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1 I. E. BUSH, *The Chromatography of Steroids*, Pergamon, New York, 1961.
2 R. NEHER, *Steroid Chromatography*, Elsevier, Amsterdam, 1964.
3 M. L. LEWBART AND J. J. SCHNEIDER, *J. Org. Chem.*, 29 (1964) 2559.
4 J. J. SCHNEIDER AND M. L. LEWBART, *Tetrahedron*, 20 (1964) 943.
5 M. L. LEWBART AND J. J. SCHNEIDER, *J. Biol. Chem.*, 241 (1966) 5325.
6 M. L. LEWBART AND J. J. SCHNEIDER, *J. Org. Chem.*, 33 (1968).
7 E. C. BATE-SMITH AND R. G. WESTALL, *Biochim. Biophys. Acta*, 4 (1950) 427.
8 D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. 13, Interscience, New York, 1965, pp. 357–433.
9 E. L. ELIEL, *Stereochemistry of Carbon Compounds*, McGraw-Hill, New York, 1962, pp. 131–132.

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Estimation of the products of DNA alkylation

A variety of nucleic acid bases are methylated both *in vivo* and *in vitro* by chemical mutagens such as N-methyl-N-nitro-N-nitrosoguanidine and N-methyl-N-nitroso-*p*-toluenesulfonamide (MNTS)[1]. As no simple convenient assay for the commonly alkylated bases (7-methylguanine, 1-methyladenine, and 3-methyladenine) was available, a thin-layer chromatographic procedure was developed.

Methylated DNA was prepared by adding 0.5 mg of ^{14}C-MNTS (New England Nuclear Corporation) and 9.5 mg of MNTS (Aldrich Chemical Co.) in 2 ml of ethanol to 10 ml of water containing 10 mg of highly polymerized DNA (Nutritional Biochemicals Corporation) and reacting for 7 days in the dark at 26°. The products were separated by two extractions with chloroform–octanol (1:1, v/v) (in which MNTS is soluble), followed by chromatography on Sephadex G-25 (Pharmacia Fine Chemicals, Ltd.). After concentration using a rotary evaporator, the DNA was hydrolyzed in 2 ml of 1 *N* HCl at 100° for 1 h to release purine bases and pyrimidine nucleotides.

Adenine, guanine, 7-methylguanosine, thymidine, and deoxycytidine monophosphoric acid were obtained from Sigma Chemical Co. The 7-methylguanosine was hydrolyzed to the free base using 1 *N* HCl at 100° for 1 h. 1-Methyladenine and 3-methyladenine were prepared according to BROOKES AND LAWLEY[2].

Thin-layer plates were prepared in the standard manner with MN cellulose (300 G containing binder, Canadian Laboratories Ltd.). The samples were applied to the plates (activated at 120° for 30 min) using 5 μl capillary pipettes. Five micrograms each of solutions of authentic samples of the methylated purines were also applied as markers. After development with methanol–HCl–H_2O (7:2:1, v/v) for a

distance of 15 cm in one dimension (1.5 h), drying at 100° for 5 min, and redevelopment in *n*-butanol–NH_3–H_2O (88:2:10, v/v) for 15 cm in the second dimension (2 h), and drying again, the compounds were visualized with a Mineralight lamp (maximum emission 254 mμ). The spots, visible under ultraviolet light, were loosened from the plate by scraping, and were then sucked into scintillation vials[3] containing approximately 0.3 g of Cab-O-Sil (Packard Instrument Corporation), and 10 ml of Liquifluor (Nuclear Chicago Corporation)[4]. Radioactivity was determined with a Nuclear-Chicago Mark I Scintillation Counter.

Ion exchange chromatography according to MAGEE AND FARBER[5] (using a Dowex 50W x2, J. T. Baker Chemical Co. 1 × 15 cm column with a continuous gradient 0.2 *N* to 2.0 *N* HCl) was done in order to check the TLC method.

In Fig. 1, a typical thin-layer chromatogram is shown. Good separation of the alkylated bases from the normal bases was found. Table I indicates the proportion

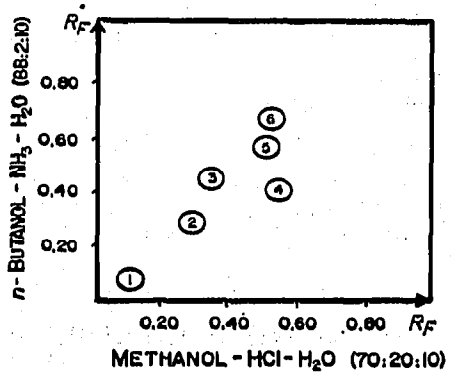

Fig. 1. Thin-layer chromatogram of acid hydrolysate. Solvents used are indicated in the figure. 1 = Pyrimidines and site of application; 2 = guanine; 3 = 7-methylguanine; 4 = 1-methyladenine; 5 = adenine; 6 = 3-methyladenine.

