RAPID LOSS OF 7-METHYLGUANINE FROM LIVER NUCLEIC ACIDS IN MICE DURING THE INITIAL STAGE OF LIVER CARCINOGENESIS INDUCED BY DIMETHYLNITROSAMINE

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Summary: Dimethylnitrosamine was administered to mice in their drinking water. The amount of 7-methylguanine in liver nucleic acids was found to increase initially and then to decrease. This decrease in 7-methylguanine was found to be due to decrease in the rate of its formation and increase in its excision from nucleic acids.

There have been many reports on the interaction of chemical carcinogens with cellular macromolecules, which have been reviewed by Miller and Miller (1), Heidelberger (2) and others. However, there have been only few studies on modification of nucleic acids under the conditions inducing tumors in animals (3,4).

Dimethylnitrosamine (DMN) induces hepatic tumors to rats, mice and hamsters (5-9), when administered over a long period at a comparatively low concentration in the diet or drinking water. We reported previously (4) that on administration of ^{3}H-DMN under conditions inducing liver tumors the incorporation of radioactivity and the formation of 7-methylguanine did not increase steadily but first increased and then decreased slightly.

This paper reports studies on the causes of the decrease in the formation of 7-methylguanine.

Materials and Methods: ^{3}H-Labeled DMN (24.9 mCi/mmole) was purchased from New England Nuclear, Boston, Mass., and unlabeled DMN from Tokyo Kasei Kogyo Co. Ltd., Tokyo. Male ICR/JCL mice, of 8 weeks old at the start of the experiment, were given drinking water containing 10 ppm DMN ad libitum (the concentration of ^{3}H-DMN solution was adjusted to 0.1 mCi/mg DMN by mixing labeled and unlabeled DMN and diluting the mixture with deionized water). DNA was extracted from purified nuclei, and RNA from the cytoplasm by modifications of the methods of Marmur and of Scherrer and Darnell, respectively, as described previously (4). Nucleic acids were hydrolyzed with hydrochloric acid and analyzed by Dowex 50 column chromatography. 7-Methylguanine was determined as reported previously (4).

The syntheses of DNA and RNA during administration of DMN to mice were measured as incorporations of radioactivity into DNA and RNA, respectively 1 hr after intraperitoneal injection of 50 μCi of ^{3}H-thymidine (12.3 Ci/mmole, Daiichi Pure Chemical) plus 2 μCi of ^{14}C-orotic acid (20.6 mCi/mmole, Radiochemical Centre, Amersham). Nucleic acids were fractionated by the method of Schmidt and Thannhauser (10). DNA was measured colorimetry with diphenylamine (11) and RNA with orcinol (12), using calf thymus DNA and yeast RNA, respectively as standards.

Results and Discussions: On continuous administration of ^{3}H-DMN solution to mice, radioactivity in liver DNA and RNA increased to a maximum after 2 weeks and then decreased to one-half the maximal level after 4 weeks (Fig. 1). Incorporation of radioactivity in the 2nd week (i.e. from day 8-14) was greater than in any other

