Carcinogenicity of Single Doses of N-Nitroso-N-methylurea and N-Nitroso-N-ethyurea in Syrian Golden Hamsters and the Persistence of Alkylated Purines in the DNA of Various Tissues

Alexei J. Likhachev, Mikhail N. Ivanov, Henriette Brésil, Ghyslaine Planche-Martel, Ruggero Montesano, and Geoffrey P. Margison


ABSTRACT

The carcinogenicity of N-nitroso-N-methylurea (NMU) and N-nitroso-N-ethyurea (NEU) has been determined in adult male Syrian golden hamsters following a single i.p. injection or two-thirds of the acute 50% lethal dose, or 30 and 60 mg/kg, respectively. The principal site of action of these agents was the forestomach, squamous cell papillomas of this organ developing in 53 and 61% of the animals receiving the higher doses of NMU and N-nitroso-N-ethyurea, respectively. NMU also induced a low incidence of liver tumors (17%). Very few tumors were seen at other sites.

The formation and removal of alkylated purines in DNA was measured in various tissues up to 50 hr after administration of [14C]NMU. Methylation products were detected in all tissues examined, the level in liver being somewhat higher than in other tissues. The removal of 7-methylguanine and 3-methyladenine from DNA occurred at approximately similar rates in all tissues examined, indicating no substantial differences in N-glycosylase activities. Removal of the promutagenic DNA lesion O6-methylguanine varied considerably from tissue to tissue; very little occurred in brain or kidney, while up to 36 and 32% was lost from DNA of intestine and testes, respectively. In the liver, there were relatively small changes in O6-methylguanine levels up to 24 hr, but by 50 hr, 38% had been removed. The persistence of O6-methylguanine relative to 7-methylguanine was highest in the DNA of the brain and intestine and lowest in that of the liver. These results indicate that in this experimental system, the formation and persistence of O6-methylguanine in DNA is insufficient alone to account for the organotropic effect of NMU.

INTRODUCTION

The Syrian golden hamster, like many other animal species, is susceptible to tumor induction by a variety of N-nitroso compounds (15). The tissues in which tumors arise depend on the carcinogen: N-nitroso-dimethylamine, given in single or multiple doses, induces liver tumors and, to a lesser extent, tumors of the nasal cavity; the principal target site of N-nitroso-N-ethyurea is the upper respiratory tract (nasal cavity, trachea, larynx, and bronchi), although tumors of the forestomach, bronchi, liver, and genital system are also produced. Hamsters given NMU i.v., i.g., or i.p. develop tumors of the small and large intestine as well as odontogenic tumors and epidermoid carcinomas (11).

Relatively few studies have examined the carcinogenic effect of NEU in Syrian hamsters. In animals exposed to NEU transplacentally, additional skin application starting at 6 weeks of age considerably increased the incidence of melanomas in comparison with animals exposed to NEU during embryogenesis only or postnatally only (25). Simultaneous p.o. administration of NEU precursors (ethyurea and sodium nitrite) to adult hamsters resulted in the development of a wide spectrum of tumors (in spleen, liver, forestomach, and genital system, and neurogenic tumors) (11).

N-Nitroso compounds are known to act only after their conversion by chemical decomposition or metabolic activation in the body into alkylating agents, which react with various nucleophilic sites in cellular macromolecules. Of the reactions, O6 alkylation of guanine in DNA has been studied most extensively in recent years, since the adduct causes incorporation of noncomplementary nucleotides during polynucleotide synthesis in vitro (1, 7) and may therefore be the molecular basis of the mutagenic and carcinogenic effects of alkylating agents (14, 19). Other DNA modification such as O6-alkylthymine have also been implicated in mutagenesis and carcinogenesis for the same reason (26).

