Comparison of Citrated and Oxalated Plasma in the Photoelectric Determination of Prothrombin Time. (19039)

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In a previous report a method for the photoelectric determination of prothrombin time was described(1) based upon the observation that the optical density of plasma increases sharply at the instant of clotting, thus producing a movement of the galvanometer lightbeam to the left. The original experiments were carried out with oxalated plasma. However, subsequently a comparison of the photoelectric mechanisms involved in the clotting of oxalated and citrated plasma revealed a more distinct "endpoint" when the latter was used. In order to investigate this fact and to properly evaluate the difference between prothrombin times obtained with oxalated and citrated plasma, the present study was undertaken.

Material and methods. One hundred specimens of venous blood were collected from normal individuals. Each specimen was distributed into two tubes in a ratio of nine volumes of blood to one volume of 0.1 M solution of sodium citrate and 0.1 M solution of sodium oxalate, respectively. The prothrombin time was determined in triplicate on each specimen of plasma according to the photoelectric modification of the one-stage Quick method(1). In another series of 50 specimens of blood of normal subjects, the same procedure was employed using, however, the conventional one-stage Quick method(2). Prothrombin times of 2 series of plasma dilutions were established photoelectrically, utilizing both citrated and oxalated plasma. The

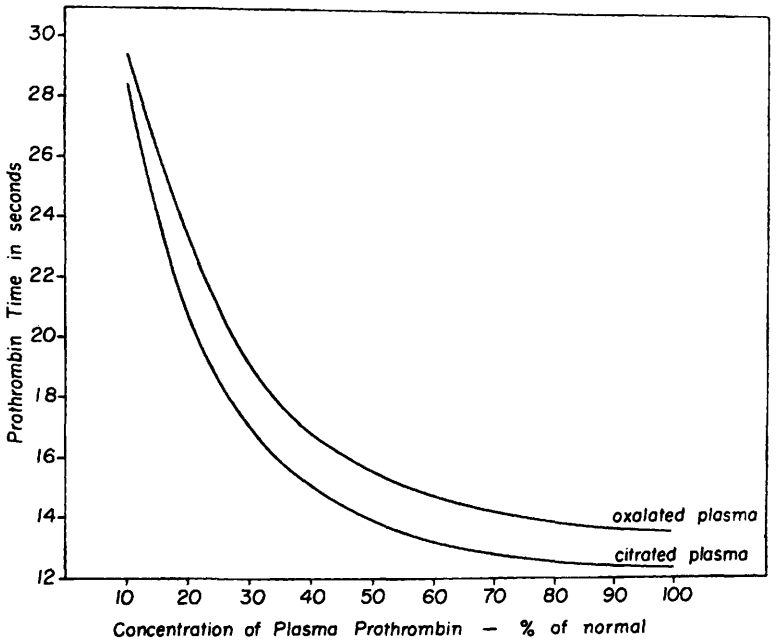

FIG. 1. Comparison of the concentration of prothrombin in serial dilutions between normal citrated and normal oxalated plasma by the photoelectric method.

1. Losner, S., Volk, B. W., Jacobi, M. and Newhouse, S., *J. Lab. and Clin. Med.*, 1950, v36, 473.

2. Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. M. Sc.*, 1935, v190, 501.

TABLE I. Comparison of Mean Values, Range and Stand. Dev. of Prothrombin Times Obtained with Citrated and Oxalated Plasma Using the One-Stage Quick Method and the Photoelectric Method.

	One-stage Quick method		Photoelectric method	
	Mean prothrombin time 50 normal individuals	Stand. dev.,* (sec)	Mean prothrombin time 100 normal individuals	Stand. dev.,* (sec)
Citrated plasma	13.1 sec; range 12.4 to 15.8; 150 determinations	.39	12.6 sec; range 11.4 to 14.6; 300 determinations	.15
Oxalated plasma	14.5 sec; range 13.2 to 16.5; 150 determinations	.43	13.8 sec; range 12.5 to 15.1; 300 determinations	.19

* Stand. dev. obtained by applying formula $\sigma = \sqrt{\frac{\Sigma d^2}{N}}$.

results were plotted against the respective concentrations (Fig. 1).

