

Citation Classics

Ogur M & Rosen G. The nucleic acids of plant tissues. I. Extraction and estimation of desoxypentose nucleic acid (DNA) and pentose nucleic acid (PNA) from plant tissue. *Archives of Biochemistry* 27:260-76, 1950.

A method is described for the analytical extraction and quantitative estimation of RNA and DNA, enabling the utilization of u.v. absorbance as well as sugar and phosphorus estimation in the validation of the assay. [The SC® indicates that this paper was cited 664 times in the period 1961-1975.]

Professor Maurice Ogur
Department of Microbiology
Southern Illinois University
Carbondale, Illinois 62901

February 4, 1977

"A paper dealing with methodology, coming at a time when a field is expanding rapidly, tends to get many citations. This particular paper emerged when interest in the nucleic acids was expanding rapidly and the relationship between the amount of DNA per cell and polidy was being demonstrated. Many biologists were interested in nucleic acid estimation, and the available extraction methods relying either on estimating phosphorus or ribose and deoxyribose, while fairly satisfactory for some cells, ran into major interferences in others.

"I had just completed a Ph.D. in Chemistry at Columbia University in 1948 and was at an important crossroad when an opportunity arose to spend a post doctoral year at the University of Pennsylvania. It was a very stimulating and productive experience for me. I remember my awe at seeing Otto Meyerhof at work, meeting Britton Chance, having many conversations with David Coddard. Ralph Erickson, Ed Cantino. Conway Zirkle. Maurice Sevag, and others, and having my naive ideas worked over by Seymour Cohen's scholarship and critical judgment.

"Ralph Erickson. in the Botany Department, had been working on *Lilium* for some years and believed that the developing pollen might provide a cell population in sufficient synchrony for the measurement by chemical methods of nucleic acid changes during development. I tackled the problem with great enthusiasm by the available methods, only to be

frustrated by the polyuronides and pentosans of plant tissues, which interfered with the orcinol-pentose estimation as a measure of RNA, and by an unknown material, which interfered with the diphenylamine reaction as a measure of DNA. This made me hesitant to rely on phosphorus estimation alone without the check of at least one more of the generic constituents of a nucleotide in molar ratio. I tried the u.v. absorbance of the hot TCA nucleic acid fraction but found that the TCA interfered. It could be removed by steam distillation or solvent extraction, but this seemed too messy. Herman Kalckar had used perchloric acid in the extraction of the acid soluble mono-nucleotide fraction in a method of estimation utilizing u.v. absorbance. It seemed reasonable therefore that hot perchloric acid might readily replace hot TCA. It also seemed possible that, if the internucleotide link in RNA was more susceptible to alkali than that in DNA, it was worth seeing if it might also be more susceptible to acid as the pre-Feulgen hydrolysis seemed to suggest. Preliminary experiments were encouraging enough to warrant attempting to establish the best conditions of concentration, time and temperature of perchloric extraction to separate RNA and DNA for analytical, though not for preparative, purposes. At this point, the collaboration with Mrs. Gloria Rosen was very helpful. We published a set of conditions which appeared to accomplish this without excessive cross contamination of the fractions in terms of the analytical criteria employed. It also seemed reasonable that our procedure might provide a more carefully standardized pre-Feulgen hydrolysis than the procedure then in use for nuclear cytology.

"Anyone who has developed and published a method must be prepared to receive not only the praise from those who have found it applicable to and useful in their work, but also the blame from those who have encountered unexpected difficulty with it on other materials. I am surprised that the method and various modifications of it appear to have been useful to so many for so long."