Several studies have shown that the ability to repair O6-alkylguanine can be a major determinant in a tissue susceptibility to tumor induction and have thus provided considerable evidence to support the critical role of O6-alkylguanine in the carcinogenicity of alkylating agents. In order to extend this approach to other systems, we have, in the present experiments, examined the carcinogenic effects of a single i.p. dose of NMU or NEU in Syrian hamsters and in addition, measured the formation and persistence of methylated purines in DNA of different organs after a single i.p. administration of radiolabeled NMU.

MATERIALS AND METHODS

Chemicals. [14C]NMU (13.5 mCi/mmol) was obtained from Amer sham International, Amersham, United Kingdom; unlabeled NMU from Ash Stevens, Inc., Detroit, Mich.; and unlabeled NEU from Schuchardt,
Munich, Federal Republic of Germany. Sephadex G-10 was obtained from Pharmacia, Uppsala, Sweden. Other biochemicals were products of the Sigma Chemical Co., St Louis, Mo., except for O-methylguanine, which was synthesized by a published procedure (28).

Alkylation Experiments. Experiments to determine the methylation of DNA by NMU were carried out at the International Agency for Research on Cancer, Lyon, France. Male Syrian golden hamsters (90 to 110 g) were obtained from the Animalerie des Essertines, Rochetaillée, France. They were allowed food pellets and water ad libitum and were housed in a room illuminated for 12 hr/day (8 a.m. to 8 p.m.).

Three groups consisting of 4 hamsters each were given [14C]NMU (30 mg/kg body weight i.p.; 8.79 mCi/mmol) dissolved in 0.9% sodium chloride/3 mM sodium citrate, pH 6.0, and killed by exsanguination 5, 24, or 50 hr later. Liver, kidney, lung, forestomach, brain, testis, and small intestine were removed, frozen in liquid nitrogen, and stored at −30°C. DNA was isolated by extraction with phenol, as described previously (18), and stored at −30°C. The DNA was hydrolyzed to release free purine bases by heating at 70°C for 30 min in 0.1 N hydrochloric acid.

After addition of unlabeled markers of methylated purines (3-methyladenine, 7-methylguanine, and O-methylguanine), the sample was adjusted to pH 2.9 and then chromatographed on a Sephadex G-10 column (85 × 1.0 cm) eluted with 0.05 M ammonium formate, pH 6.75, containing 0.02% (w/v) sodium azide. The radioactivity present in the fractions corresponding to methylated bases was determined; the amounts of guanine and adenine in the sample were calculated from the absorbance of the fractions containing these bases; and the amounts of methylated bases were then calculated as μmol/mol of parent base. Further details of this analytical procedure were given in recent publications (18, 22).

Carcinogenicity Experiments. Experiments on the carcinogenic effects of NMU and NEU were carried out at the N. N. Petrov Research Institute of Oncology, Leningrad, U. S. S. R. Male Syrian golden hamsters (90 to 110 g) were obtained from the Rappolovo Animal Farm of the U. S. S. R. Academy of Medical Sciences, Leningrad, U. S. S. R. They were housed in stainless steel cages, 4 to 6 animals to a cage, and fed natural foods (by standards used in the U. S. S. R. for laboratory animals of this species). One kg of this diet contains 550 g of pearl barley porridge or millet, 83 g of mixed feed, 250 g of bread, and 117 g of meat; minced vegetables (carrots and cabbage) were added. Animals received food and drinking water ad libitum. In the spring and summer, the room was illuminated naturally; in the autumn and winter, the room was illuminated for 12 hr/day (8 a.m. to 8 p.m.).

For injection, NMU and NEU were dissolved in 0.9% sodium chloride solution, pH 6.0, and used within 1 hr. The 50% lethal doses of NMU and NEU administered i.p. were determined according to the method of Weil (29); animals were observed for 7 days. The values obtained were 110 mg/kg body weight and 411 mg/kg body weight for NMU and NEU, respectively. The principal causes of death appeared to be extensive liver cell necrosis, circumscribed necroses in the kidneys, and hemorrhagic lesions of the intestine.