Results. The prothrombin times of 100 normal individuals as determined with the photoelectric method using citrated plasma ranged from 11.4 to 14.6 seconds with a mean value of 12.6 seconds and a standard deviation of 0.15 second (Table I). When oxalated plasma was used the prothrombin times varied from 12.5 to 15.1 seconds with a mean value of 13.8 seconds and a standard deviation of 0.19 second. The prothrombin times of 50 normal individuals as determined with the conventional one-stage Quick method ranged from 12.4 to 15.8 seconds with a mean value of 13.1 seconds, and a standard deviation of 0.39 second using citrated plasma. The prothrombin times varied from 13.2 to 16.5 seconds, with a mean value of 14.5 seconds, and a standard deviation of 0.43 second when oxalated plasma was employed.

The plasma dilution curve obtained with citrated plasma showed uniformly lower prothrombin times than those with oxalated plasma, the difference being slightly more than one second at each plasma concentration (Fig. 1).

Discussion. This study demonstrates the advantage of the use of citrated plasma in the photoelectric determination of prothrombin time. It was observed that the "endpoint" of clotting is thus more distinctly established as compared with oxalated plasma. Moreover, a greater accuracy has been achieved, as evidenced by the standard deviation of only 0.15 second. When oxalated plasma is used in the photoelectric determination of prothrombin time, the standard deviation has been found to be 0.19 second. This approximates the value previously reported with this method (1). The disadvantage of oxalated plasma, it is believed, is probably due to the fact that upon addition of calcium chloride, a precipitate of calcium oxalate is formed which causes the galvanometer lightbeam of the spectrophotometer to move slowly to the left before the actual endpoint is reached. On the other hand, no precipitate but a soluble complex of calcium citrate(3) is formed upon the addition of calcium chloride if citrated plasma is used. Correspondingly, at the onset of clotting, the swing of the lightbeam indicating the "endpoint" is therefore more distinct with citrated than with oxalated plasma.

It has been known that sodium citrate very quickly removes ionic calcium, whereas the action of sodium oxalate is relatively slow(4). After recalcification of citrated plasma the prothrombin time may be prolonged if lower concentrations of calcium are used as compared with the values obtained with oxalated plasma(5). The addition of .01 M solution of calcium chloride or higher concentrations of calcium chloride to citrated plasma, however, yields an optimal prothrombin time according to Quick(5). In the present study recalcification was performed with .02 M solution of calcium chloride. The resulting prothrombin

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times seem to warrant the conclusion that citrated plasma yields optimal values as compared with oxalated plasma. This, in conjunction with the aforementioned factors would make citrated plasma more suitable for the photoelectric determination of prothrombin time than oxalated plasma.

Summary. (1) In a series of 600 photoelectric determinations of the prothrombin time of 100 normal individuals, the mean value for citrated plasma was 12.6 seconds as compared to 13.8 seconds for oxalated plasma. (2) The standard deviation was 0.15 second for citrated as compared to 0.19 second for oxalated plasma. (3) The onset of clotting is more easily determined photoelectrically with citrated than with oxalated plasma. This is evidenced by a more distinct swing of the galvanometer lightbeam because no precipitate but a soluble complex of calcium citrate is formed upon addition of calcium chloride.

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Blood, Plasma and "Globulin" Space of Guinea Pigs Determined with I^{131} Rabbit Globulin. (19040)

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Iodine^{131} trace-iodinated-proteins have been used to determine vascular volumes in the dog(1), mouse(2) and man(3). In the course of studies of I^{131} trace-iodinated-rabbit antibody(4) it was necessary to determine the volume of guinea pig plasma in which this protein was diluted after intravenous administration. Since blood and plasma volumes have not been published for the guinea pig in the recent literature(5-7) except for a report of the plasma volume of 4 guinea pigs using T-1824(8), it was felt desirable to present the data.

TABLE I. Data on I^{131} Rabbit Globulin.

Amt protein nitrogen injected (mg)	12.63
" I^{131} injected (μc)	3.94
" protein bound iodine injected (μg)	200
No. iodine atoms per protein molecule	3.2
% activity not precipitable with trichloracetic acid	.51
Volume globulin injected (cc)	2.5

Methods. The globulin studied was obtained from immune rabbit serum and iodinated by a method described previously(9). Table I presents the composition and pertinent data of the preparation used in this study. The guinea pigs received the I^{131} rabbit globulin intravenously, and blood was obtained from the ear with a 20 mm^3 Sahli pipette at the times indicated in Table II. The blood was immediately delivered into an ointment tin sample dish lined with lens

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