibrin was collected on a siliconized glass rod by carefully winding the rod against the side of the glass tube. The fibrin on the glass rod was then dialyzed free of salts by repeated changes of distilled water and was then transferred from the glass rod into the bottom of a tared volumetric flask. The fibrin was then dehydrated by the addition of 10-ml volumes of a mixture of ethanol and ether (v/v, 3 parts to 2 parts) 5 times. The flasks containing the fibrin were dried to constant weight at 110°. Freshly prepared alkaline urea (40% urea in 0.2 *N* NaOH) was added to each flask to dissolve the fibrin, and the optical density of samples containing different amounts of fibrin was measured in 1-cm quartz cuvettes at 282 mμ in a Beckman DU spectrophotometer (Beckman Instrument Co., Fullerton, Calif.).

The mean percentage coagulability of the purified fibrinogen from the 4 dogs was 95.3%.

A plot of the 30 measured optical densities against the concentration of fibrin in alkaline urea is shown in Fig. 1. Least square analysis reveals that the equation $y = 1.743x + 0.015$ (solid line in Fig. 1) best describes the data (dots) and the correlation coefficient between optical density and fibrin concentration is 1.0 (0.9998). The standard deviation of a single value from the calculated curve is ±0.009.

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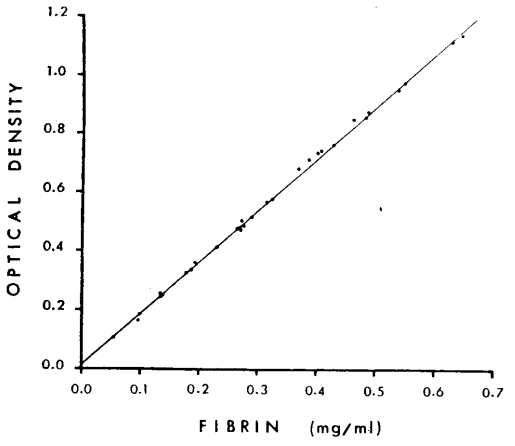

FIG. 1. Optical density values plotted against canine fibrin concentration in alkaline urea; the line represents least square analysis of the 30 data points.

Discussion. The extinction coefficient of a 0.1% solution of canine fibrin dissolved in alkaline urea has a value of 1.743 determined by least square analysis of the data. This analysis of the data reveals, however, that the points do not pass through the origin but show an intercept on the y-axis which is equal to 0.015 optical density units. An intercept on the y-axis has also been reported by Blombäck and Blombäck (2), for both human and bovine fibrin in alkaline urea if the optical densities were determined more than 30 min after the addition of alkaline urea. The curve passes through the origin only if the optical density is determined within 10 min after addition of the alkaline urea. In the present study the optical density values were obtained several hours after dissolving the fibrin and this probably is the cause of the y-axis intercept.

The extinction coefficient for canine fibrin in alkaline urea is higher than the values reported for man, cow (2), and rabbit (1) which have a mean value of 1.617. It is interesting to note that canine fibrinogen also differs from the fibrinogen obtained from the other species in that the fibrinopeptides released following the addition of thrombin contain two unesterified tyrosine residues (4, 5). The use of the directly determined extinction coefficient in the dog will enable us to calculate the canine fibrinogen concentrations determined by the isotope dilution method (1) and to compare the results with those determined by a colorimetric procedure (6).

Summary. Highly purified (95% coagulable) canine fibrinogen was converted to fibrin by thrombin (8–10 NIH units) and the fibrin, collected on a siliconized glass rod, was subsequently dialyzed free of salts (distilled water), dehydrated (ethanol–ether 3v/2v) and dried to constant weight. Following solution of the dried fibrin in freshly prepared alkaline urea (40% urea in 0.2 *N* NaOH) the optical density of samples containing different amounts of fibrin was determined at 282 mμ in 1-cm quartz cuvettes. Least square analysis of the data reveals that the equation $y = 1.743x + 0.015$ best fits the data points with a correlation coefficient between optical density and fibrin concentration of 1.0 (0.9998). The extinction coefficient for a 0.1% solution of canine fibrin in alkaline urea is 1.743. This result is discussed with regard to extinction coefficients obtained from the study of other species.

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