Hamsters were divided into 5 groups of 32 to 47 animals each. Animals of Groups 1 and 3 were given a single i.p. injection of NMU or NEU at the doses 30 and 60 mg/kg body weight, respectively. Hamsters of Groups 2 and 4 were given a single injection of NMU or NEU at doses which represented two-thirds of the 50% lethal dose. Animals of Group 5 were given an i.p. injection of the vehicle (0.9% sodium chloride/3 mM sodium citrate, pH 6.0). The animals of Groups 1 to 4 were observed until they died. In the control group, all hamsters survived for the period of the experiment, apart from 5 animals which were lost due to cannibalism between 149 and 160 days. The surviving animals in the control group were killed between 330 and 370 days, when most of the treated animals had died. Complete autopsies were performed on all animals which could be used in the study (those not cannibalized or autolyzed), and all organs were fixed in 10% buffered formalin. Tumors and areas in which tumor growth was suspected were excised, and material was embedded in paraffin-celloidin; sections were stained with hematoxylin and eosin.

Statistics. The Fisher exact test (5) was used to compare tumor incidence rates in the treated groups with that in control groups, and the Cochran-Armitage test (2) for positive trend was performed using rates for the control, low dosage, and high-dosage groups for both compounds. In these experiments, the single control group is used for each of the experimental groups. Therefore, in order to maintain an overall significance level of 0.05, statistical significance of a single test is taken as 0.01.

Half-lives were calculated as 

\[
\frac{\log 2}{b}
\]

where \( b \) is the slope estimate in the regression curve, and \( y = a \cdot e^{-t} \) is estimated by least square regression after logarithmic transformation. In cases in which the linear correlation based on the 3 observations was below 0.95, the estimates were not considered as reliable, and the half-lives are not given.

RESULTS

Methylation of DNA by NMU and Persistence of Methylated Purines. The amounts of 7-methylguanine, O-methylguanine, and 3-methyladenine in the DNA of various hamster tissues after injection of [14C]NMU, 30 mg/kg, are shown in Table 1. At 5 hr, the highest level of 7-methylguanine was in the DNA of the liver, followed by intestine, kidney, lung, and brain. By 50 hr, the levels had decreased in all tissues at similar rates; but while the calculated half-lives for the loss (Table 1) were slightly shorter in intestine and testes than in the other tissues, these differences may not have been significant.

The persistence of other methylation products can be expressed relative to 7-methylguanine (see "Discussion"). Of the tissues examined, the liver had the lowest initial and slowest increase in the O-methylguanine/7-methylguanine ratio (Chart 1). The ratios in brain, intestine, and lung were initially high, and they continued to increase markedly during the period of the experiment. Testis and kidney occupied intermediate positions. In the case of the forestomach, because of a poor yield of DNA, it was not possible to determine the amounts of adenine and guanine and hence, the absolute amounts of methylated purines. In addition, radioactivity "tailing" from the pyrimidine oligonucleotide peak prevented determination of 3-methyladenine. However, the initial (5-hr) value and the increase in the O-methylguanine/7-methylguanine ratio were similar to those of testis, lung, and kidney (Chart 1).

Furthermore, in another experiment in which only liver, lung, kidney, and forestomach DNAs were examined at 5 and 24 hr after NMU injection, the O-methylguanine/7-methylguanine ratios were very close to those obtained in the present experiment.4

In comparison to the O-methylguanine/7-methylguanine ratios, the 3-methyladenine/7-methylguanine ratios showed only slight tissue differences both at the initial values and in the rate and extent of decrease, with the possible exception of the intestine, which had slightly elevated values at all times (Chart 1). The changes in these ratios suggest that the removal of 3-methyladenine and 7-methylguanine occurred at similar rates in all tissues and that only the liver had some capacity to remove O-methylguanine but that this was itself limited.

Carcinogenesis in Hamsters after a Single Dose of NMU or NEU. Chart 2 shows the survival curves of the 4 treated groups; survival in Group 1 animals was better than that in the other 3 groups.

