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DNA-PURINE METHYLATION IN HEPATIC CHROMATIN FOLLOWING EXPOSURE TO DIMETHYLNITROSAMINE OR METHYLNITROSOUREA

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Abstract—The investigations reported in this paper were designed to analyze the patterns of DNA-purine methylation in hepatic chromatin following *in vivo* exposure to the carcinogenic alkylating agents dimethylnitrosamine (DMN) or methylnitrosourea (MNU). Male Sprague–Dawley rats were exposed to [^{14}C]DMN (8 μmoles, 1.0 μCi per μmole per 100 g) or [^{3}H]MNU (15 μmoles, 10 μCi per μmole per 100 g) via gastric intubation. Hepatic chromatin was fractionated into portions having characteristics of template-active euchromatin (S2) and template-repressed heterochromatin (P2) by digestion with DNase II followed by $MgCl_2$ precipitation. Specific DNA purines were identified at 24 hr post-intubation using an isocratic high pressure liquid chromatographic system. A qualitatively similar pattern of 7-methylguanine, O^6-methylguanine, 1-methyladenine and 3-methyladenine alkylation was observed in DNA from total chromatin versus heterochromatin at 24 hr following exposure to either carcinogen. These assessments were made at times following carcinogen exposure which produced maximal quantitative differences in alkylation of euchromatin versus heterochromatin DNA. Similar patterns of DNA purine alkylation were observed in total chromatin and heterochromatin. These observations suggest that, once the reactive species is generated and access to chromatin DNA occurs, a similar pattern of DNA-purine alkylation is produced in different regions of the genome.

The studies presented here focus on two methylating carcinogens, methylnitrosourea (MNU) and dimethylnitrosamine (DMN). DMN has been shown to produce hepatocellular carcinomas [1–3]. Methylnitrosourea can initiate liver carcinogenesis but is not a complete carcinogen for this organ [4–7]. The formation and persistence of "pro-mutagenic" methylated purines in DNA following exposure to these *N*-nitroso compounds has been proposed as one of the critical initial events in carcinogenesis [5, 8–10]. The differences in the formation and persistence of O^6-methylguanine (O^6-MeG) in liver following MNU versus DMN exposure have been suggested as the key to the differences in their abilities to produce liver carcinomas [5].

Less evidence concerning the possible biological significance of 1- and 3-methylations of adenine is present in the literature, but the possibility of mutation by 3-alkyladenine has been suggested via AT to GC transitions [11, 12]. Removal of 3-methyladenine from DNA appears to be faster than solely spontaneous depurination and, therefore, enzymatic mechanisms were also proposed for this base [13, 14]. A 3-methyladenine DNA-glycosylase has been partially purified from human lymphocytes [15].

Tew [16] discusses the theory that the site of chemical interaction within the cell may be as important or more important than the chemical lesion. Although some studies on the intragenomic distribution of carcinogen–DNA interactions have been conducted, the question of whether or not carcinogen-induced alkylation is selective for specific regions of the genome remains to be definitively answered.

Ramanathan *et al.* [17] studied the methylation of rat liver chromatin DNA by DMN. Dnase I and micrococcal nuclease were used to selectively digest chromatin DNA. Results from their experiments [17] in which either nuclease was employed revealed that 70% of the methylated products was associated with the nuclease sensitive regions of chromatin DNA 4 hr after DMN exposure.

Galbraith and Itzhaki [18] examined the distribution of 7-methylguanine (7-MeG) in chromatin DNA isolated from hepatic tissue of rats injected with [^{14}C-*methyl*]dimethylnitrosamine (2 mg/kg). The level of 7-MeG in DNase I degradable DNA was 1.3 times that of whole DNA.

Cox [19] has observed that the specific activities of MNU-induced alkylation products were higher in DNase I sensitive regions of DNA isolated from rat brains 4 hr after *in vivo* exposure. Berkowitz and Silk [20] have also observed a non-random distribution of MNU alkylation in rat liver nuclear DNA.

