The Extinction Coefficient of Fibrinogen from Man, Dog, Elephant, Sheep, and Goat at 280 mμ[1] (34792)

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The plasma concentrations (1), metabolism and distribution (2), and the transfer from plasma to lymph (3) of the fibrinogen molecule, have been studied in this laboratory in recent years. In addition, the influence of fibrinogen on blood viscosity has been investigated in man, dog, elephant, sheep and goat (4–6). The concentration of purified bovine fibrinogen has been quantitatively determined by the use of the characteristic protein absorption of this molecule at 280 mμ (7). To the knowledge of the authors, such an extinction coefficient (optical density at 280 mμ) has not been measured for the species studied in this laboratory. Although the assumption has been made (7, 8) that the bovine extinction coefficient can be used to quantitate purified fibrinogen preparations from other species, there has been a need of experimental evidence to substantiate this supposition. Species difference does exist in the chemical nature of the peptides split off from fibrinogen following thrombin addition (9). In the case of the dog, it has been shown that three peptides are released (10, 11) instead of the two normally liberated from the fibrinogen of other species. These findings further indicate that the determination of the optical density at 280 mμ in each species studied is necessary for accurate quantitation of purified preparations.

Methods and Results. Fibrinogen from the plasma of man, dog, elephant, sheep, and goat was isolated and purified following the method of Atencio *et al.* (12). Following the last wash of the precipitate with ¼ saturated $(NH_4)_2SO_4$ (1 vol of $(NH_4)_2SO_4$ saturated at room temperature + 3 vol of distilled water) the plastic centrifuge tubes were inverted onto a paper towel, the rims were wiped free of solution and the precipitated fibrinogen was dissolved in 1.0–1.5 ml of 0.005 *M* sodium citrate. The contents of all tubes were combined, and the resulting 10–12 ml of fibrinogen concentrate was pipetted into a prewashed dialysis tubing and dialyzed against 4–1 liter portions of 0.005 *M* sodium citrate over a 40-hr period to remove the $(NH_4)_2SO_4$. At the end of the 40 hr the fibrinogen solution was removed from the dialysis tubing, centrifuged (4000 rpm for 10 min at 4°) and transferred to a clean tube. The purified fibrinogen solution as well as a portion of the last dialysis diffusate (to be used as the diluent and blank) were allowed to come to room temperature. A series of samples were prepared by mixing the purified fibrinogen and the dialysis diffusate in different proportions. The samples were greater than 90% clottable by the addition of thrombin (Parke, Davis & Co., Detroit, Mich.).

The absorbance at 280 mμ was determined by pipetting 0.50 ml of each sample into 10.0 ml of dialysis diffusate and reading the optical density on a Beckman DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) against a dialysis diffusate blank. No correction was made for turbidity by determining the optical density at 320 mμ. The total optical density at 280 mμ of each sample was calculated by correcting for the 21-fold dilution.

The total nitrogen of an aliquot of each sample was determined by the micro-Kjeldahl (13) procedure. Duplicates of each

[1] This investigation was supported by U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2272 and by Public Health Service Research Grant HE-06139 from the National Heart Institute.

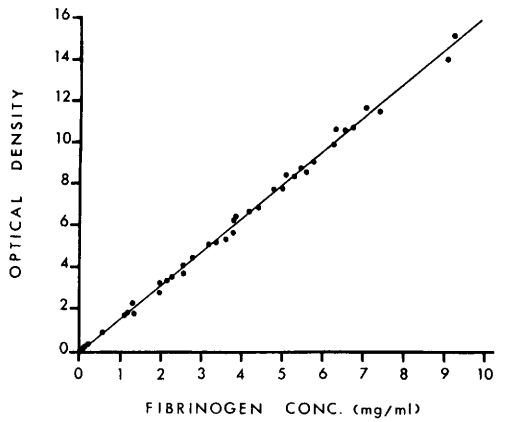

FIG. 1. Optical density values plotted against canine fibrinogen concentration in sodium citrate; the line represents least square analysis of the 38 points.

sample, containing 0.4–1.4 mg of nitrogen, were pipetted into Kjeldahl flasks containing concentrated H_2SO_4 and approximately 500 mg of a mixture of K_2SO_4 and mercuric sulfate (as a catalyst). The samples were digested for 3 hr. Following the adjustment to room temperature and dilution with distilled water, the contents were steam distilled and finally titrated with 0.01 *N* HCl. The total nitrogen was converted to fibrinogen by multiplication by the factor 5.92 (7, 14), which corresponds to a nitrogen content for fibrinogen of 16.9%.

