Evaluation of Three Methods of Protein Analysis for Serum and Heart Homogenates

JONATHAN E. SCHWARTZ, B.S.* and BEATRICE C. DURHAM, M.A.

William Likoff Cardiovascular Institute, Hahnemann Medical College and Hospital, Philadelphia, PA 19102

ABSTRACT

The Biuret, Ultraviolet and Lowry techniques for the determination of total protein content were compared in 31 samples of patient serum and in 30 rat heart tissue homogenates. Statistically significant differences were found to exist between values obtained with each method. Human serum and heart homogenate protein were both lowest when assayed by the Lowry procedure, while serum and homogenate values were highest with the Ultraviolet and Biuret method, respectively. Possible sources of these discrepancies are discussed. Although the Biuret method may not be as sensitive as the other two methods, it proved to be the most reproducible technique for determining total protein concentration in the particular types of samples used in this study.

Introduction

Studies utilizing tissue enzymes to measure rates of metabolic activity must have some means of normalizing values so that various samples can be compared. The most direct method is to utilize wet weight of the tissue. Dry weight is another means of relating enzymatic activity to the tissue sample. However, because the active portion of the tissue may differ from organ to organ, weight may not be a very accurate reference value, and protein content is often preferable.

Nitrogen determinations have been employed for the quantitation of tissue proteins, but since these are time-consuming, many investigators now use one of the various spectrophotometric methods for assaying protein. Peters reported the relative popularity of a variety of spectrophotometric methods and showed that some form of the biuret reaction was being used by the majority of laboratories surveyed. All soluble proteins give the biuret reaction which occurs upon the treatment of a peptide or protein with CuSO₄ and alkali. The resulting purple complex of Cu²⁺ can be quantitatively measured by spectrophotometry. The method of Gornall et al was somewhat preferred over that of Weichselbaum in a 1964 survey. Other laboratories use a modification of the Folin-Ciocalteu or Lowry phenol technique which combines the biuret reaction with the reduction of the Folin

* Present address: Temple University School of Medicine, Philadelphia, PA 19140.
phenol reagent by the copper-treated protein, relative to its tyrosine and tryptophan content.

Ultraviolet (UV) spectrophotometric methods are also used in the quantitative estimation of protein concentrations. Proteins exhibit an absorption maximum in the region of 280 nm. This absorption is caused by the aromatic amino acids present in protein, specifically tyrosine and tryptophan. The intensity of absorption at this wavelength depends on the proportions of these amino acids in the protein. However, the base components of nucleic acids or nucleotides, purine and pyrimidine, also absorb strongly at 280 nm. Fortunately, nucleic acids absorb light more strongly at 260 nm than does protein. Warburg and Christian capitalized on this fact and devised a series of calculations which are meant to eliminate the interference owing to nucleic acids.

Lowry is the most sensitive of the three methods, capable of measuring as little as 200 ng protein per sample. Its sensitivity is 10 to 20 times that of the UV determination and 100 times more than biuret. Parvin et al have reported that the Lowry method is preferable to the biuret reaction for determining protein in crude tissue extracts and Gonyea et al have determined that the Lowry method is most appropriate for estimating protein in isolated ferritin preparations. On the other hand, Lubran recommended the biuret method for the determination of total serum protein. Since both the nature of the sample and the level of protein concentration to be determined can affect the results, the biuret, Lowry and UV techniques were compared by us on the same samples of both human serum and rat heart disease homogenates.

Materials and Methods

Patient serum was obtained from 31 patients admitted to the Coronary Care Unit at Hahnemann Medical College and Hospital. Three tissue homogenates were prepared from each heart of ten male Wistar rats, 200 to 400 g. The rats were sacrificed by a sharp blow on the head, following which the heart was immediately excised and placed in cold saline. Pieces of heart tissue were weighed, minced with scissors and homogenized by a motor-driven teflon pestle in medium (25 ml per g) containing 0.25 M sucrose, 0.001 M mercaptoethanol and 0.0001 M neutralized sodium ethylenediaminetetraacetic acid. The homogenate was centrifuged at 16,000 g for 10 min at 4°. The supernatant fraction was removed and centrifuged for an additional 10 min.