We have also examined the intragenomic distribution of carcinogen-induced methylation [21]. A limited digestion with DNase II followed by selective $MgCl_2$ precipitation was used to separate chromatin regions having characteristics of template-active regions (euchromatin) from template-inactive chromatin regions (heterochromatin). At times of peak alkylation following exposure to either MNU or DMN, the euchromatin fractions were selectively

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alkylated. The rates of loss of alkylation from the heterochromatin fractions were less than that observed from euchromatin; therefore, differences between euchromatin and heterochromatin were probably a result of actual increases in the initial alkylation of euchromatin DNA rather than faster repair capacities of heterochromatin DNA. The euchromatin/heterochromatin DNA alkylation ratio for MNU-exposed rates was 2.7 and for DMN-exposed rats was 1.3 [21].

In this paper, we report on assessments of the specific methylated purines in heterochromatin and compare the pattern of purine methylation to the pattern observed in total chromatin following exposure to MNU or DMN.

EXPERIMENTAL PROCEDURES

Materials. The carcinogens *N,N*-dimethylnitrosamine and *N*-methyl-*N*-nitrosourea were obtained from the Aldrich Chemical Co. (Milwaukee, WI) and K & K Rare and Fine Chemicals (Plainview, NY) respectively. [^{14}C-*methyl*]*N,N*-Dimethylnitrosamine was purchased from the Amersham Corp. (Arlington Heights, IL). [^{3}H-*methyl*]-Methylnitrosourea was obtained from the New England Nuclear Corp. (Boston, MA). Deoxyribonuclease II, protease (Pronase P) and ribonuclease A were purchased from the Sigma Chemical Co. (St. Louis, MO). Authentic standards of O^6-MeG and 3-methyladenine (3-MeA) were gifts from Drs. G. Bochert and J. Webb (Berlin, Federal Republic of Germany), Dr. G. H. Hitchings (Wellcome Research Laboratories, NC, U.S.A.) and Dr. P. D. Lawley (Pollards Wood Research Station, Buckinghamshire, England). 7-MeG and 1-methyladenine (1-MeA) were purchased from the Sigma Chemical Co.

Animals, carcinogen administration. Male Sprague–Dawley rats (230–260 g), obtained from Spartan Farms (Haslett, MI) and King Animal Laboratories, Inc. (Madison, WI), were used for these experiments. Rats were exposed to either carcinogen, [^{14}C]DMN (8 μmoles, 1.0 μCi per μmole per 100 g) or [^{3}H]MNU (15 μmoles, 10 μCi per μmole per 100 g) via gastric intubation at 9:00 a.m. ± 1 hr. All animals were fasted for 14–16 hr prior to intubation, and food was withheld for an additional 3 hr after carcinogen administration. DMN was diluted with 9% NaCl, whereas MNU was dissolved in an 11 mM sodium citrate buffer, pH 6.0. All solutions of carcinogens were prepared in a glovebox to limit human exposure. Animals were killed at 3 and 24 hr post-intubation.

Isolation of chromatin. Details of the procedure for chromatin isolation from hepatic nuclei were presented previously by Schwartz and Goodman [22]. Hepatic nuclei are isolated and layered over a discontinuous sucrose gradient (1.3 M sucrose/1.6 M sucrose). The pellet formed after centrifugation at 112,000 *g* for 2 hr in a Beckman SW 27 rotor represents the chromatin fraction. Chromatin isolated in this manner contains 1.28(±0.09) mg DNA/g liver [21]. This agrees with the values reported in the literature [23, 24].

Chromatin fractionation. Chromatin was fractionated into putative euchromatin and heterochromatin fractions by DNase II digestion and selective $MgCl_2$ precipitation [25]. This fractionation procedure results in the separation of a euchromatin fraction (S2) which represented 10.5 ± 1.7% of the DNA recovered [26]. The heterochromatin fraction (P2) contained 74.0 ± 5.8% of the recovered DNA while the nuclease resistant fraction (P1) represented 15.4 ± 5.4% of the DNA recovered. Following fractionation, 84% of the total DNA content of unfractionated chromatin was recovered [21].

Purification of DNA for purine isolation. To identify specific methylated DNA purines, a method to isolate DNA was required that would result in a purified polymeric DNA fraction that was free of RNA and protein contamination. A modified Marmur procedure was used to isolate DNA from hepatic chromatin and chromatin fractions [26–29]. This procedure involves a sodium dodecyl sulfate incubation, RNase and protease digestion, chloroform:isoamyl alcohol extractions, phenol washes, and ethanol precipitations.

