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This Week's Citation Classic

Johns E W. Studies on histones 7. Preparative methods for histone fractions from calf thyumus. *Biochem. J.* **92**:55-9, 1964.

The author describes two preparative methods for the isolation of the four main groups of histories, F1, F2A, F2B and F3, from calf thymus. [The *SCI*® indicates that this paper has been cited over 625 times since 1964.]

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"The methods developed for the isolation of histones by E.W. Johns and his colleagues, particularly D.M.P. Phillips, have provided the major impetus to most of the chemical and physico-chemical studies on histones. The reason for this success lay in the development of simple clean methods for the isolation and purification of large quantities of the individual histones. In this paper two methods were given for the large scale isolation of four histone groups in one preparation. These were called F1, F2A, F2B, and F3. A subsequent paper[1] showed how F2A could be separated into two components to give F2A1 and F2A2. Thus complete separation of the five histone classes, now called H1, H2A, H2B, H3 and H4, was achieved. Approximately 400 mg of each of these fractions could be isolated from 100 g of calf thymus. and the histones could be further purified by conventional techniques. Other methods used at the time employed column separation, and these were unable to separate the arginine-rich histones H3 and H4 and gave only a few mgs of the other histones.

"Serendipity always plays a part in major scientific achievements and some of the procedures established were the results of happy accidents. Method 1 came about because histones were being tested for RNase activity. Samples of histones were incubated with RNA, and perchloric acid (PCA) added to precipitate all high molecular weight materials. As a control, PCA was also added to solutions of the individual histones. All precipitated except the very lysine-rich histone H1. PCA was then used for the specific isolation of H1. Method II was discovered because ethanol and acid were being used to inhibit proteases and to make other proteins less soluble. However, only about half of the histone was extracted. This proved to be a specific method for the separation of histones H2A, H3 and H4 from H1 and H2B. Another feature of this paper is that it contained the first reference to the now very interesting HMG chromosomal proteins as a contaminant of H1.

"It is quite clear that these methods formed the basis for the successful histone sequencing and for many of the detailed chemical and physical studies of the properties of histones. In a decade from now I wouldn't be surprised to be writing about the HMG group of histones."

REFERENCE

1. **Johns E W.** A method for selective extraction of histone fractions F2(A)1 and F2(A)2 from calf thymus deoxyribonucleoprotein at pH 7. *Biochem. J.* **105**:611-4,1967.[The *SCI*® indicates that this paper has been cited over 140 times since 1967.]