This Week's Citation Classic

Itzhaki R F & Gill D M. A micro-biuret method for estimating proteins.
Anal. Biochem. 9:401-10, 1964.
[Department of Radiotherapeutics, University of Cambridge, England]

This method of estimating proteins depends on their interaction with alkaline copper sulphate. It is rapid, fairly sensitive, and reasonably independent of type of protein. It is unaffected by the presence of high concentrations of DNA. [The *SCI*® indicates that this paper has been cited in over 830 publications since 1964.]

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"Mike Gill and I developed the microbiuret method in the department of radiotherapeutics, University of Cambridge. I was struggling then to characterise chromatin. The few publications on that subject were of little help, appearing to bear no relationship to one another. Also, I was engaged in a constant battle with some refractory equipment, homemade, for an esoteric technique known as electric birefringence. It was a 'do-it-yourself' laboratory with a tradition of *laissez-faire*. Even if one was a newly fledged PhD, one managed on one's own; there was no question of being guided by a senior worker nor of any technical assistance. But on the whole a technician would have been an encumbrance as I was feeling my way slowly and painfully in a subject which at that time interested no one in Cambridge apart from the head of my department. The general attitude was understandable; chromatin was demonstrably messy in its properties—unlike whiter-than-white DNA—and the fact that in the living cell the latter was yoked to proteins and RNA was immaterial.

"Initially, a technician would have been useful. I was trying to analyse the effects of radiation on chromatin—though I soon realised that it was necessary to characterise the chromatin first and irradiate it after. My early efforts involved giving the chromatin a dose of X rays and dashing to my laboratory to look at the birefringence properties, apparatus permitting, before they went too far into decline because of postirradiation effects. Another pair of hands would have been invaluable, if only to beat the birefringence equipment into submission.

"The main problems with chromatin were that there was neither a standard method of preparation nor a defined product. Even the gross composition was uncertain. It was obviously necessary to have a quick method for estimating protein in the presence of large amounts of DNA. Gill, who was working with nuclei, had a similar requirement and so he and I devised the micro-biuret method. It was less sensitive than the Folin-Lowry[1] but was much quicker and simpler.

"I suppose that its ease of use accounts for its popularity. But I would have preferred my subsequent studies on chromatin structure—the first to use DNAases[2] and polylysine[3] as probes—and on distribution of carcinogen-bound sites in chromatin,[4] to be better known. However, it is rewarding that both approaches triggered off a number of studies by others, even if circumstances precluded my continuing them myself.

"I cannot help feeling nostalgic for Cambridge. The city was—and is—so beautiful. Also, one was able to do the work one thought necessary; there was no pressure to move off the topic from any higher authorities sublimely indifferent to, or ignorant of, its nature. Lastly, there was more scope for individualism, as opposed to teamwork, and for simple experimentation. Techniques are becoming increasingly complex. Engaged now in Southern blots, *in situ* hybridisation, and embarking on recombinant DNA work, I am highly dependent on a continuity of scientific assistance—not easy to maintain in days of financial constraints. The consolation is, of course, that one can now ask far more searching questions."

1. Lowry O H, Rosebrough N J, Farr A L & Randall R J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265, 1951. [Citation Classic. *Current Contents* (1):7, 3 January 1977.]
2. Itzhaki R F. The arrangement of proteins on the deoxyribonucleic acid in chromatin. *Biochemical J.* 125:221-4, 1971.
[The *SCI* indicates that this paper has been cited in over 60 publications since 1971.]
3. ————. Structure of deoxyribonucleoprotein as revealed by its binding to polylysine. *Biochem. Biophys. Res. Commun.* 41:25-32, 1970.
[The *SCI* indicates that this paper has been cited in over 50 publications since 1970.]
4. Cooper H K, Maughan G P, O'Connor P J & Itzhaki R F. Heterogeneous distribution of DNA alkylation products in rat liver chromatin after *in vivo* administration of *N, N*-D[^{14}C]methylnitrosamine. *Chem.-Biol. Inter.* 11:483-92, 1975.
[The *SCI* indicates that this paper has been cited in over 35 publications since 1975.]