Isolation of purines. Isolated DNA was resuspended in 10 mM Tris–HCl, pH 7.9. This resuspended DNA solution consistently had an A_{280}/A_{260} equal to 0.5 to 0.6. HCl (1.0 N) was added to the supernatant fraction to obtain a final concentration of 0.1 N HCl. Purines were released from the DNA by incubating this solution at 60° for 20 min [30–32]. Under these conditions, it is known that 80% of the O^6-MeG is stable, and greater than 90% of the 7-MeG and 3-MeA residues are stable. At the end of the hydrolysis, the solution was placed on ice and centrifuged at 12,100 *g* for 10 min at 0–4°. The supernatant fraction was removed and evaporated to dryness at 35° under a stream of N_2. These samples were dissolved in 0.03 M $NH_4H_2PO_4$ (pH 4.6) at $\frac{1}{4}$ the pre-evaporation volume. This solution was then analyzed for methylated purine content by high pressure liquid chromatography as previously described [26]. This system simultaneously separates the following four methylated bases: 7-MeG, O^6-MeG, 1-MeA and 3-MeA, using an isocratic buffer (0.03 M $NH_4H_2PO_4$, pH 4.6) and a Whatman partisil 10 SCX (strong cation exchange resin) column. Fractions corresponding to the portions of the column effluent in which the specific bases elute were combined and evaporated under a stream of N_2, and the amount of radioactivity was determined using a liquid scintillation fluor prepared from toluene and Triton X-100 [33]. Quench correction was performed by internal standardization.

RESULTS

Quantitative assessments of purine methylation in specific chromatin fractions were made. 7-MeG, O^6-MeG, 1-MeA and 3-MeA contents in putative heterochromatic genome regions were compared to the content of the four methylated bases in total chromatin. Assessments were made at 24 hr following MNU or DMN intubation. The time point of 24 hr was chosen since this is the time where maximum differences between putative euchromatic and heterochromatic regions were observed in our previous studies [26].

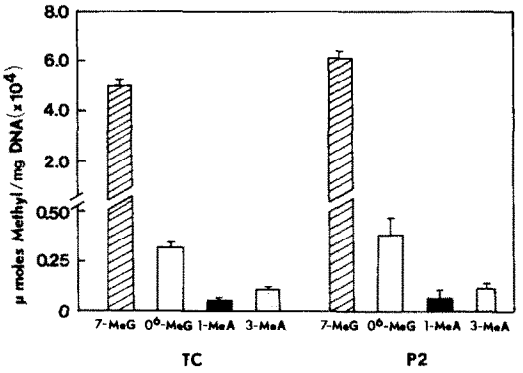

Fig. 1. Alkylation of specific methylated bases in total chromatin versus heterochromatin DNA following exposure to [^{14}C]DMN. Chromatin fractions were isolated from rats exposed to [^{14}C]DMN (8 μmoles, 1.0 μCi per μmole per 100 g) 24 hr previously. Amounts of the four methylated bases 7-MeG, O^6-MeG, 1-MeA, and 3-MeA in total chromatin and heterochromatin (P2) were assessed by HPLC. Total chromatin DNA alkylation represented 0.903(± 0.2) × 10^{-3} μmoles methyl/mg DNA and total heterochromatin alkylation equalled 1.12(±0.11) × 10^{-3} μmoles methyl/mg DNA. A recovery of 99(±2.0)% of the radioactivity put onto the HPLC column was obtained. The purinic hydrolysate represented 74(±16)% and 60(± 1.8)% of the total chromatin and heterochromatin DNA alkylation respectively. The values in this figure represent the mean ± S.E. for three independent determinations.

