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Probable methylation of nucleic acids of mouse colon by 1,2-dimethylhydrazine *in vivo*

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ADMINISTRATION of 1,2-dimethylhydrazine produces a high incidence of multifocal tumours of the large gut in both rats[1] and mice.[2] Preussmann *et al.*[3] have postulated that the mechanism of action of 1,2-dimethylhydrazine might involve oxidation to azomethane followed by removal of one methyl group by microsomal enzymes with the production of formaldehyde and a highly reactive methylating species, most probably a carbonium ion. It is known that 1,2-dimethylhydrazine is metabolized by rat liver microsome preparations to yield formaldehyde, molecular oxygen and an NADPH generating system being required for maximal activity.[4,5] The similarities between 1,2-dimethylhydrazine and methylazoxymethanol have been pointed out[3] and it has also been suggested[6] that azomethane, an intermediate postulated by Preussmann *et al.*,[3] may be oxidized to azoxymethane which on hydroxylation would yield methylazoxymethanol. Methylazoxymethanol and its glycoside cycasin are hepatotoxic, teratogenic and neurotoxic.[7] These compounds produce tumours of the liver as well as at other sites including the large intestine.[8] Methylazoxymethanol methylates phenols[9] and RNA and DNA both *in vitro*[10] and *in vivo*.[11,12]

Furthermore, it has been shown that the cytostatic agent 1-methyl-2-(*p*-isopropylcarbamoyl) benzylhydrazine hydrochloride (NSC 77213) (the hydrochloride of Procarbazine (Ibenzmethyzin) and sometimes called Natulan) methylates nucleic acids of mouse leukaemic cells[13,14] and ascites tumour cells.[15] In all cases 7-methylguanine was the predominant methylated base. By contrast the unsymmetrical isomer of 1,2-dimethylhydrazine, 1,1-dimethylhydrazine, is a weak carcinogen in mice[16] and either inactive or only weakly active in rats,[17,18] producing no tumours of the colon in either species. It is also metabolized by rat liver microsomes[4] but does not alkylate rat liver RNA *in vivo*.[19] There was no experimental evidence for the reactions proposed for 1,2-dimethylhydrazine by Preussmann *et al.*,[3] and the present work was undertaken to discover whether an active methylating agent was produced during the metabolism of this compound.

1,2-Dimethylhydrazine was synthesized from [^{14}C]methyl iodide (Radiochemical Centre, Amersham) as the starting material from which [^{14}C]methyl methanesulphonate was prepared by reaction with silver methanesulphonate. 1,2-[^{14}C]Dimethylhydrazine dihydrochloride was then prepared by reaction of 1,2-diformylhydrazine with [^{14}C]methyl methanesulphonate followed by removal of the formyl groups with conc. HCl.[20] The dihydrochloride was kept under N_2 at 0–4°.

The 1,2-[^{14}C]dimethylhydrazine (specific radioactivity 0·56 mc/m-mole) was administered subcutaneously in a dose of 15 mg/kg body weight to twelve 22 g female NMRI mice, each animal receiving 2·95 μc. The animals were kept in a metabolism cage with food and water and the expired air was collected in 2 N NaOH. Six animals were killed at 6 hr and a further six at 24 hr after in-

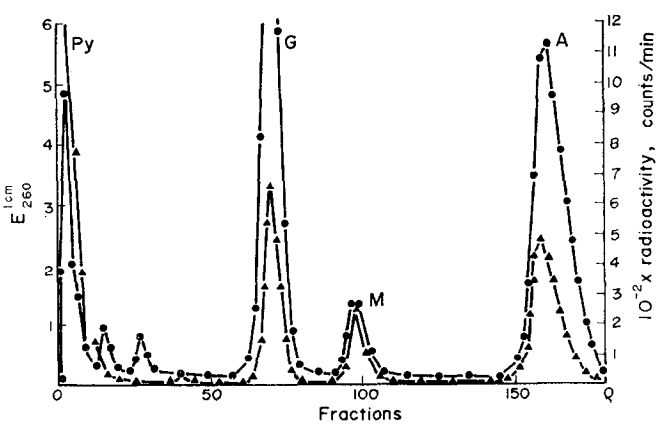

FIG. 1. Ion-exchange chromatography of hydrolysed nucleic acids obtained from animals killed at 24 hr as described in the text. 7-Methylguanine was added as marker: ●, E^{1cm}_{260}; ▲, radioactivity; Py, pyrimidine nucleotides; G, guanine; M, 7-methylguanine; A, adenine.

jection. The livers and colons were quickly removed, washed in 0·9% (w/v) NaCl and frozen in liquid N_2. DNA and RNA were extracted from the livers by a phenol method[21] and then hydrolyzed in 1 N HCl for 1 hr at 100°. The total nucleic acids were extracted from the colonic tissue by the method of Schneider[22] using 5% (w/v) trichloroacetic acid at 90° for 15 min. The sample was centrifuged and the supernatant made 1 N with respect to HCl and then heated for 1 hr at 100°. The pyrimidine nucleotides and purine bases released by hydrolysis were chromatographed on a Dowex 50 (10 × 1 cm) column with a 1–3 N exponential HCl gradient after the addition of authentic 7-methylguanine (Sigma, London) as marker. Fractions of 4·5 ml were collected, E_{260nm} measured and then evaporated to dryness. The residue was dissolved in Hyamine before radioactivity assay in a conventional toluene scintillation fluid. The samples were counted in a Packard TriCarb 3320 liquid scintillation counter with an efficiency of 78 per cent.

The chromatographic profiles of the hydrolysates of RNA and DNA of liver and of the mixed nucleic acids from colon all showed a peak of radioactivity in the region between guanine and adenine which co-chromatographed exactly with the added authentic 7-methylguanine (Fig. 1). The radioactivity appearing with the guanine and adenine probably represents incorporation into the purine rings by normal biosynthetic pathways from the one-carbon intermediate pool, which would be expected to be labelled by the [^{14}C]methyl groups of the 1,2-[^{14}C]dimethylhydrazine.

It is concluded that the results of this experiment support the hypothesis that 1,2-dimethylhydrazine can act as a methylating agent *in vivo* and thus shares a common feature with some carcinogenic nitroso compounds, cycasin and carcinogenic alkylating agents.

The 1,2-[^{14}C]dimethylhydrazine had a small amount of 1-methylhydrazine present as impurity (approx. 6 per cent of radioactivity) and thus the specific activity quoted earlier is only an approximation. Purification of this material is currently in progress. There appear to be no published reports of the carcinogenic potential of monomethylhydrazine. Experiments to test this are currently in progress in this laboratory.

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