Standard curves for the Lowry and biuret methods were prepared from bovine serum albumin (Fraction V); control serum and protein standard solution were assayed in order to assure continued accuracy. The biuret protein assay was performed according to the method described by Gornall et al. Protein estimation by UV absorption was carried out according to the method of Warburg and Christian as outlined by Layne. The Folin phenol technique was followed according to the method of Lowry et al. All samples were analyzed in duplicate by each method. Patient sera were analyzed first by the UV method, then by the biuret and Lowry procedures. Tissue homogenates were analyzed by the biuret, then by UV and Lowry methods, except for six samples on which biuret values were obtained following the other two values. When samples were not processed on the same day by all three methods, they were stored frozen.

Results

The mean estimates ± standard error of the mean of total protein for each method are given in tables I and II. Human serum assayed by the biuret method had a 75.42 ± 0.73 mg protein per ml, while the mean values obtained with UV and Lowry were
81.54 ± 2.06 and 54.94 ± 0.83, respectively. One-way analysis of variance between the values obtained on serum using the three methods was significant at p < 0.001. Using a Student's t-test for related samples, the mean differences between biuret and Lowry and between UV and Lowry were significant at p < 0.001. Biuret was found to be significantly lower than UV (p < 0.01).

Biuret determination of rat heart homogenates (table II) yielded a mean of 1.20 ± 0.07 mg protein per ml while UV and Lowry were 0.82 ± 0.04 and 0.34 ± 0.004, respectively. Mean differences between the values obtained from homogenates by the three methods and one-way analysis of variance of the three groups of values were all significant at p < 0.001.

With both types of samples, Biuret and UV values were higher than those obtained with the Lowry method (p < 0.001). However, biuret yielded considerably higher values with homogenates and somewhat lower values with serum than did the UV method.

Discussion

The results stress the importance of considering the relative advantages and limitations of any method used to analyze protein concentrations, as well as the nature of the samples to be assayed. Significantly different mean values for total protein of both tissue and serum were obtained with each of the three methods. However, the differences between the UV and biuret method for human serum may be within the limits of the determination since the difference represents only 8 percent of the value. This was not the case with the heart homogenates, where the differences were 30 percent. Results obtained by the Lowry method were consistently lower than those obtained by either of the other methods. In addition, control serum values (acceptable range 29 to 32 mg per ml) were lower using the Lowry method (26 mg per ml) but were 32 and 33 mg per ml for the UV and biuret methods, respectively. The coefficient of variation for duplicate determinations was 0.95 percent for biuret, 5.1 percent for Lowry and 13.4 percent for the UV method.

Various drawbacks which have been described in the literature could partially account for our disparate results.6,7,10,15,21 Many substances have been shown to interfere with color development in the Lowry method,4 including sucrose which was present in our homogenates. While ethylene diaminetetraacetic acid (EDTA) is known to reduce the Folin-Ciocalteu phenol reagent,12 its presence in the samples would only have tended to increase the values, not to decrease them as we observed. Difficulty was encountered by us in preparing the cupric sulfate-sodium potassium tartrate reagent such as reported by Zishka and Nishimura.21 When
the present authors assayed control serum using their procedure, higher values were obtained than when using reagent prepared according to Lowry.

Although the UV method produced results which were within a reasonable range, certain disadvantages became evident as our study progressed. The relatively high dilutions necessary to read the values in a suitable scale on the spectrophotometer may have contributed in part to the greater variation of values seen with human serum. Also, since albumin contains comparatively few aromatic amino acids responsible for UV absorption, the determination of total serum protein by this method has been discouraged when the albumin concentration is unknown.\textsuperscript{19} While Warburg and Christian attempted to minimize interference due to nucleic acids, the higher values obtained with serum and the lower ones with homogenates may be indicative of improper correction for their presence in the two types of samples. In addition, simple peptides exhibit maximum absorption at 190 nm rather than at 280 nm.\textsuperscript{19} Their presence or absence would affect the biuret more than the UV values.

Of the methods used in this study, biuret has yielded consistent results with relative speed and ease. It does not require the high dilution necessary for the UV determination and nucleic acids do not interfere. Although biuret is not as sensitive as Lowry,\textsuperscript{10} the color yield per weight of peptide is more constant from protein to protein,\textsuperscript{2} and the presence of sucrose or EDTA does not affect the reaction. While the final choice of method rests with the investigator and the special needs of a particular study, it would appear that the biuret method has more versatility and accuracy when dealing with a variety of samples with different components, in which the quality of protein available for assay is not a limiting factor.

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