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This Week's Citation Classic

Panyim S & Chalkley R. High resolution acrylamide gel electrophoresis of histones. *Arch. Biochem. Biophys.* **130**:337-46, 1969.
[Dept. Biochemistry, Univ. Iowa, Iowa City, IA]

A high resolution gel electrophoresis of histones is described, capable of distinguishing five major groups of calf thymus histones and histone fractions whose mobilities differ by as little as one percent. The applicability of this technique for a comparison of histones from a wide variety of species is discussed. [The *SCI*® indicates that this paper has been cited over 1,440 times since 1969.]

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"When I was a graduate student in the laboratory of Roger Chalkley at the University of Iowa, the structure and function of histones was one of the exciting areas of research. The conjecture by Stedman and Stedman[1] that histones were gene repressors led many investigators to search for tissue and species specific histones. I was persuaded by Chalkley to look for specific histones in various tissues and animals along the evolutionary scale. I was then trying to find a simple method which could resolve histones into a minimum of five types as shown by the chemical fractionation of Phillips and Johns.[2] I started by trying various existing methods including ionic exchange (IRC-50) chromatography, electrophoresis, and chemical fractionation. We came to a conclusion that polyacrylamide gel electrophoresis would best suit our need. However, there were conflicting reports about the number of histone bands on polyacrylamide gel electrophoresis. In the course of the study we realized that one of the reasons for the conflicting reports was proteolysis of histones, and fortunately we found that sodium bisulfite was a very effective inhibitor against the proteolysis.

"With intact histones in our hands, I tried all available polyacrylamide gel systems and found that the best procedures were that of Bonner *et al.*[3] and that of Johns.[4] However, the Bonner *et al.* procedure failed to separate H2B from H2A while Johns's method was incapable of resolving H2B from H3. By careful analysis of these two techniques, it became apparent to us that they mainly differed in urea concentration. We thought that by changing the urea from 6.25 M (Bonner *et al.*[3]) to 0 M (Johns[4]) there should be the urea concentration capable of resolving H2B from H2A from H3 and thus, capable of resolving histones into five main types (H1, H3, H2B, H2A, and H4). To our delight I found that at 2.5 M urea histones were resolved into five bands, some of which still showed microheterogeneity. Our delight turned to frustration when the manuscript was rejected. Fortunately, the editor of *Archives of Biochemistry and Biophysics* promptly considered it worthy of publication. For a recent review of this field the reader can refer to I. Isenberg.[5]

"Trying to rationalize why this paper became a *Citation Classic*, I believe that this paper has been highly cited because it was published at the peak of a need for a simple and reliable method capable of separating histones into five main types. There were a very large number of reprint requests as soon as the paper appeared. I wonder if the paper had been published in 1971, when I finished my PhD, would it still be highly cited?"

1. **Stedman E & Stedman E.** Cell specificity of histones. *Nature* **166**:780-1, 1950.
2. **Phillips D M P & Johns E W.** A fractionation of the histones of group F2a from calf thymus. *Biochemical J.* **94**:127-30, 1965.
3. **Bonner J, Chalkley G R, Dakmus M, Fambrough D, Fujimera F. Huang R C, Huberman J. Jensen R. Marnshige K, Ohleabnscb H, Olivera B & Widholm J.** Isolation and characterization of chromosomal nucleoproteins. *Method. Enzymol.* **12B**:32-7, 1968.
4. **Johns E W.** The electrophoresis of histones in polyacrylamide gel and their quantitative determination. *Biochemical J.* **104**:78-82, 1967.
5. **Isenberg I.** Histones. *Annu. Rev. Biochem.* **48**:159-91, 1979.