to express protein concentrations, either in defatted or in whole milk, is essential. We prefer to express protein concentrations in whole milk, because this is what the infant consumes. In this case protein concentrations in defatted milk have to be corrected for the "creamaticrit" (4, 6), the volume ratio of cream in human milk determined analogously to the hematocrit, as follows: protein in whole milk = protein in defatted milk \times (1 - creamaticrit).

In conclusion, using the biuret method we found: straight calibration lines, high sensitivity and precision, and linearity up to 25 g/L, a correction factor for the interfering lactose, and an excellent correlation with the Kjeldahl method.

References

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Keller and Neville respond:
To the Editor:

We undertook the comparison of protein methods only after intensive investigation of the biuret assay. Extensive unpublished analysis revealed that the quantity of "interfering substance(s)" varied widely between milk samples, and that it could be removed only by precipitating the milk proteins with trichloroacetic acid before the biuret assay. This procedure had its own set of problems, which gave very erratic protein estimates. In our hands we were unable to obtain good precision with the biuret method and had to repeat the analysis of over 1500 milk samples, using the BCA method. The results of the two assays were, in many instances, not comparable. For this reason we continue to advise against using the biuret assay for determining concentrations of human milk total protein.

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Verheul and Cornelissen comment further:

We do not doubt the care taken by Keller and Neville when they evaluated the four methods of determining total protein in human milk. However, the extensive number of milk samples that they mention to have analyzed is not documented in their paper.

We agree with them that precipitating milk proteins using trichloroacetic acid in order to get rid of the "interfering substances" does not give satisfactory results. The absorbivity and notably the linearity of the biuret assay depend on the composition of the biuret reagent (1). Their lack of success with the biuret method might be attributed to the biuret variant they have used. With ours, good precision and linearity is assured, and for that reason we state that with this biuret reagent, taking the interference of lactose into account, a simple, rapid, and reliable determination of protein in human milk can be achieved.

Reference

Densitometric of Serum Protein Electrophoreograms

To the Editor:

Kahn and Strong (1) reported on the imprecision of electrophoretic quantification of serum protein fractions. We agree entirely with the author's conclusions that only results based on visual inspection should be reported and that densitometry should only be used to measure monocolonal components. For many years this procedure has been performed in our hospitals to the complete satisfaction of the clinical staff. We would like to add some comments.

The evaluation of the 95% confidence intervals of densitometric quantification of the five electrophoretic zones shows that the scatter is such as to unravel their clinical usefulness.

We think that the error of such an evaluation is deeply-rooted, because it stems from ignorance of the fact that, with some experience, it is possible to detect minor changes of mobility and concentration of nine to 13 proteins by visual inspection of a good-resolution electrophoretic separation in agarose gel or cellulose acetate. This evidence was presented many times over the years (2–7).

The densitometric "zonal terminology" has no clinical value. Let us consider the cases of the alpha and beta zones. A recent report (8) showed that in five cases of severe deficiency of $\alpha_1$-antitrypsin the densitometric values were apparently normal or subnormal, while on visual inspection the zone appeared "empty."

The two main components of the alpha-2 zone ($\alpha_2$-macroglobulin and haptoglobin) are usually easily detected visually, especially with cellulose acetate as a support. A densitometric normal value for the alpha-2 zone can obscure two possibilities: a decrease or an increase of haptoglobin in the presence of often physiologically high or low $\alpha_2$-macroglobulin. The two missed clinical events are respectively a hemolytic or an inflammatory condition.

These same remarks may apply to the beta zone, where the two main components, transferrin (beta 1) and C3 (beta 2), have quite different pathophysiological significance and show opposite behavior in inflammatory diseases.

Moreover, it is worth remembering that the most important clinical indication for electrophoresis is the detection of monocolonal components (MC). It has been already shown that the "Microzone" technique, commonly used for densitometric scanning, does not permit recognition of about half of the MCs detectable by visual inspection of high-resolution electrophoreograms (9). We can now add that, in a recent survey by the Italian Protein Commission, only 22 of 78 general laboratories were able to detect the presence of a small MC (10). The professional societ-