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Letter to the Editor

An appreciation of the Folin-Lowry protein assay

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1991 marked the fortieth anniversary of arguably the most quoted paper in biomedical science. In 1951, Oliver Lowry et al described an improvement of an existing colorimetric technique for protein determination. The method was sensitive, robust and comparatively easy to use. In the intervening years the Lowry (or Folin-Lowry) method for total protein has become a benchmark assay in the field, though it does not escape criticism, principally for its variable response to different proteins.

There is a danger that this degree of familiarity can make it seem unnecessary to ask the normal questions of 'how' and 'why?' the mechanism operates. This was illustrated to me recently by a considered reply from a fellow researcher, who asserted that one of my experiments was flawed because the Lowry method only measures phenolic residues, such as tyrosine and tryptophan. I was a little surprised by this since I recalled measuring collagen and gelatin (which contain only traces of tyrosine) and producing standard curves for polylysine! I feel it is important then, on this anniversary, to try to stimulate a little extra awareness of what is involved in the 'simple' measurement of protein with this familiar and trusted technique.

Standard, classical tests for proteins include the biuret, ninhydrin and Folin reactions. The ninhydrin reaction is distinct in requiring prior hydrolysis of the protein. The biuret reaction is the most specific for proteins, giving a violet-red complex with copper ions and amide linkages (—CO—NH—) of proteins. Requiring a minimum of a tetrapeptide in length, this reaction provides reliable evidence for the presence of polypeptides, yet caution in calibration is still advised and for many applications its sensitivity is limiting.

Folin & Denis (1912) described the use of a phosphotungstic-phosphomolibdic acid reagent to produce a blue colour with substances containing phenolic groups. With development, the reaction was applied to the measurement of plasma proteins (Wu 1922) and subsequent use of the Folin-Ciocalteu reagent (containing lithium salts (Greenberg 1929)) improved the reagent solubility. Pre-treatment of proteins with alkaline copper was found to increase the reaction sensitivity by 3- to 15-fold (Herriot 1941) and this effect was utilized by Lowry and his colleagues. However, in taking advantage of this additional sensitivity, the assay relies on a complex chromogenic response, recognizing a number of features in the protein.

Tyrosine and tryptophan residues give a colour in the absence of copper ions. The rest of the protein contributes colour entirely through the binding of copper, and this represents about 75% of the colour yield. Chou & Goldstein (1960), analysing the contribution of a range of peptides, found that only tyrosine, tryptophan and cysteine contributed significantly to the reaction after complete hydrolysis of the protein. Any dipeptide was found to produce some colour but the response was greatly enhanced by the presence of amino or carboxylic amino acid side chains. Consequently, a variety of features contribute to the final colour, giving the technique a broad specificity but also producing considerable variability between proteins.

More recent examples of protein assays include such techniques as the Bradford dye-binding assay (Bradford 1976). Though sensitive and simple to use, this method also works by a complex mechanism which can give different responses for different proteins (Macart & Gerbaut 1982). This can be largely overcome by addition of the detergent sodium dodecyl sulphate, though with a reduction in sensitivity.

In conclusion, we shall, I think, be using the Lowry method well past its fiftieth anniversary and there can be little glamour in learning about something which is as familiar as that. Yet it is essential to maintain an interest and comprehension of the basic mechanisms. After all, blunders in the basic, everyday techniques tend to be the most damaging; they are certainly the most embarrassing.

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