

This Week's Citation Classic

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Mazia D, Brewer P A & Alfert M. The cytochemical staining and measurement of protein with mercuric bromphenol blue. *Biol. Bull.* 104:56-67, 1953. [Department of Zoology, Univ. of California, Berkeley, CA]

Mercuric bromphenol blue, which has been used to visualize protein spots on filter paper, was studied with respect to its suitability as a quantitative cytochemical stain for tissue sections. The stain permits sharp visual differentiation of structural details and it follows the Beer-Lambert laws and can be used for microphotometry. It reacts with various sites in proteins, to the extent of about one dye binding group per ten amino acid residues. [The *SCI*[®] indicates that this paper has been cited over 495 times since 1961.]

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"The 1950s were a period of rapid progress in cell biology and of an intense search for new methods to study cell structure and the composition of cell parts. Before a diverse set of new analytical methods became available (such as, for instance, high resolution radioautography), various efforts were made to extend Caspersson's microscopic absorption technique to cytological stains and to measure amounts of stained substrates in various cellular compartments. With respect to nuclear DNA, the Feulgen reaction served this purpose very effectively, but there was no comparably useful color reaction to demonstrate general proteins and measure them quantitatively in microscopic preparations. The Millon reaction was used to some extent, but it produces poor visual contrast and therefore did not find wide application as a cytological stain.

"I was at that time already a staff member at U.C. Berkeley and engaged in developing and applying quantitative cytochemical procedures. The paper on bromphenol blue staining resulted from a cooperative effort between two graduate students, working in the laboratory of my colleague Dan Mazia, and myself. One of these students was Phil Brewer, who was at that time also my teaching assistant in cytology (and who unfortunately succumbed to leukemia a couple of years later). The other one was Gertrude Blumenthal, who is now Gertrude Kasbekar, a NSF program officer in Washington. Phil Brewer initiated the project and tested the usefulness of mercuric bromphenol blue as a general tissue stain; Blumenthal checked the stoichiometry of dye binding to various protein model systems. I studied the absorption properties of the dye in the free state and when combined to cellular substrates, and compared it to the Millon reaction, which had already been standardized for such purposes.

"What proved to be a very pretty and quantitative staining reaction was, however, subsequently used not as much by microscopists as by biochemists for the detection of peptides and proteins in the course of chromatographic procedures. This method, as we had developed it for intended cytological use, thus owes its many citations mainly to chromatographic applications, whose earliest attempts had drawn our attention to that dye. It is gratifying when some work turns out to be widely and generally useful and when its applicability extends into an area beyond that which was originally envisaged."