

**Alfert M & Geschwind II.** A selective staining method for the basic proteins of cell nuclei. *Proc. Nat. Acad. Sci. US* **39**:991-9, 1953.

**Treatment of formafin-fixed tissue sections with Fast Green at pH 8 after extraction of nucleic acids results in a specific chromatin stain. Model experiments indicate that histones or pro-famines are responsible for the staining picture. Some quantitative relationships between DNA and their associated histones are reported. [The SCI® indicates that this paper has been cited 494 times since 1961.]**

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"My doctoral thesis research in the late forties dealt with quantitative studies of DNA in individual cell nuclei, by staining and light absorption methods. The genetic role of DNA was then being established and there were speculations about the function of other nuclear components. Basic proteins were known to be associated with DNA and suspected to be involved in the regulation of gene expression; however, biochemical knowledge of those proteins was rudimentary and there existed no cytochemical test for their selective visualization, comparable to the Feulgen test, for DNA

"Against this background, I began to do a series of staining experiments on nuclear proteins and to quantitate them by light absorption measurements. I also engaged the advice and cooperation of my friend and colleague, Dr. I. I. Geschwind; as a professional biochemist, he was able to devise staining tests on natural and modified protein model systems which were necessary to

elucidate the mechanism of the nuclear staining reaction. We were then able to publish the first cytochemical method for the direct visualization of histones (and related basic proteins), explain how it worked and provide some quantitative data, relating DNA to histone content of nuclei.

"Since then, histones have continued to attract the attention of cell biologists and they now figure prominently in the most recent theories of chromatin organization, involving nucleosomes. Much of the recent work is done at the fine structure level, supplemented by biochemical and biophysical studies. At the same time, many cytologists have remained interested in visualizing histones by staining and they have used and referred to our method in their numerous publications. (But despite the many citations of this method, it has managed to escape the attention of some authors of recent reviews on nuclear changes during the cell cycle, a subject to which this technique has been often applied )

"Soon after the publication of this paper, Dr. Geschwind and I had mostly, but not exclusively favorable comments on it. A late, well-known biochemist from New York expressed his disdain for this and similar quantitative methods when they are used in conjunction with a microscope, rather than a test tube. However, subsequent applications of this method by us and many others have yielded interesting results on changes in nuclear composition, especially during spermiogenesis and in early embryonic development.

"This work was done at the University of California, with which we both remained associated until Dr. Geschwind's untimely death in July 1978. When we did that work, it was difficult to obtain funds for basic research; the same is true again today."