The individual values of 38 determinations using fibrinogen solutions purified from the plasma of 7 mongrel dogs are plotted in Fig. 1. Least square analysis of these data reveals that the equation $y = 1.583x - 0.044$ is the equation of best fit. The extinction coefficient for a 0.1% solution of canine fibrinogen is thus 1.583. The correlation coefficient between the fibrinogen concentration (mg/ml) and the optical density at 280 mμ is 0.998 and the standard deviation of any measured value from the calculated value is ±0.023 OD units.

In another series of experiments, the extinction coefficients were determined from man, dog, elephant, sheep, and goat fibrinogen solutions. In each case the ratio of optical density to fibrinogen concentration was calculated and these values are listed in Table I for the five species studied. The average values for man, dog, elephant, sheep and goat are shown to range from 1.55 for man to 1.58 for the dog with an average value for all species ($n = 15$) being 1.56. The mean value for the dog ($n = 3$) of 1.58 in this series of experiments agrees closely with that of 1.583 determined earlier from least square analysis of the 38 data points (Fig. 1). In Table I are also listed the ratio of the extinction coefficient of each species ($E_{species}$) to the average

TABLE I.

Species	$E^{0.1\%}_{1.0\text{ cm},\,280\text{ m}\mu}$	Mean	$E_{Species}/E_{av}$	% Diff.
Man	1.56			
	1.56			
	1.53	1.55	0.994	−0.6
Dog	1.57			
	1.57			
	1.58	1.58	1.013	+1.3
Elephant	1.54			
	1.60			
	1.59	1.57	1.006	+0.6
Sheep	1.58			
	1.53			
	1.55	1.55	0.994	−0.6
Goat	1.55			
	1.57			
	1.55	1.56	1.000	0.0
E_{av} ($n = 15$)		1.56		

extinction coefficient for all five species. The percentage difference between the two is within ±1%.

Discussion. The extinction coefficients for 0.1% solutions of fibrinogen from man, dog, elephant, sheep, and goat ranged from 1.55 to 1.58, with an average value for the five species being 1.56. This average value agrees closely with the value of 1.57 reported earlier (7) for purified bovine fibrinogen, and provides experimental evidence that the extinction coefficient shows negligible differences among species. The use of this value E (0.1%) will thus allow easy conversion of optical density measurements at 280 mμ of purified fibrinogen solutions into concentration, at least for the five species in this study, plus the cow.

The data of this study were uncorrected for turbidity which amounts to no more than 1% of the reading (7). In the low concentration range the turbidity would not be as large as in the higher concentration range because of the increased dilution. In the data presented in Fig. 1 this concentration-dependent effect would lower the slope of the optical density–concentration relation and possibly raise the intercept (—0.044 OD units) closer to zero. For practical purposes a value of 1.56 could be assumed for the slope and the calculated concentration would be in error by only ±1% (Table I).

Summary. Fibrinogen from the plasma of man, dog, elephant, sheep, and goat was isolated, purified, and dialyzed free of $(NH_4)_2SO_4$ against 0.005 M sodium citrate. The optical density at 280 mμ and the total nitrogen (micro-Kjeldahl) were determined on serial dilutions from each specie and the extinction coefficient E (0.1%) calculated. In the case of the dog, least square analysis of 38 determinations reveals that the equation $y = 1.583x - 0.044$ is the line of best fit, with correlation coefficient of 0.998. The extinction coefficient for a 0.1% solution of dog fibrinogen in 0.005 M sodium citrate is thus 1.583. Extinction coefficients, calculated as the ratio of optical density to fibrinogen concentration without least square analysis, for fibrinogen from other species have also been determined. These extinction coefficients show mean values of 1.55 for man, 1.58 for dog, 1.57 for elephant, 1.55 for sheep and 1.56 for goat, with the average value for all five species being 1.56. The use of this average value for these species as well as the cow introduces an error of only ±1%.

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Received Jan. 23, 1970. P.S.E.B.M., 1970, Vol. 134.