Journal of Supramolecular Structure
and Cellular Biochemistry 17:259–273 (1981)
Mechanisms of Chemical Carcinogenesis 183–197

Alkylation of DNA and Tissue Specificity in Nitrosamine Carcinogenesis

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A peculiarity of nitrosamines is the high degree of cell and organ specificity in inducing tumors. There is substantial evidence that the initiation of the carcinogenesis process by carcinogens of this group is linked to the metabolic competence of the target tissue or cell to convert these carcinogens into mutagenic metabolites and to the binding of those metabolites to cellular DNA. Alkylation occurs in the DNA at the N-1, N-3, and N-7 positions of adenine; the N-3, N-7, and O^6 of guanine; the N-3, and O^2 of cytosine; and the N-3, O^4, and O^2 of thymine; and the phosphate groups. The initial proportion of each DNA adduct depends upon the alkylating agent used. The various DNA adducts are lost to a variable extent from DNA in vivo by spontaneous release of bases and/or by specific DNA repair processes. Studies conducted in vitro and in vivo indicate that alkylation at the oxygen atoms of DNA bases is more critical than alkylation at other positions in the mutagenesis and carcinogenesis induced by N-nitroso compounds. In particular, tissues in which tumors occur more frequently after a pulse dose of nitrosamine are those in which O^6-alkylguanine persists longest in DNA, presumably resulting in an increased probability that a miscoding event (mutation) will take place during DNA synthesis. The more rapid removal of O^6-methylguanine from the DNA of liver (as compared with extrahepatic tissues) of rats has been associated with the absence of tumor production in this organ by a single dose of dimethylnitrosamine; however, a significant incidence of liver tumors is observed if the same dose is given 24 hr after partial hepatectomy, and tumors are induced by such a dose of dimethylnitrosamine in the liver of hamsters, which has a low capacity to remove O^6-methylguanine from its DNA. These data also indicate that the rate of disappearance of 7-methylguanine from the liver or extrahepatic tissues is independent of the dose of dimethylnitrosamine; whereas O^6-methylguanine is lost from DNA more rapidly after a low dose of this nitrosamine. It has been shown that in liver the removal of O^6-methylguanine, but not of other DNA adducts, from DNA can be affected by pretreating the animals with N-nitroso compounds. The modulation of DNA repair processes observed after a single dose and after chronic treatment with nitrosamines is discussed in relation to the tissue-specific carcinogenic effect of this group of carcinogens.

Key words: DNA alkylation, nitrosamines, carcinogenesis, O^6-methylguanine, DNA repair

Received June 15, 1981; accepted July 10, 1981.

0275-3723/81/1703-0259$04.50

Within the limits of the present knowledge of carcinogenesis, the process leading to the appearance of neoplasms has been interpreted as a sequence of successive events. The two most widely studied are "initiation" and "promotion" [1,2]. Of the various classes of chemical carcinogen, the nitrosamines have probably contributed to a greater extent than most to the understanding of the process of initiation of carcinogenicity. These carcinogens belong to the chemical class of N-nitroso compounds, comprising the N-nitrosamines and the N-nitrosamides. A major difference between the two groups is that the nitrosamides (eg, methylnitrosourea, MNU) are unstable at physiological pH and decompose nonenzymatically to reactive intermediates; whereas the nitrosamines (eg, dimethylnitrosamine, DMN) are chemically stable under physiological conditions, and their adverse biological effect is mediated through the formation of reactive metabolites after enzymatic conversion, mainly by microsomal mixed-function oxidases. The N-nitroso compounds have been shown to be toxic [3], teratogenic [4], mutagenic (see [5]) and carcinogenic ([3,6] see [7]) in various animal species. In addition, the carcinogenic effect of these agents shows a high degree of species, tissue, and cell specificity, and many are carcinogenic when administered in a single dose. For instance DMN or MNU are no longer detected in the blood of rats a few hours or minutes after the administration of a dose that results in the appearance of kidney tumors many months later [8]; similar observations have been made with ethylating agents such as diethylnitrosamine and ethylnitrosourea [9]. This implies that the molecular or cellular changes that eventually lead to tumor production must occur during the short period after administration of the carcinogen.

Various experimental systems have been developed which permit examination of the relevance of the cellular or biochemical changes induced by N-nitroso compounds to their capacity to induce tumors in a specific animal species, tissue and/or cell. The tissue- or cell-specific carcinogenesis of nitrosamines appears to be governed by six major determinants: systemic distribution of the carcinogens, the metabolic capacity of the target tissue or cell, the specificity and extent of DNA damage, the efficiency of DNA repair processes, the accuracy of DNA polymerases, and cell turnover rate. For a greater insight into the roles played by tissue distribution and metabolism in the carcinogenicity of N-nitroso compounds, the reader is referred to several recent publications [7,10–13]. It has been shown that metabolic activation (in the case of nitrosamines) or chemical breakdown (in the case of nitrosamides) into reactive mutagenic metabolites is a necessary, although not sufficient, condition for the carcinogenic action of this group of compounds. This article reviews the evidence that the probability that an organ will develop a tumor after exposure to N-nitroso compounds is the result of the interplay between the formation and persistence of specific DNA alkylation products, the efficiency of the various processes that deal with repair of those DNA lesions, and DNA replication.

