Short Communications

The Reaction of *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine with Deoxyribonucleic Acid

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The biologically potent compound MNNG* has been very widely used as a mutagen since the discovery of its mutagenicity for bacteria (Mandell & Greenberg, 1960), and more recently it has been shown to be a very active carcinogen (Schoental, 1966; Sugimura, Nagao & Okada, 1966; Druckrey *et al.* 1966). Very recently, much interest has arisen

* Abbreviation: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

in the chemical nature of the reactions that account for these biological effects, in particular its reactions with nucleic acids (Chandra, Wacker, Süssmuth & Lingens, 1967; Singer & Fraenkel-Conrat, 1967), but no definite chemical evidence for a particular reaction product seems to have been published. The present communication gives evidence for the methylation of guanine of DNA by MNNG.

As preliminary experiments showed that the

Fig. 1. Ion-exchange chromatography on Dowex 50 (X12; H⁺ form) of DNA isolated from the intestine of a rat that had been treated with [¹⁴C]formate during the first few days of life. (a) Control sample; (b) treated with MNNG as described in the text. ○, *E*₂₆₀; △, radioactivity. T, Thymidylic acid and CMP; G, guanine; M, carrier 7-methylguanine; A, adenine.

extent of reaction of DNA with MNNG was not very great under physiological conditions, radioactive DNA was used to increase the sensitivity of tests for reaction products. The DNA used had been isolated from the intestine of a rat previously treated with [^{14}C]formate during the first few days of life (Craddock & Magee, 1967a) and had received a total of $205\text{ }\mu\text{C}$ (1.29 mg.); the animal was killed at 6 months. DNA (10 mg.) was dissolved in $0.2\text{ M-NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ buffer, pH 7.5, and incubated at 37° with shaking. Finely ground MNNG (30 mg.) was added, the mixture was incubated for 30 min. and the same addition and incubation procedure were repeated twice. The control sample of DNA was incubated without the addition of MNNG. The DNA was precipitated with 95% (v/v) ethanol, washed with ethanol and dried with acetone. The preparations were hydrolysed with 1 N-HCl at 100° for 1 hr., with standard 7-methylguanine carrier added, and the hydrolysates were run on a Dowex 50 (X12) column ($1\text{ cm.} \times 10\text{ cm.}$) with 1 N-HCl and then a $1\text{-}4\text{ N-HCl}$ linear gradient. The E_{260} of the effluent was determined, and 8 ml. portions of the 10 ml. fractions were evaporated to dryness and radioactivities determined as in previous experiments (Craddock & Magee, 1967b).

The remainder of each fraction was used for paper chromatography. The solutions were pooled corresponding to the radioactive peaks, concentrated by use of a rotary evaporator and spotted on to Whatman no. 1 chromatography paper. The solvent systems used were butan-1-ol-aq. NH_3 (sp.gr. 0.88)-water (85:2:12, by vol.) and propan-2-ol-conc. HCl -water (34:9:7, by vol.). The chromatograms were cut into sections, eluted with 1 N-HCl , the eluates evaporated to dryness and radioactivities determined as described above.

The results are shown in Fig. 1. As the DNA was derived from rats treated with [^{14}C]formate, radioactivity was in guanine, adenine and thymine. Two minor peaks of radioactivity occurred in the MNNG-treated DNA that did not appear in the control sample, and one of these corresponded to the E_{260} profile of the carrier 7-methylguanine. The

fractions in this peak were pooled and studied by paper chromatography. Approximately half of the radioactivity accompanied the carrier 7-methylguanine in each solvent system, which corresponded to 1-2% of the guanine having been converted into 7-methylguanine.

These results suggest that 7-methylguanine is formed in DNA as a result of treatment with MNNG. It is possible that 7-methylguanine is not the major product of the reaction, but that the mechanism is similar to the proposed by Henry (1950) for the reaction of MNNG with amines:

Any guanidino compounds formed would probably have been destroyed by the acid hydrolysis. Such a reaction mechanism might explain the occurrence of two reactions of MNNG in *Escherichia coli*, only one of which is subject to repair processes (Cerdá-Olmedo & Hanawalt, 1967).

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