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tumor-bearing animals. The first tumor, a papilloma of the
not given.
been determined (see "Results").
forestomach, was found in a hamster of Group 2 at 125 days
after exposure. The majority of the animals in each group
was the site affected in 12 to 61% of animals (Table 2). No
64% of survivors, and the principal site of tumor production,
control group, are given in Table 2. The Cochran-Armitage test
incidence rates of certain tumors in a treated group with the
Lung

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time and half-life (hr)$^{b}$</th>
<th>3-Methyladenine</th>
<th>7-Methylguanine</th>
<th>O6-Methylguanine</th>
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</thead>
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<tr>
<td>Liver</td>
<td>5</td>
<td>17.0</td>
<td>504</td>
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<td></td>
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<td>Intestine</td>
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<td>69</td>
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<tr>
<td>Kidney</td>
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<td>Half-life</td>
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</table>

$^{a}$ For the forestomach, the absolute amounts of methylated purines have not been determined (see "Results").
$^{b}$ In cases in which the linear correlation was below 0.95, the half-lives are not given.

The pattern of survival in all 5 experimental groups does not
bias the comparison of the tumor incidence by the rates of
tumor-bearing animals. The first tumor, a papilloma of the
forestomach, was found in a hamster of Group 2 at 125 days
after exposure. The majority of the animals in each group
survived for longer than this period. Tumors developed in 16 to
64% of survivors, and the principal site of tumor production,
independent of the carcinogen used, was the forestomach; this
was the site affected in 12 to 61% of animals (Table 2). No
tumors were found in the animals of the control group.

The p values of the Fisher exact test (5), comparing the incidence rates of certain tumors in a treated group with the
control group, are given in Table 2. The Cochran-Armitage test
(2) showed, for both NMU and NEU, highly significant ($p <
0.0001$) positive trends of the incidence, with increasing dose
both for the total tumor yield and for tumors of the forestomach.
For liver tumors, the $p$ value of the trend statistics was 0.002
for NMU and not significant for NEU.

The findings clearly demonstrate a dose-dependent induction
of tumors of the forestomach by both compounds. In almost all cases, these tumors were squamous cell papillomas.
A squamous cell carcinoma was found in one hamster of Group
4. In addition, some hamsters developed cystocholangiomas.

Tumors of the respiratory tract were found in only 3 animals.
Two developed in animals of Groups 1 and 4 in the lung tissue,
and the other developed in a hamster of Group 2 in the lumen
of the trachea. These tumors were classified as alveolar ade
nomas. One animal of Group 1 developed a basal cell papilloma,
and another developed a squamous cell papilloma of the
esophagus. A third hamster of this group developed a carcinoma
in the back paw. A reticulosarcoma was found in one animal
of Group 2, and a reticuloleukemia was found in another animal
of the same group. In one hamster from Group 3, a poorly
differentiated adenocarcinoma of the kidney was detected, and
polymorphous cell sarcoma of abdominal cavity was found in
one animal from Group 4.

DISCUSSION

The mechanism by which alkylating agents produce tumors in
experimental animals has been the subject of extensive
investigation. Considerable progress has been made over the
last decade, since the suggestion (14) that one particular DNA
alkylation product, O6-alkyguanine, might be responsible for
the mutational event which is thought to initiate malignant
transformation. In several experimental carcinogenesis sys
tems, the principal target organ appears to be that in which the
perfusion of O6-alkyguanine in DNA is greatest. While alkyl
ation also takes place in nontarget organs, they may be pro
ected from initiation by repair processes which remove this
"promutagenic" lesion from DNA before replication produces a
permanent heritable change in the base sequence (8, 13, 14,
17, 23, 24). In the present study, we examined whether a
similar correlation exists in Syrian hamsters given a single dose
of the direct-acting alkylnitrosourea NMU.

