

This Week's Citation Classic

Wyatt G R. The purine and pyrimidine composition of dcoxyptentose nucleic acids. *Biochemical J.* 48:584-90, 1951.
[Agricultural Research Council Plant Virus Research Unit,
Molteno Institute, University of Cambridge, Cambridge, England]

A technique for the determination of the purine and pyrimidine composition of DNA is described, using acid hydrolysis, separation of the bases by chromatography on filter paper, detection in UV light, and spectrophotometric estimation. Results for DNA from eight animal and one plant source are reported. [The SC² indicates that this paper has been cited over 865 times since 1961.]

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"I am delighted to learn that my 1951 paper has been cited so often, so many years after its publication. Reading this paper now, one feels that it stems from another era, both because of its primitive view of DNA and its perambulating style which would horrify present-day editors.

"I had gone from Canada to Cambridge to pursue a PhD in insect virology in the Agricultural Research Council Virus Unit. My attempts to obtain meaningful results from sick caterpillars proved frustrating, but I fell under the influence of Roy Markham, a young biochemist working on plant viruses (who unfortunately died of cancer in 1979). Markham held the then radical view that understanding of viruses would come through study of their nucleic acids, and he was developing a technique for quantitative analysis of RNA by separation of bases and nucleotides on paper chromatograms, detection with UV light, and estimation in the newly arrived Beckman spectrophotometer. Since the insect viruses in which I was interested contained DNA, Markham suggested that I might try adapting the method for DNA.

"Analysis of DNA by paper chromatography

had already been reported from the laboratories of Rollin Hotchkiss¹ and Erwin Chargaff,² but the techniques seemed open to improvement. I found that the purine and the pyrimidine bases could be obtained in good yield from a single hydrolysis in formic or perchloric acid. For the chromatographic solvent to overcome the low solubility of guanine, I tested many mixtures of various alcohols with strong acids and selected a combination of isopropanol and HCl that gave compact spots and a well-spaced separation of guanine, adenine, cytosine, and thymine. Animal and plant DNA hydrolysates showed an unexpected fifth spot, which was identified as 5-methylcytosine.³ I analyzed such DNAs as I could easily obtain and wrote up the method and first results before turning to look at the DNA of some insect and other viruses.

"I believe that this paper has often been cited for methods: conditions for quantitative hydrolysis of DNA, a solvent useful for thin-layer as well as paper chromatography, and a table of extinction coefficients of the DNA bases. In addition, it reported the compositions of several DNAs long before the simpler methods of determination using T_m and buoyant density were developed. This information was useful to Watson and Crick⁴ and many others. The discussion in my paper is extraordinarily cautious, however. The remark, 'It is tempting to speculate whether DNA composition may bear some direct relation to genetic structure,' is sufficient to remind us of the DNA revolution that separates 1981 from 1951.

"Those reading my recipe for the isopropanol HCl solvent may have wondered why 16°C is specified. Working in Britain in 1948-1950, I had come to accept this as a normal room temperature; one day, my notebook records 11°C!

"My sole authorship of this paper reflects the generosity of my advisors; Markham declined to add his name though he gave much help. Data on the base compositions of DNAs from numerous organisms are now available."⁵

1. Hotchkiss R D. The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. *J. Biol. Chem.* 175:315-32, 1948.
2. Chargaff E, Vischer E, Doniger R, Green C & Misani F. The composition of the desoxyptentose nucleic acids of thymus and spleen. *J. Biol. Chem.* 177:405-16, 1949.
3. Wyatt G R. Recognition and estimation of 5-methylcytosine in nucleic acids. *Biochemical J.* 48:581-4, 1951.
4. Watson J D & Crick F H C. Molecular structure of nucleic acids. *Nature* 171.737-8, 1953.
5. Shapiro H S. Distribution of purines and pyrimidines in deoxyribonucleic acids. (Fasman G D. ed.) *CRC handbook of biochemistry and molecular biology.* Cleveland, OH: CRC Press. 1976. Vol. 2. p. 241-83.