TABLE I

DISTRIBUTION OF RADIOACTIVITY

Method of Analysis	*Amount of label applied*	*Recovery, c.p.m. (%)*		
		7-Methylguanine	*1-Methyladenine*	*3-Methyladenine*
TLC	4200	760 (18)	40 (0.9)	55 (1.3)
Dowex 50W x2 column	5600	1200 (21.5)	135 (2.4)*	

* These two bases were not separated on the column.

of radioactivity found in these compounds, and demonstrates that the distribution is quite similar to that found using column chromatography (Fig. 2).

This method will facilitate analysis of the purine content of DNA isolated from mutagen treated organisms.

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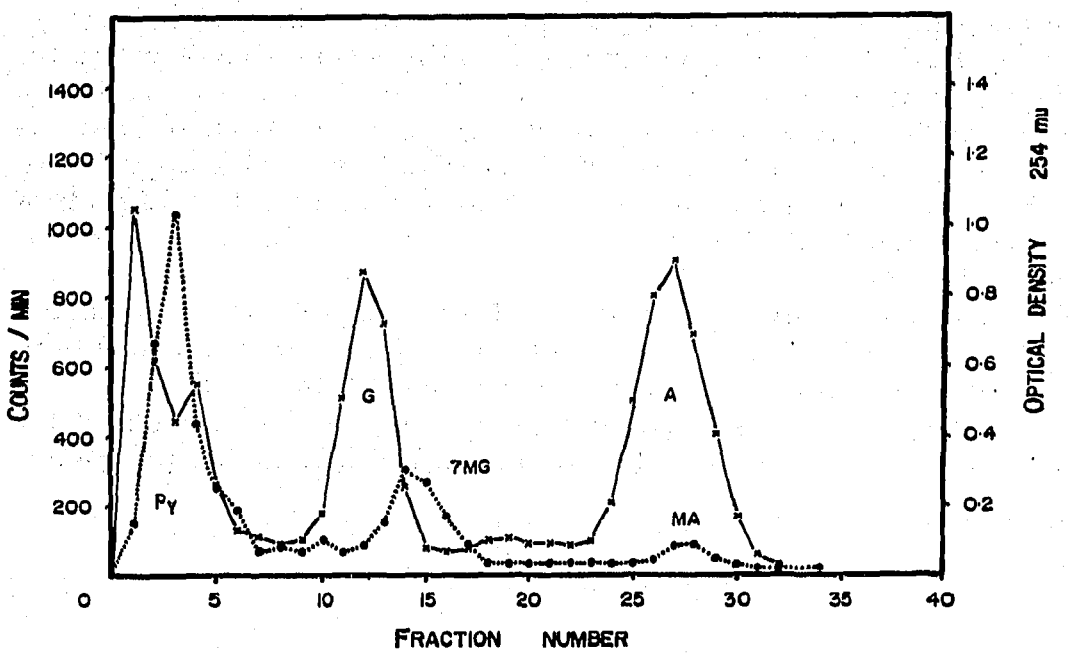

Fig. 2. Ion exchange chromatography of acid hydrolysate of DNA (Dowex 50W x2, H^+ form, continuous gradient 0.2 *N* to 2.0 *N* HCl) (5 ml fractions were collected). (×—×—×) Optical density at 254 mμ; (●...●...●) radioactivity (c.p.m.). Py = Pyrimidines; A = adenine; G = guanine; 7MG = 7-methylguanine; MA = methylated adenine.

of 1-methyladenine and 3-methyladenine, and the Medical Research Council for support in the form of a Post-Doctoral Fellowship.

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1 D. R. MCCALLA, *Biochim. Biophys. Acta*, 155 (1968) 114.
2 P. BROOKES AND P. D. LAWLEY, *J. Chem. Soc.*, (1960) 539.
3 G. R. BJORK AND I. SVENSSON, *Biochim. Biophys. Acta*, 112 (1966) 346.
4 F. SNYDER AND N. STEPHENS, *Anal. Biochem.*, 4 (1962) 128.
5 P. N. MAGEE AND E. FARBER, *Biochem. J.*, 83 (1962) 144.

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