THE DETERMINATION OF SERUM TOTAL PROTEIN, ALBUMIN, AND GLOBULIN BY THE BIURET REACTION

By GEORGE R. KINGSLEY

(From the Division of Biochemistry, Laboratories, Philadelphia General Hospital, Philadelphia)

(Received for publication, August 28, 1939)

The biuret reaction for the estimation of protein in urine was first introduced by Riegler (1). Autenrieth (2, 3), Hiller (4), and Fine (5) modified and improved the method. All agreed that the biuret reaction was reliable, the error usually not exceeding 5 per cent in experienced hands. It was also found that equal quantities of albumin and globulin yielded violet colors of practically the same intensity. However, these investigators found it difficult to obtain a satisfactory standard. Biuret was generally abandoned because of impurities and because when treated its color tint differed from that of proteins. Gavrilov and Ginzburg (6) who employed a peptone standard believed that the biuret reaction was suitable only for the estimation of total peptide linkages and not protein. Fine (5) used serum protein preserved in chloroform as a standard which was said to be stable for 6 months. This is questionable, since a deposit often separates from protein solutions on standing.

The older methods have been simplified and made more accurate by the elimination of the preliminary precipitation of protein. The procedure has been further shortened by the use of copper sulfate and sodium hydroxide in such proportion that precipitate formation is avoided. The need for a standard was eliminated in the method herein described by use of a photoelectric colorimeter.[1] Standardization was accomplished by diluting pooled blood serum with 0.85 per cent sodium chloride to give solutions of various

[1] The Mass photoelectric colorimeter was used, but any other reliable instrument can be adapted to the method.

TABLE I

Comparison of Serum Total Protein and Albumin by Biuret and Kjeldahl Methods (Per Cent)

Biuret		Kjeldahl	
Total protein	Albumin	Total protein	Albumin
8.2	4.3	8.4	4.5
8.2	4.1	8.2	4.2
8.0	4.4	8.0	4.6
7.9*	5.0	7.9	5.0
7.6	5.0	7.5	4.9
7.6	5.1	7.5	4.9
7.5	4.7	7.3	4.4
7.5	4.3	7.3	4.2
7.4	4.0	7.4	3.9
7.3	4.7	7.3	4.9
7.0*	4.5	6.9	4.6
6.9	2.8	6.9	2.9
6.6	3.3	6.6	3.3
6.4	3.3	6.5	3.5
6.3	4.9	6.5	4.7
6.3	3.9	6.5	4.0
6.2	4.2	6.3	4.2
6.0	3.5	6.0	3.5
6.0	3.0	6.2	2.8
5.8	3.7	5.9	3.4
5.7	2.9	5.7	2.8
5.3	3.0	5.1	2.8
5.3*	3.7	5.3	3.7
5.2	2.7	5.4	2.8
5.2	3.4	5.0	3.5
5.0	2.4	5.2	2.4
4.6	2.8	4.6	2.7
3.8†	2.5	4.0	2.5
3.6	2.4	3.7	2.3
3.5†	1.2	3.5	1.5
3.6†	1.6	3.6	1.6
3.2†	1.4	3.4	1.4
Average..6.08	3.52	6.11	3.51

* Jaundiced.

† Lipemic (lactescent).

total protein and albumin concentrations, and plotting the readings produced in the biuret reaction against the total protein or

albumin content as determined by the Kjeldahl method. The data in Table I represent analyses of a variety of pathological sera by the biuret and Kjeldahl methods. It is apparent that the biuret method is in good agreement through a wide range of total protein concentrations and albumin to globulin ratios. Constancy in total N to peptide linkage ratios thus is demonstrated. It was found that an olive-green filter (No. 401, Corning) gave satisfactory light transmission for the purple biuret color and also absorbed the blue color of the copper reagent. Error caused by the latter is unimportant even when no filter is used except at protein concentrations so low as to be rarely encountered. The olive-green filter will also diminish small errors produced by jaundiced serum. Lipemic and jaundiced sera when treated with biuret reagents become turbid on standing. Formation of precipitate is readily avoided by extraction with ether just after the biuret color is developed.

Total Protein—To exactly 4 cc. of 10 per cent sodium hydroxide in a photoelectric colorimeter tube add 0.1 cc. of fresh serum (free from cells) with a Folin micropipette. Rinse out the pipette three times with sodium hydroxide solution. Mix by rotating and add 0.5 cc. of 1 per cent copper sulfate. Shake vigorously five to six times. If the serum is jaundiced or lipemic, add 2 cc. of ether (U.S.P.) and shake vigorously about 20 seconds. Allow to stand for 25 minutes and read in a photoelectric colorimeter. Tap the tube gently before reading if all gas bubbles have not separated into the ether layer. The readings remain stable until opalescence appears, usually about 1 hour, or several hours when ether is used. As the ether is somewhat soluble in the reaction mixture, a correction of 8 per cent must be added to the result.

Albumin—Globulin may be precipitated from 0.5 cc. or 1 cc. of fresh serum (free from cells) with 7.5 or 15 cc. of 23 per cent sodium sulfate respectively. After standing for 3 hours at 37.5°, the globulin is removed by filtering through a single sheet of Whatman No. 5 paper; it is refiltered if necessary. (Losses of albumin due to absorption have been negligible in our experience.) When all of the solution has filtered, mix and measure 2 cc. of filtrate into a photoelectric colorimeter tube containing 2 cc. of 20 per cent sodium hydroxide. Mix by rotating and add 0.5 cc. of 1 per cent copper sulfate. Shake vigorously five to six times. Allow to

stand for 25 minutes and read. If the serum is jaundiced or lipemic, treat with ether in the same manner as for total protein and correct the results by adding 8 per cent to the final result.

Globulin—Total protein minus albumin equals globulin.

Deterioration of copper sulfate solution due to contamination or standing several weeks may give low results.

SUMMARY

A rapid simplified method for application of the biuret reaction directly to serum for the determination and fractionation of protein is described. The need for standard solutions is avoided by use of a photoelectric colorimeter.

BIBLIOGRAPHY

1. Riegler, E., *Z. anal. Chem.*, **53**, 242 (1914).
2. Autenrieth, W., and Mink, F., *Münch. med. Woch.*, **62**, 1417 (1915).
3. Autenrieth, W., *Münch. med. Woch.*, **64**, 241 (1917).
4. Hiller, A., *Proc. Soc. Exp. Biol. and Med.*, **24**, 385 (1927).
5. Fine, J., *Biochem. J.*, **29**, 799 (1935).
6. Gavrilov, N. I., and Ginzburg, E. I., *Arch. sc. biol.*, U.S.S.R., **39**, 549 (1935).