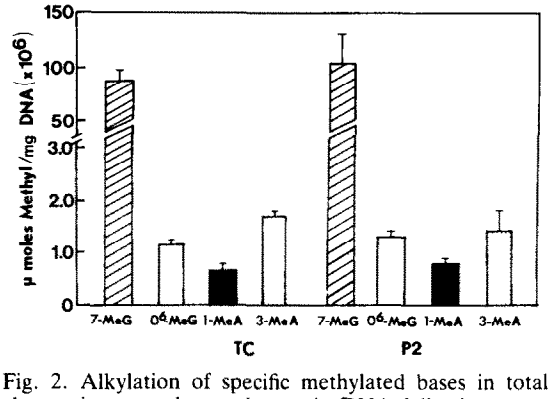

Fig. 2. Alkylation of specific methylated bases in total chromatin versus heterochromatin DNA following exposure to [^{3}H]MNU. Chromatin fractions were isolated from rats exposed to [^{3}H]MNU (15 μmoles, 10 μCi per μmole per 100 g) 24 hr previously. Amounts of the four methylated bases 7-MeG, O^6-MeG, 1-MeA and 3-MeA in total chromatin and heterochromatin (P2) were assessed by HPLC. Total chromatin DNA alkylation represented 1.30(± 0.18) × 10^{-4} μmoles methyl/mg DNA. Heterochromatin DNA alkylation equalled 1.5(±0.24) × 10^{-4} μmoles methyl/mg DNA. A recovery of 86(±2.1)% of the radioactivity put onto the HPLC column was obtained. The purinic hydrolysate represented 83(±8.8)% and 75(± 4.1)% of the total chromatin and heterochromatin DNA alkylation. These values represent the mean ± S.E. for three independent determinations.

The alkylation of specific methylated purines in total chromatin DNA versus heterochromatin DNA following exposure to [^{14}C]DMN is presented in Fig. 1. Similar patterns of purine alkylation were observed in both chromatin fractions. This is evident when the alkylation of the specific bases is expressed as a percentage of total DNA alkylation (Table 1).

Similar comparisons were made when assessing specific methylated purines in total chromatin versus heterochromatin following [^{3}H]MNU exposure. The methylation of DNA purines observed 24 hr after [^{3}H]MNU intubation is presented in Fig. 2. These data are presented as percentages of total DNA alkylation in Table 2. As observed following DMN exposure, MNU patterns of methylation for the two chromatin fractions were very similar.

The patterns of alkylation observed following [^{3}H]MNU and [^{14}C]DMN exposures are presented in Fig. 3. Data from these experiments have been expressed as ratios of the methylated bases to 7-

Table 1. Purine methylation in DNA from total chromatin and heterochromatin following exposure to [^{14}C]DMN*

Fraction	Base	μmoles Methyl (× 10^4) / mg DNA	% of total DNA alkylation
Total chromatin	7-MeG	5.06 ± 0.31	61.9 ± 13.5†
	O^6-MeG	0.32 ± 0.03	3.8 ± 0.7
	1-MeA	0.06 ± 0.01	0.8 ± 0.3
	3-MeA	0.11 ± 0.03	1.4 ± 0.6
Heterochromatin	7-MeG	6.20 ± 0.35	55.7 ± 2.1‡
	O^6-MeG	0.38 ± 0.07	3.4 ± 0.3
	1-MeA	0.07 ± 0.03	0.6 ± 0.3
	3-MeA	0.11 ± 0.03	1.0 ± 0.2

* Chromatin fractions were isolated from rats exposed to [^{14}C]DMN (8 μmoles. 1.0 μCi per μmole per 100 g) 24 hr post-intubation. Amounts of the four methylated bases 7-MeG, O^6-MeG, 1-MeA and 3-MeA in total chromatin and heterochromatin (P2) were assessed by HPLC separation and radioactive scintillation counting. A recovery of 99(±2.0)% of the radioactivity put onto the HPLC column was observed. The values in this table represent the mean ± S.E. of the data obtained from three individual determinations.

† Total chromatin DNA alkylation represented 0.903(±0.2) × 10^{-3} μmoles methyl/mg DNA. The purine hydrolysate from total chromatin represented 74(±16)% of the total chromatin DNA alkylation.

‡ Total heterochromatin DNA alkylation represented 1.12(±0.11) × 10^{-3} μmoles methyl/mg DNA. The purine hydrolysate from heterochromatin represented 60(±1.8)% of the total heterochromatin DNA alkylation.