Administration of NMU to hamsters resulted in DNA methyl
ation in all tissues examined. Since NMU decomposition and
the formation of methylated species takes place spontaneously
(11) and the compound is detectable in the blood for only 15
min after administration (27), the nonuniform initial levels of
DNA methylation as measured by the formation of 7-methyl
guanine in DNA is greater. While alkylation also takes place in
target organs, they may be protected from initiation by repair processes which remove this
"promutagenic" lesion from DNA before replication produces a
permanent heritable change in the base sequence (8, 13, 14,
17, 23, 24). In the present study, we examined whether a
similar correlation exists in Syrian hamsters given a single dose
of the direct-acting alkylnitrosourea NMU.

The loss of 7-methylguanine from DNA occurred with a half
life of between about 26 hr in the intestine and testis to about
37 hr in lung (Table 1) which, by comparison with the in
vitro rate of depurination (16, 17), is indicative of an active excision
process. Enzymic removal of 7-methylguanine from DNA has
previously been implicated in Chinese (21) and Syrian (17)
hamsters after administration of DMN, and recently 7-methyl
guanine N-glycosylase activity has been demonstrated in Syr
ian hamster liver extracts (20); our results indicate that this
activity is not confined to the liver. Tissue differences in DNA
turnover will contribute to the range of 7-methylguanine half

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**Table 1**

Methylated purines in the DNA of various Syrian hamster tissues up to 50 hr after administration of [14C]NMU (30 mg/kg; 8.79 mCi/mmol) and half-lives for their loss rates.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time and half-time (hr)$^{b}$</th>
<th>3-Methyladenine</th>
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**Chart 1.** Methylated purine ratios in DNA of various Syrian hamster tissues after administration of [14C]NMU (30 mg/kg; 8.79 mCi/mmol). ○, O6-methylguanine/7-methylguanine; ●, 3-methyladenine/7-methylguanine.
lives; hence, those for intestine and testes which contain proliferating and end cell populations are shorter than those of other tissues. The present data thus also suggest that there are no substantial tissue differences in 7-methylguanine N-glycosylase activities.

The loss of 3-methyladenine from DNA also occurred at similar rates in all tissues, the half-lives being less than that for the loss of this base in vitro (17) and therefore also indicating active removal. The similarity of the 3-methyladenine/7-methylguanine ratios is consistent with the suggestion that hamster tissues like those of the rat (19) vary little in their glycosylase activities. Consequently, the persistence of O<sub>6</sub>-methylguanine can be reasonably expressed relative to that for 7-methylguanine. These O<sub>6</sub>-methylguanine/7-methylguanine ratios increased in all tissues but to a lesser extent in liver than in all other organs, again indicating that the hepatic O<sub>6</sub>-methylguanine repair system was the most active, while those in brain, intestine, and forestomach were least active.

The principal target tissue for the carcinogenic effect of NMU was the forestomach, and tumors developed in other organs only at very low incidences. During the period of investigation, the forestomach had a very limited capacity to remove O<sub>6</sub>-methylguanine from DNA after the dose of NMU, but this was also true of the kidney, brain, and intestine. Since these latter tissues are not major targets for tumor production by NMU, this indicates that the persistence of O<sub>6</sub>-methylguanine in DNA cannot be considered in isolation as the critical determinant in carcinogenesis. Because DNA synthesis also appears to be an essential factor in the initiation of malignant transformation (6), the frequency of cell division in the target cell population and the effect of tumor-promoting agents which might be ingested after initiation should also be taken into consideration.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals at start</th>
<th>No. of animals completing study</th>
<th>Total</th>
<th>No. of animals with each tumor type</th>
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<td>1</td>
<td>NMU (30 mg/kg body wt)</td>
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<td>30</td>
<td>9 (30)&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>NMU (71 mg/kg body wt)</td>
<td>32</td>
<td>30</td>
<td>18 (60)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>NEU (60 mg/kg body wt)</td>
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<td>4 (16)&lt;sup&gt;n&lt;/sup&gt;</td>
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<tr>
<td>4</td>
<td>NEU (274 mg/kg body wt)</td>
<td>42</td>
<td>28</td>
<td>18 (64)&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Control (vehicle only)</td>
<td>47</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.
* <sup>a</sup> p = 0.0001.
* <sup>b</sup> p = 0.0006.
* <sup>c</sup> Not significant; p > 0.05.
* <sup>d</sup> Basal cell papilloma of esophagus, squamous cell papilloma of esophagus, and sarcoma of back paw.
* <sup>e</sup> p < 0.0001.
* <sup>f</sup> p = 0.0072.
* <sup>g</sup> Reticulosarcoma of abdominal cavity and reticuloleukemia.
* <sup>i</sup> Poorly differentiated adenocarcinoma of kidney.
* <sup>n</sup> Polymorphous cell sarcoma of abdominal cavity.

