DETERMINATION OF PLASMA FIBRINOCEN WITH OUR BIURET REAGENT

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In the preceding paper[1] our modification of Levin-Brauer's biuret reagent was presented together with its application to the determination of albumin and globulin in blood serum. Shortly after the completion of this work a new routine method to estimate plasma fibrinogen has been developed by coupling our reagent with Campbell-Hanna's technique[2] which employs sodium sulfite to salt out fibrinogen.

METHOD

Reagent

1) Aqueous solution of sodium sulfite, 12.5 g./dl. in concentration. Katayama's anhydrous sodium sulfite (chemically pure; Na_2SO_3) is used for its preparation.

2) Biuret reagent described in the preceding paper.[1]

3) Standard protein solution. Blood serum of healthy person is determined for its protein concentration, and it is diluted to 500 mg./dl. with physiological salt solution.

4) 10 g./dl. aqueous solution of sodium hydroxide.

Procedure

(1) Pipette 0.5 ml. of plasma which has been separated from oxalated blood* into a round-bottomed centrifuge tube A, and 9.5 ml. of sodium sulfite solution, mix, and incubate at 37 C for ten minutes to salt out fibrinogen as flocculence.

(2) Centrifuge at 3000 r.p.m. for ten minutes, discard the supernatant by decantation and rinse thoroughly its inner surface with about 6 ml. of sodium sulfite solution. Rotating the tube round and round on its axis, slowly pour down about 1 ml. of the solution from Komagome pipette along its orifice, whirl up the precipitate (fibrinogen) by tapping, and then wash down the protein which still adheres to the inner surface of the tube from orifice to interior with the remaining 5 ml. of the solution.

(3) Centrifuge (3000 r.p.m. for fifteen to twenty minutes) again, and decant. Invert the centrifuge tube with a filter paper at its orifice to absorb all the fluid which drips down. Transfer 1.0 ml. of sodium hydroxide solution and 0.5 ml. of distilled water into the tube, place it in boiling water bath and stir gently with a glass rod to dissolve the precipitate (fibrinogen).

(4) Prepare test tubes B, C and D as follows.

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* Transfer two ml. of venous blood into a vial in which two drops of 2 g./dl. potassium oxalate solution have been evaporated to dryness at 60 C, stir gently, and centrifuge to obtain plasma.

	Standard protein solution (ml.)	sodium hydroxide solution (ml.)	distilled water (ml.)
B	0.50	1.00	
C	0.20	1.00	0.30
D		1.00	0.50

Add 2.0 ml. of biuret reagent to each as well as to the centrifuge tube A which has been treated in the steps (1) to (3), mix, and measure in a photoelectric colorimeter at 570 mμ (Filter S 57), using D as a blank, during the period of five to fifteen minutes after mixing. Plot the absorbance obtained against the concentration of protein in which B and C represent 500 and 200 mg./dl. Read the concentration of plasma fibrinogen from the calibration curve thus constructed.

The procedure was checked by modified Arnold-Gunning's nitrogen determination. An aliquot of 0.1 ml. of the fibrinogen suspension, which was obtained by adding 1.0 ml. of sodium hydroxide solution to the precipitate in the centrifuge tube A in step (3), was transferred into a digestion tube containing 1.0 ml. of distilled water** and heated with 0.7 ml. of digetion mixture. Fibrinogen concentration (mg./dl.) was computed as 6.25 N (mg./dl.)***

Results and Discussion

Heretofore either addition of calcium salt or vigorous stirring with glass beads has been commonly employed for the separation of fibrinogen from plasma. In our experience Campbell-Hanna's technique which salted out fibrinogen with sodium sulfite had the advantage of simplicity of procedure. The flocculent fibrinogen was so easy to wash that it was completely separated from the other protein which was coincident in plasma by washing two times, dispensing further purification. The precipitate of fibrinogen thus purified was readily soluble in hot 10 g./dl. solution of sodium hydroxide, yielding a limpid solution which suited biuret method.

The following figures which were picked up from the record of duplicate determination reveal that divergence remains almost within ten per cent of the estimation.

262, 269; 276, 279; 368, 381; 376, 401; 456, 511; 518, 527; 658, 662 (mg./dl.)

However biuret method seems to be three per cent lower, on the average, than nitrogen determination and the discrepancy between them occasonally surpassed ten per cent of the determination.

Biuret method	(mg./dl.)	262,	276,	279,	286,	401,	527, 662
Nitrogen determination	(mg./dl.)	265,	317,	317,	259,	428,	501, 681

This does not necessarily imply special defectiveness of the accuracy in our procedure, since error of ten per cent is also usual in other mothods.[3]

** The remainder of fibrinogen suspension which was 0.9 ml. in volume was subjected to biuret method after the addition of 1.1 ml. of distilled water. The fibrinogen concentration read from calibration curve was multiplied by $\frac{10}{9}$ to get true concentration of plasma fibrinogen.

*** To determine N concentration, aliquots of 0.2, 0.4 and 0.6 ml. of standard N (10 mg./dl.) solution were used to represent 40, 80, and 120 mg./dl. N on the calibration curve.

Summary and Conclusion

A new method for the determination of plasma fibrinogen was developed by the application of our biuret reagent to Campbell-Hanna's technique which salted our fibrinogen with sodium sulfite. The procedure was simple and especially suitable for routine work, although extreme accuracy could not be attaied.

References

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3) Ratnoff, O.O., and Menzie, D. : A new method for the determination of fibrinogen in small samples of plasma. *J. Lab. & Clin. Med.* **37** : 316–320, 1954.