Table 2. Purine methylation in DNA from total chromatin and heterochromatin following exposure to [^{3}H]MNU*

Fraction	Base	μmoles Methyl (× 10^6) / mg DNA	% of total DNA alkylation
Total chromatin	7-MeG	89.6 ± 10.5	69.4 ± 5.1†
	O^6-MeG	1.2 ± 0.1	0.9 ± 0.2
	1-MeA	0.7 ± 0.1	0.5 ± 0.1
	3-MeA	1.7 ± 0.1	1.3 ± 0.1
Heterochromatin	7-Meg	105.0 ± 11.8	71.2 ± 5.1‡
	O^6-MeG	1.2 ± 0.1	0.9 ± 0.1
	1-MeA	0.7 ± 0.1	0.5 ± 0.1
	3-MeA	1.4 ± 0.4	1.0 ± 0.3

* Chromatin fractions were isolated from rats exposed to [^{3}H]MNU (15 μmoles, 10 μCi per μmole per 100 g) 24 hr post-intubation. Amounts of the four methylated bases 7-MeG, O^6-MeG, 1-MeA and 3-MeA in total chromatin and heterochromatin (P2) were assessed by HPLC separation and radioactive scintillation counting. A recovery of 86(±2)% of the radioactivity put onto the HPLC column was observed. The values in this table represent the mean ± S.E. of the data obtained from three individual determinations.

† Total chromatin DNA alkylation represented 1.3(±0.18) × 10^{-4} μmoles methyl/mg DNA. The purine hydrolysate from total chromatin represented 82(±8.8)% of the total chromatin DNA alkylation.

‡ The purine hydrolysate from heterochromatin represented 75(±4)% of the total heterochromatin DNA alkylation. Total heterochromatin DNA alkylation represented 1.5(±0.24) × 10^{-4} μmoles methyl/mg DNA.

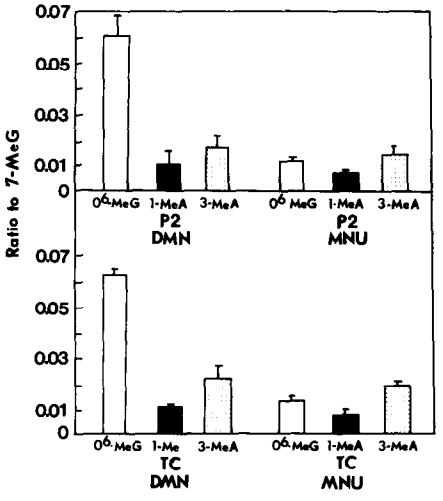

Fig. 3. Comparison of MNU- and DMN-induced patterns of purine alkylation. Chromatin fractions were isolated from the livers of rats 24 hr after exposure to [^{3}H]MNU (15 μmoles, 10 μCi per μmole per 100 g) or [^{14}C]DMN (8 μmoles, 1.0 μCi per μmole per 100 g). Assessments of 7-MeG, O^6-MeG, 1-MeA and 3-MeA content in heterochromatin (P2) and total chromatin were made. Following MNU exposure total chromatin and heterochromatin DNA alkylation equalled 1.3(±0.18) × 10^{-4} and 1.5(±0.24) × 10^{-4} μmoles methyl/mg DNA respectively. The 7-MeG content of total chromatin represented 69(±5.1)% of total chromatin DNA alkylation. The 7-MeG content of heterochromatin equalled 71(±5)% of total heterochromatin DNA alkylation. Following DMN exposure, total chromatin and heterochromatin DNA alkylation equalled 0.903(±0.2) × 10^{-3} and 1.12(±0.11) × 10^{-3} μmoles methyl/mg DNA respectively. The 7-MeG content of total chromatin represented 62(±13)% of total chromatin DNA alkylation. In heterochromatin, 7-MeG content represented 56(±2)% of total heterochromatin DNA alkylation following DMN exposure. Values represent mean ± S.E. for three rats.

MeG. Comparisons can be made between P2 and total chromatin DNA alkylation at 24 hr following DMN or MNU exposure. No statistical differences could be ascertained in the patterns of purine alkylation of total chromatin versus heterochromatin following exposure to either carcinogen.