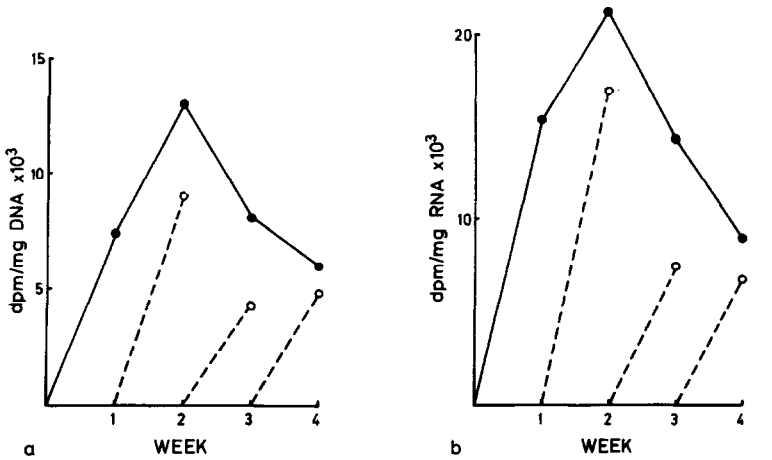

Fig. 1 - Changes in radioactivity in nuclear DNA (a) and cytoplasmic RNA (b).
Radioactivity in nucleic acids on continuous administration of ^{3}H-DMN solution (solid line).
Radioactivity in nucleic acids on continuous administration of unlabeled DMN and then of ^{3}H-DMN for 1 week before sacrifice (dotted line).
Values are means of those in 2 animals.
Values in pairs of animals differed by less than 10%.

week. It is of interest that during the 3rd and 4th weeks radioactivity was not accumulated but rapidly eliminated, although incorporation during this period was quite high, being about one-half of that during the 2nd week.

The nucleic acids were treated with 1 N HCl for 1 hr at 100° and the hydrolyzate was subjected to Dowex ion-exchange chromatography with an HCl gradient. The radioactivity was mainly eluted in the fraction of 7-methylguanine. The radioactivities in the fractions of guanine and adenine gradually increased during administration of ^{3}H-DMN in the drinking water, as reported earlier (4). Table 1 shows that maximal methylation of nucleic acids occurred during the 1st week, while labeling was highest in the 2nd week.

Experimental period (week)	Guanine residues methylated (% of total)	
	DNA	RNA
0 - 1	0.063	0.122
0 - 2	0.058	0.097
0 - 3	0.028	0.052
0 - 4	0.017	0.029
2	0.042	0.107
3	0.021	0.034
4	0.015	0.026

Table 1 - Formation of 7-methylguanine in liver nucleic acids during administration of DMN.
Hydrolysates of nucleic acids were fractionated by Dowex 50 ion-exchange chromatography, and the extent of methylation of guanine residues was calculated from the radioactivity eluted in the 7-methylguanine fraction and the absorbance of guanine at 260 nm, assuming that tritium labeling was equally distributed between the two methyl groups of DMN and that under acid conditions E_{260} of guanine was 8000.

Experimental period (week)	7-Methylguanine	
	Half-life of decrease (day)	Calculated amount formed (% of total guanine)
2	3.5	0.092
3	2.3	0.036
4	1.8	0.023

Table 2 - Half-life of 7-methylguanine in DNA and total amount of 7-methylguanine formed per week.
The mean half-lives of decrease of 7-methylguanine and the actual quantities of 7-methylguanine formed per week were calculated from the results in Table 1.

The amount of 7-methylguanine decreased rapidly, being one-quarter as much in the 4th week at in the 1st week. Results on the amount of 7-methylguanine formed per week, suggested that turnover of 7-

methylguanine in vivo was very rapid. For example, 0.063% of the guanine residues in DNA were methylated in the 1st week but only 0.016% remained methylated in the 2nd week. The figures in Table 1 do not indicate the total amount of 7-methylguanine formed but the amounts of 7-methylguanine remaining in DNA and RNA. The actual amounts of 7-methylguanine formed per week and mean half-lives of decomposition of 7-methylguanine in DNA, calculated from the results, are listed in Table 2. The quantity of 7-methylguanine formed in the first week was not calculated as its half-life was not known, but results indicated that the capacity to produce a methylating agent from DMN decreased. Similar results were obtained by den Engelse and Emmelot (13), by comparison of the methylation of DNA before and after treatment with DMN for 6 weeks. The activity of microsomal protein to metabolize DMN also tended to decrease during administration of DMN (our unpublished results). The half-life of 7-methylguanine decreased on DMN administration being around 3.5 days in the 2nd week, 2.3 days in the 3rd week and 1.8 days in the 4th week. Margison et al. (14) reported that the half-life of methylated guanine in rat liver DNA in vivo was 3 days. Recently, Capps et al. (15) also calculated a similar value for the half-life of 7-methylguanine in DNA in regenerating liver, and found that the value was not related to the rate of cell division.