Chart 2. Survival of Syrian hamsters after administration of NMU (O, 30 mg/kg body weight; —, 71 mg/kg body weight) or NEU (—, 60 mg/kg body weight; ——, 274 mg/kg body weight).

Tumor incidence in Syrian golden hamsters exposed i.p. to NMU or NEU

Animals with tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals completing study</th>
<th>Total</th>
<th>Foregut</th>
<th>Liver</th>
<th>Lung, trachea</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>9 (30)&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7 (23)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 (7)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1</td>
<td>3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>18 (60)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16 (53)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5 (17)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1</td>
<td>2&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>4 (16)&lt;sup&gt;n&lt;/sup&gt;</td>
<td>3 (12)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1</td>
<td>1&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>18 (64)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>17 (61)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1</td>
<td>1&lt;sup&gt;i&lt;/sup&gt;</td>
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<tr>
<td>5</td>
<td>47</td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses, percentage.
<sup>b</sup> p = 0.0001.
<sup>c</sup> p = 0.0006.
<sup>d</sup> Not significant; p > 0.05.
<sup>e</sup> Basal cell papilloma of esophagus, squamous cell papilloma of esophagus, and sarcoma of back paw.
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<sup>i</sup> Reticulosarcoma of abdominal cavity and reticuloleukemia.
<sup>n</sup> Poorly differentiated adenocarcinoma of kidney.
Carcinogenesis and DNA Alkylation in Hamsters

Of all tissues studied, the concentration of O\textsuperscript{2}-methylguanine over the period of observation was most stable in brain DNA. This was also the case in the experiment, where hamsters were exposed to a single dose of NEU.\textsuperscript{8} These results are similar to those from experiments with NNU and NEU in rats (8, 13) in which O\textsuperscript{2}-alkylguanine persisted to the greatest extent in the DNA of brain. However, in rats, the central nervous system is the principal target organ, and no such tumors were seen in any of the Syrian hamsters exposed to NEU or NNU. In this context, the results for Syrian hamsters are similar to those for mice, which also have a low capacity to repair O\textsuperscript{2}-alkylguanine but are not susceptible to the induction of brain tumors (12).

Cystocholangiomas developed in the livers of some animals. However, while O\textsuperscript{6}alkylguanine in DNA may be an essential factor in the carcinogenic effects of alkylating agents, the tissue specificity of tumor production is not necessarily determined exclusively by the formation and/or persistence of this lesion in tissue DNA. Carcinogenesis is probably initiated as a consequence of a complex interaction between the alkylation, repair, and replication of DNA in those cell types within a tissue which eventually give rise to tumors.

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REFERENCES

8. Goth, R., and Rajewsky, M. F. Persistence of O\textsuperscript{6}-ethylguanine in rat brain DNA. Correlation with nervous system-specific carcinogenesis by ethylnitro-
Carcinogenicity of Single Doses of N-Nitroso-N-methylurea and N-Nitroso-N-ethylurea in Syrian Golden Hamsters and the Persistence of Alkylated Purines in the DNA of Various Tissues

Alexei J. Likhachev, Mikhail N. Ivanov, Henriette Brésil, et al.


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