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This Week's Citation Classic™

Hurlbert R B, Schmitz H, Brumm A F & Potter V R. Nucleotide metabolism. II. Chromatographic separation of acid-soluble nucleotides. *J. Biol. Chem.* **209**:23-39, 1954.
[McArdle Memorial Laboratory, Medical School, Univ. Wisconsin, Madison, WI]

A chromatographic method employing gradient elution of Dowex-1 (formate) columns was developed to systematically resolve nucleoside mono-, di-, and triphosphates. Extracts of tissues were discovered to contain all these derivatives of cytidine, guanosine, and uridine, as well as of adenosine. Related work showed these to be potentially direct metabolic precursors of RNA purines and pyrimidines. [The *SCI*® indicates that this paper has been cited in over 875 publications since 1961.]

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"Several factors converged at the time of this work to make development of the methodology feasible. First was the commercial availability of the Dowex line of ion-exchange resins, originally developed for nonbiological purposes and then shown by Waldo E. Cohn at the Oak Ridge National Laboratory to be useful for separations of nucleic acid derivatives.[1,2] Second was our purposeful use of the new principle of gradient elution to fan out smoothly *all* the small molecular weight anionic components of tissue extracts, combined with the use of concentrated volatile eluants (of rather low eluting ability but high buffering capacity) to permit complete resolution of co-eluting compounds by rechromatography at a different pH. Third was our suspicion that the immediate precursors (not yet known) of the nucleic acids ought to be lurking in there somewhere and might be related to the nucleotides whose chemistry had recently been described.[3,4] Fourth was the availability of radioisotopes which helped us sort out relationships by following label from ^{14}C-orotic acid to pyrimidine nucleotide peaks to RNA.[5] It is gratifying that the resolution obtained then was as good as obtained now with modern HPLC, with two differences: what took two days then can be done in two hours with greater sensitivity now.

"I especially want to mention Hanns Schmitz and Anne Brumm (both now deceased, Anne a victim of cancer). We predicted by analogy that triphosphates of cytidine and guanosine ought to exist and should appear just before and after the ATP emerged. I recall vividly Hanns's excitement when a bulge in the ultraviolet 275/260 ratio first revealed CTP to exist in tissues.[6] Also, his almost tearful disappointment after he reported that discovery (last paper on the program at the 1953 American Association for Cancer Research meeting) because the meager audience after the main exodus consisted almost entirely of our lab associates. Anne quietly and with dedication did much of the analytical work on this and related papers;[5,6] both she and Hanns would feel rewarded to learn of this recognition by *Citation Classics*™, that all those overtime hours did help measurably in the massive struggle to gain control over cancer. Van Potter was astute as usual; he let us play around with the procedure at first when it would have been more expedient to stick to business on his original plans as funded, which were quite different. Waldo was somewhat ambiguous when he remarked that he had thought about applying his techniques to tissue extracts but regarded it as 'pearl diving.' It is even harder now to get a grant application funded for such ventures.

"Although then the procedure discovered new nucleotides[7] and helped establish order in the field of nucleic acid biosynthesis, I think its current significance is that it gives a picture, a snapshot, of the metabolic status of the tissue. The simple ribo- and deoxyribonucleotides are not only intermediates in RNA and DNA synthesis, but they and their derivatives are involved as reactants or regulators in almost all metabolic pathways, serving as systemic interconnectors. Thus, their relative and absolute concentrations both reflect and control the physiological function of a cell, its stage in the cell cycle, and its growth potential. Combined with pulse-labeling and treatments by antimetabolites, the method (in modern form) can provide more dynamic insights into control of cell growth. I don't believe it has even yet been fully exploited for this purpose."

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2. **----------.** Some results of the applications of ion-exchange chromatography to nucleic acid chemistry. *J. Cell. Comp. Physiol.* **38**(Suppl. 1):21-40, 1951.
3. **Baddiley J.** Chemistry of nucleosides and nucleotides. (Chargaff E & Davidson J N, eds.) *The nucleic acids: chemistry and biology*. New York: Academic Press, 1955. Vol. I. p. 137-90.
4. **Brown D M & Todd A R.** Evidence on the nature of the chemical bonds in nucleic acids. (Chargaff E & Davidson J N, eds.) *The nucleic acids: chemistry and biology*. New York: Academic Press, 1955. Vol. I. p. 409-45.
5. **Hurlbert R B & Potter V R.** Nucleotide metabolism. I. The conversion of orotic acid-6-C^{14} to uridine nucleotides. *J. Biol. Chem.* **209**:1-21, 1954.
6. **Schmitz H, Hurlbert R B & Potter V R.** Nucleotide metabolism. III. Mono-, di-, and triphosphates of cytidine, guanosine, and uridine. *J. Biol. Chem.* **209**:41-54, 1954.
7. **Mandel P.** Free nucleotides in animal tissues. *Progr. Nucleic Acid Res. Mol. Biol.* **3**:299-334, 1964.