At 24 hr following MNU exposure, the ratio of O^6-MeG/7-MeG was 0.012 for both total chromatin and heterochromatin. The 1-MeA/7-MeG ratios in total chromatin and heterochromatin were 0.0075 and 0.0070 respectively (Fig. 3). The 3-MeA/7-MeG

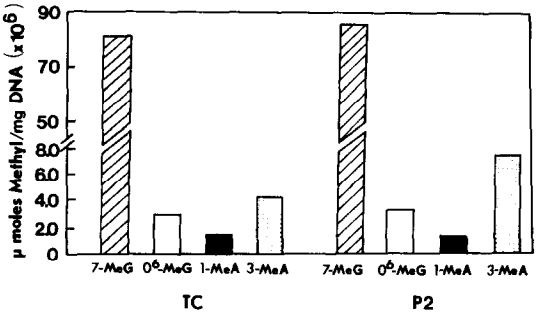

Fig. 4. Identification of methylated bases in chromatin fractions at 3 hr post-intubation of [^{3}H]MNU. Chromatin fractions were isolated from rats exposed to [^{3}H]MNU (15 μmoles, 10 μCi per μmole per 100 g) 3 hr previously. The 7-MeG, O^6-MeG, 1-MeA and 3-MeA content in total chromatin and putative heterochromatin (P2) was assessed by HPLC. Total chromatin DNA alkylation represented 12.5 × 10^{-4} μmoles methyl/mg DNA. Total heterochromatin DNA alkylation represented 1.18 × 10^{-4} μmoles methyl/mg DNA. A recovery of 80% of the radioactivity put onto the HPLC column was obtained. The purine hydrolysate represented 86 and 96% of the total chromatin and heterochromatin DNA alkylation respectively. The values in this figure represent samples from one rat.

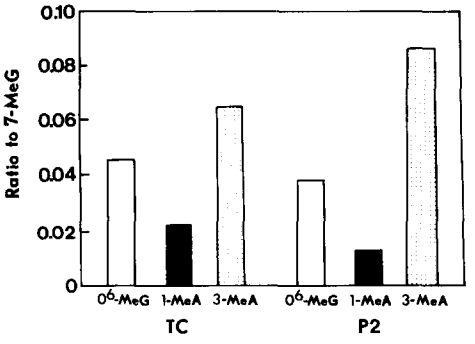

Fig. 5. Analysis of methylated bases in chromatin fraction at 3 hr after [^{3}H]MNU exposure. Chromatin fractions were isolated from rats exposed to [^{3}H]MNU (15 μmoles, 10 μCi per μmole per 100 g) 3 hr previously. Amounts of the four methylated bases 7-MeG, O^6-MeG, 1-MeA and 3-MeA in total chromatin and putative heterochromatin (P2) were assessed by HPLC. Total chromatin DNA alkylation represented 1.25×10^{-4} μmoles methyl/mg DNA. Total heterochromatin DNA alkylation represented 1.18×10^{-4} μmoles methyl/m gDNA. A recovery of 80% of the radioactivity put onto the HPLC column was obtained. The purine hydrolysate represented 86 and 96% of the total chromatin and heterochromatin DNA alkylation respectively. 7-MeG content represent 66 and 72% of the total DNA alkylation of total chromatin and heterochromatin respectively. The values in this figure represent samples from one rat.

ratios in total chromatin and heterochromatin at 24 hr following MNU exposure equalled 0.019 and 0.014 respectively.

At 24 hr following DMN exposure, the ratio of 1-MeA/7-MeG in total chromatin was 0.11 and was equal to the ratio observed in heterochromatin. The O^6-MeG/7-MeG ratios in total chromatin and heterochromatin following DMN exposure were 0.063 and 0.061 respectively. The 3-MeA/7-MeG ratios were 0.022 and 0.018, respectively, for total chromatin and heterochromatin at 24 hr following DMN exposure (Fig. 3).

Specific methylated purines have also been assessed at 3 hr post-MNU exposure in total chromatin versus heterochromatin (Fig. 4). Similar patterns of alkylation were observed in total chromatin and heterochromatin when the relative amounts of the various bases were expressed as a ratio to 7-MeG (Fig. 5).

DISCUSSION

The objectives of these investigations were to examine both quantitatively and qualitatively the methylation of hepatic DNA isolated from specific chromatin fractions following exposure to MNU or DMN.

