

Munro H N & Fleck A. The determination of nucleic acids.

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This review article is a detailed critique of the various methods recommended from 1945 to 1965 for estimating RNA and DNA, particularly in tissue samples. A considerably modified form of one method, the Schmidt-Thannhauser procedure, is finally selected as likely to be reliable under most circumstances. The review provides a table for trouble-shooting when applying this procedure in new circumstances. [The *SCI*® indicates that this paper has been cited in over 960 publications.]

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September 9, 1986

When it was shown in 1941 by histological means that cells most active in protein synthesis are richest in RNA, it became apparent that biochemical procedures for measuring cell and tissue concentrations of RNA and DNA needed to be developed. In 1945 two such methods were published: that of Schmidt and Thannhauser¹ based on phosphorus estimation following separation of RNA and DNA and Schneider's method² using the orcinol reaction for RNA and the diphenylamine reaction for DNA applied to the same tissue extract. During the next decade, our experience with these methods in the Biochemistry Department of Glasgow University showed considerable limitations, so my colleague, W.C. Hutchison, and I set about reviewing the expanding methodology of nucleic acid estimations using the extensive collection of reprints on nucleic acids assembled by the department head, J. Norman Davidson. This involved going through more than 10,000 papers to see whether the investigators had quantitated the

nucleic acids, what their experiences were, and what modifications they had found profitable. I well remember going each weekend during the summer of 1959 to join my vacationing family on the nearby island of Arran, armed with several hundred heavy reprints, and returning each Monday morning with the relevant information on index cards. The end product—a comprehensive review with 404 references—was published in 1961³ and demonstrated that, up to that time, no universally applicable procedure had emerged.

This conclusion created a challenge, so Hutchison and I began by tackling the limitations of the Schneider procedure. At the same time, I had the good fortune to have as a PhD candidate a physician, Adam Fleck (now professor of chemical pathology at the University of London), who was studying plasma protein synthesis. In the course of his work, Adam applied his keen interest in quantitation to improving the Schmidt-Thannhauser procedure. Choosing ultraviolet absorption at two wavelengths instead of phosphorus to measure the extracted RNA, he authenticated each step in his modified procedure, which was published in a widely cited paper.⁴ Meanwhile, others in my group explored various applications of nucleic acid estimations to tissue analysis. It thus became timely to assemble a second review—the article cited above—integrating the new information with the old to provide a comprehensive view of the state of nucleic acid estimations. A short updated successor appeared in 1969⁵ as part of a chapter on tissue analysis. Our continued confidence in the modified Schmidt-Thannhauser procedure is implied by our recent use of it⁶ for measuring the RNA and DNA content of intestinal mucosal cells.

Was all this work worth the considerable effort? I believe it fulfilled a timely need for investigators to have a sound basis on which to apply these techniques with confidence and insight. Finally, I would note that the process of creating such a review was made infinitely easier by having access to a large collection of reprints.

1. Schmidt G & Thannhauser S J. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. *J. Biol. Chem.* 161:83-9, 1945. (Cited 3,110 times since 1955.)
2. Schneider W C. Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxyribose nucleic acid and of pentose nucleic acid. *J. Biol. Chem.* 161:293-303, 1945. [See also: Schneider W C. Citation Classic. *Current Contents* (8):12, 21 February 1977.]
3. Hutchison W C & Munro H N. The determination of nucleic acids in biological materials. *Analyst* 86:768-813, 1961. (Cited 350 times.)
4. Fleck A & Munro H N. The precision of ultraviolet absorption methods in the Schmidt-Thannhauser procedure for nucleic acid estimations. *Biochim. Biophys. Acta* 55:571-83, 1962. (Cited 1,085 times.)
5. Munro H N & Fleck A. Analysis of tissues and body fluids for nitrogenous constituents. (Munro H N, ed.) *Mammalian protein metabolism*. New York: Academic Press, 1969. Vol. 3. p. 423-525. (Cited 265 times.)
6. Bronstein A D, Leleiko N S & Munro H N. RNA synthesis by villus and crypt cell nuclei of rat intestinal mucosa. *Biochim. Biophys. Acta* 739:334-43, 1983.

1-24