The values reported by both these groups were calculated from results obtained after a single injection of DMN. If the metabolism of nucleic acid changed greatly during continuous administration of DMN to mice, this would affect the rate of loss of 7-methylguanine

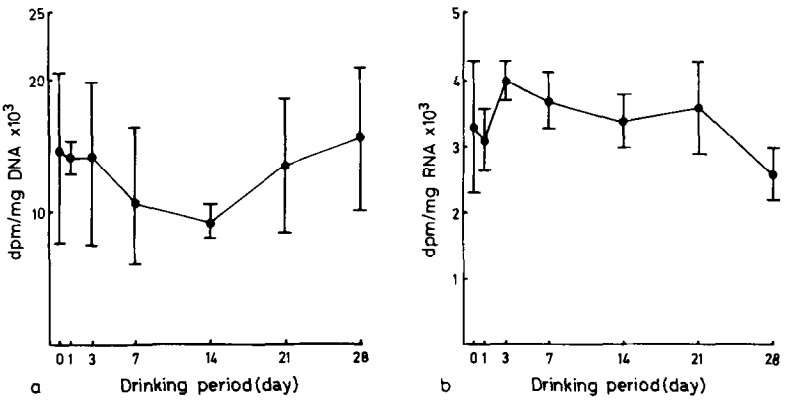

Fig. 2 - Synthesis of nucleic acids during liver carcinogenesis induced by DMN.
In corporation of radioactivity into DNA (a) and RNA (b) 1 hr after intraperitoneal injection of ^{3}H-thymidine and ^{14}C-orotic acid into mice receiving 10 ppm of DMN in the drinking water.
Points are means ± S.D. of values in 4 aminals (or 8 animals for the control).

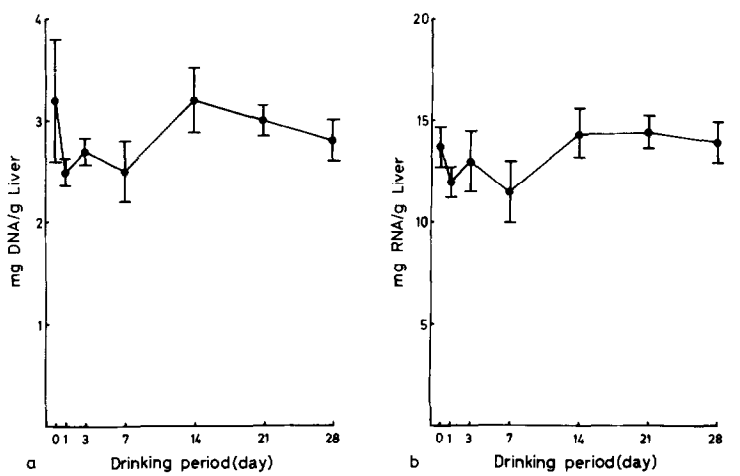

Fig. 3 - Nucleic acid content of the liver during carcinogenesis induced by DMN.
The amount of nucleic acids was determined after fractionation by the method of Schmidt-Thannhauser (10). The same animals were used as for Fig. 2.

from nucleic acid. However, as illustrated in Fig. 2, the syntheses of DNA and RNA did not alter significantly from those in control mice during the experimental period. Moreover, Fig. 3 shows that the content of nucleic acids per unit wet weight of liver tissue did not vary during the experiment. These results indicate that the decrease in the amount of 7-methylguanine was not caused by a change in metabolism of nucleic acids.

A possible cause of the rapid decrease of 7-methylguanine could be chemical depurination. However, this possibility cannot be adequately discussed until information is available on the intracellular concentration of hydrogen ion during administration of DMN in the drinking water. The mostly probable cause of the decrease is an active mechanism for excision of 7-methylguanine from DNA, as suggested by several other authors (15-17).

A rapid decrease in 7-methylguanine in RNA was also observed. In particular, the 7-methylguanine formed in the first week entirely disappeared in the 2nd week. The 7-methylguanine residues in RNA can not be removed from the polynucleotide chain by depurination under mild acid conditions, so detailed analysis is required of the mechanism of excision of methylated bases from RNA.

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