When the specific alkylated purines were examined in total chromatin versus the putative heterochromatin fraction at 24 hr post-carcinogen intubation, MNU appeared to induce qualitatively similar patterns of 7-MeG, O^6-MeG, 1-MeA and 3-MeA alkylation in these two chromatin fractions. The patterns of alkylation of specific DNA purines also appeared to be qualitatively similar when putative heterochromatin was compared to total chromatin 24 hr following DMN exposure. A limited analysis of specific patterns of purine alkylation in total chromatin versus heterochromatin was made at 3 hr post-MNU exposure; in this instance, as well, similar patterns of purine alkylation were observed in total chromatin and the heterochromatin fraction.

Observations shown in Figs. 1–3 and Tables 1 and 2 were made at 24 hr post-carcinogen intubation. Previous studies [21] have shown that, at this time following carcinogen exposure, maximum differences between euchromatin and heterochromatin DNA alkylation exist. Direct comparisons between the specific patterns of alkylation of euchromatin and heterochromatin were not feasible for these initial studies due to the small amount of DNA in the euchromatin fraction (10% of total DNA content) and the limitation this imposes due to the high cost of the radioactively labeled carcinogens. Assessment of the alkylation patterns in heterochromatin was possible since this fraction represented 75% of total chromatin DNA and approximately one-half of the total DNA alkylation following MNU or DMN exposure. Comparisons between the patterns of alkylation observed in heterochromatin and total chromatin would have the potential to reveal differences in alkylation patterns of DNA. However, subtle differences in the patterns of purine alkylation of smaller chromatin regions would not be detectable in these studies. Therefore, our findings that qualitatively similar patterns of DNA alkylation occur in heterochromatin and total chromatin following DMN or MNU exposure do not rule out the possibility that differences in patterns of alkylation of various genome regions may exist.

Our observations that methylation of euchromatic DNA occurred to a greater extent than alkylation of heterochromatin DNA is compatible with our observation of qualitatively similar patterns of specific DNA-purine methylation in total chromatin as compared to heterochromatin regions [21]. It is possible that once the reactive species is generated and access to chromatin DNA occurs, a similar pattern of DNA-purine alkylation is produced.

Following DMN exposure, we observed higher O^6-MeG/7-MeG ratios in both total chromatin and heterochromatin than the O^6-MeG/7-MeG ratios observed following MNU exposure (Fig. 3). DMN is a complete carcinogen in liver and, since the presence of O^6-MeG in various organs has been correlated with organ susceptibility to DMN-induced carcinogenesis, these findings are not surprising [1, 5]. Dose-dependent differences in the removal of O^6-MeG have been observed [34, 35]. The dose of DMN used in our study, 5.9 mg/kg, is in a dosage region where removal of O^6-MeG was less efficient. Since the MNU dose used in our studies produced O^6-MeG levels which fell in the range of more efficient removal rates, this might account for the differences in the relative amounts of O^6-MeG/7-MeG ratios that were observed following MNU versus DMN exposure.

There appears to be some degree of heterogeneity with regard to the distribution of cytochrome P-450 mono-oxygenase activity among liver cells [36]. In view of the fact that DMN requires metabolic acti-

vation while MNU does not, these chemicals could methylate different cells within the hepatic lobule. This might account, in part, for some of the differences in alkylation observation.

Selective damage of euchromatin could increase the possibility, and probability, of an alteration of phenotype following exposure to alkylating agents. This might occur directly through an alteration of transcription. DNA damage in heterochromatin regions would not be expected to be expressed. In addition, an alteration of phenotype could occur as a result of a mutation in the transcriptionally active region of the genome. DNA damage could lead to a mutation as a result of base mispairing during DNA replication or possibly through an error-prone repair process.

Although DNA damage in heterochromatin would not be expected to be expressed, the biological consequences of such alkylation could be significant. Carcinogen-induced modification of heterochromatin or heterochromatin could be manifested if derepression of previously quiescent regions of the genome occurred through an alteration of gene expression (possibly during the promotion stages of carcinogenesis). Thus, DNA damage or mutations in heterochromatin, as well as damage in euchromatin, could increase the possibility and probability of phenotypic alterations.

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