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Kissane J M & Robins E. The fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system.

J. Biol. Chem. 233:184-8, 1958.

[Depts. Pharmacology and Psychiatry and Neurology, Washington Univ. Sch. Medicine, St. Louis, MO]

This method is based upon the reaction between α -methylene aldehydes (e.g., deoxyribose) and 3,5-diaminobenzoic acid to form a fluorescent product. The reaction is applied to a hydrolysate of tissue residue after acid precipitation and lipid extraction. Ribose does not interfere. [The SCI® indicates that this paper has been cited in over 1,150 publications.]

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The basis for this method is a very old reaction (from the German dye literature) between diaminobenzoic acid (DABA) and α -methylene aldehydes to form bright yellow-green products subsequently shown to be fluorescent. The reaction had been used in a method for acetaldehyde.^{1,3} We hoped to apply it to DNA in samples of biologic material of sizes made accessible by Lowry's micromethods.⁴ It had just recently become accepted as a biologic principle that the amount of DNA per diploid nucleus is constant within a species and that the measurement of DNA afforded a cell count in biologic material. If that measurement could be performed on the final residue after other studies, so much the better.

Robins placed in my hands the basic reaction, the fact that the reaction generated a fluorescent product with deoxyribose (recall that deoxyribose is an α -methylene aldehyde

whereas ribose is not), and a brown glass bottle of DABA di-HCl that he and an organic chemist had synthesized. Fearing photo-decay, we wrapped that bottle in a square of black cambric and enclosed it in a cardboard mailing tube. I don't know how many times I opened that tube, unwrapped the black cloth like a vaudeville magician, laded out enough reagent for a few days' experiments, and then hurriedly replaced the whole assembly. Incidentally, the last time I inquired, that brown glass bottle of DABA di-HCl still existed in Robins's laboratory. Its contents, thanks to Lowry's miniaturization, have been little depleted after three decades.

Several technical aspects, ill-acknowledged in the final publication, emerged. The final hydrolysis in small volumes required immersion of microtubes in a very shallow water bath in an oven to minimize "creeping" up the inside of the hydrolysis tube. Second, the reaction is so sensitive that it should not be attempted in an environment of organic fumes. Third, the final chalky precipitate must be thoroughly dispersed in the acid. The fourth is my only intellectual contribution to the procedure. Since our DABA was in the form of the dihydrochloride, strongly acid in solution, it occurred to me to use that acidity to hydrolyze the DNA in the same step during which generation of the fluorochrome occurred.

I have rather lost touch with quantitative histochemistry, but to my knowledge this reaction is still the most sensitive applicable to quantitation of DNA. (For a recent review, see reference 5.) Acid precipitation and extraction of lipids precede any measurement of DNA, and a single step applied to the residue affords quantitation of DNA. Lowry's microinstrumentation is not essential. The method is readily applicable with commercially available glassware and any fluorometer. DABA di-HCl of high quality is now readily available, and I think you can forget about that square of black cambric.

1. Velluz L, Amlard G & Pesez M. Aldéhydes et réaction de Doebner-Miller. I. Méthyl-2 carboxy-5 amino-7 quinoléine. *Bull. Soc. Chim. France* 15:678-9, 1948.
2. Velluz L, Pesez M & Amlard G. Aldéhydes et réaction de Doebner-Miller. II. Caractérisation du groupe $-CH_2-CHO$. *Bull. Soc. Chim. France* 15:680-1, 1948.
3. Velluz L, Pesez M & Herblain M. Aldéhydes et réaction de Doebner-Miller. III. Titration de l'acétaldéhyde. *Bull. Soc. Chim. France* 15:681-2, 1948.
4. Lowry O H. The quantitative histochemistry of the brain. Histological sampling. *J. Histochem. Cytochem.* 1:420-8, 1953. (Cited 505 times since 1955.)
5. Thomas P S & Farquhar M N. Specific measurement of DNA in nuclei and nucleic acids using diaminobenzoic acid. *Anal. Biochem.* 89:35-44, 1978. (Cited 80 times.)