SITES OF ALKYLATION IN DNA

Some 12 sites in DNA have been shown to be alkylated by various methylating and ethylating agents. Table I shows the relative extents of alkylation at the various sites with nitrosamides, methylnitrosourea, and ethylnitrosourea, and with the alkylalkane sulfonates, methyl- and ethylmethanesulfonate. It is evident that the two alkylalkane sulfonates react much less with the oxygens of DNA (1.13 and 14.3%) than do MNU or ethylnitrosourea (21 and 77%). It is also apparent that ethylnitrosourea

TABLE I. Extent of Alkylation of DNA by Methylnitrosoura (MNU), Methylmethanesulfonate (MMS), Ethylnitrosourea (ENU), and Ethylmethanesulfonate (EMS) (Data From [17–21])

Site of alkylation in DNA	Total DNA alkylated (%)			
	MNU	MMS	ENU	EMS
7-Gua	66–70	81–85	11–14	58
O^6-Gua	5.9–7.7	0.31	7.6–10	2
3-Gua	0.63–1.0	0.62–0.7	0.61	0.27
3-Ade	8–8.44	9.4–11.3	2.78–5	4.24
1-Ade	0.7–0.87	1–1.89	0.29	1.68
7-Ade	0.8–1.98	0.9–1.83	0.38	1.88
O^2-Thy	0.11	ND	7–7.79	ND
O^4-Thy	0.67	ND	0.95–4.3	ND
3-Thy	ND[a]	ND	ND	ND
O^2-Cyt	0.1	ND	2.85–4	0.30
3-Cyt	0.51	ND	0.24	0.35
Total phosphotirester	12.1	0.82	49–56	12

[a]ND, not detectable ($\ll$0.02% of DNA-bound products)

preferentially alkylates the oxygen atoms resulting in a O^6-alkyl:N-7-alkylguanine ratio of 0.7; the corresponding ratio for methylnitrosourea was 0.1 and that for the ethyl- and methylmethanesulfonates much lower, indicating that the latter two compounds react preferentially with the nitrogen atoms of DNA. This differential reactivity with oxygen and nitrogen atoms is dependent on the mechanism by which the different alkylating agents react with DNA: agents that react via unimolecular nucleophilic reaction (S_{n1}) show a higher O^6/N-7 guanine ratio than agents that react via a bimolecular substitution reaction (S_{n2}) [14]. The O^6/N-7-alkylguanine ratios resulting from the reactions of these four alkylating agents with native DNA in vitro correlate well with their overall carcinogenic activity in vivo, since methyl- and ethylmethanesulfonate are very weak carcinogens as compared with ethylnitrosourea and MNU [15,16]. However, this correlation holds only for alkylating agents that react very differently with the oxygen atoms of the DNA: it is not observed within the group of N-nitroso compounds. In fact, in the case of MNU and ethylnitrosourea, which do not require metabolic activation, no difference is observed in *initial* O^6/N-7 alkylguanine ratio among target and nontarget tissues (see below.)

Despite the different conformational structure of the DNA, the relative extent of alkylation of DNA observed in vitro (Table I) (see [132]) parallels remarkably well the *initial* level of alkylation at the various sites of DNA observed in cell cultures or in vivo after exposure to these agents [17–20]. DMN is metabolized to the same methylating intermediate as that resulting from the chemical decomposition of MNU, the initial percentage of DNA alkylation at the various sites is similar with thse two nitroso compounds (see [17]).

Another important generalization that can be made on the basis of these studies in vitro and in vivo is that methylating agents have a much greater capacity than equimolar doses of ethylating agents in alkylating DNA [8,9].

BIOLOGICAL RELEVANCE OF DNA ALKYATION PRODUCTS

The hypothesis of Loveless [22] was that alkylation at the O^6-position of

guanine should cause a mutation during DNA replication resulting in a miscoding with thymine instead of with the complementary base cytosine. Since then, there has been substantial evidence to show that alkylation at the oxygen atoms of DNA bases is biologically more relevant than modifications at the nitrogen atoms. In fact, it has been shown [23,24] that only those alkylating agents that preferentially alkylate the oxygen atoms of DNA (MNU, ethylnitrosourea, and ethylmethanesulfonate but not methylmethanesulfonate) were able to mutate bacteriophages.

Table II lists the miscoding properties of various methylated bases. The presence of O^6-methylguanine or O^4-methylthymine (or uracil) in synthetic polymers results in the incorporation of noncomplementary bases during polyribonucleotide or polydeoxyribonucleotide synthesis in vitro using a bacterial RNA or DNA polymerase. On structural grounds, it is possible to construct a hydrogen-bonded base-pair between O^6-methylguanine and thymine and between O^4-methylthymine and guanine (but not adenine) [25]. The presence of other methylated bases, such as 7-methylguanine, 3-methylcytosine, 3-methylguanine, and O^2-methylthymine does not result in appreciable misincorporation when Escherichia coli DNA polymerase is used in the assay. The high specificity of methyl-nitro-nitroso-guanidine and ethylmethanesulfonate for inducing GC → AT transition is in keeping with the above observation [26].

Various data obtained from experiments in mammalian cells or bacteria confirm that the mutagenic activity of simple alkylating agents is directly related to their capacity to alkylate the oxygen atoms in DNA. Newbold et al [20] observed a good parallel between the marked difference in the mutagenic activity (forward mutation of hypoxanthineguanine phosphoribosyltransferase (HGPRT, $HGPRT^+ \rightarrow HGPRT^-$) of MNU, and of dimethylsulfate in Chinese hamster V79 cells and the difference in the level of O^6-methylguanine formed by those alkylating agents in the DNA of the cells. On the basis of experiments using the same type of cells, but a reversed mutation system ($HGPRT^- \rightarrow HGPRT^+$), Fox and Brennand [27] suggested that oxygen alkylation products other than O^6-methylguanine could contribute to the mutagenicity of MNU. In Drosophila melanogaster, the efficiency of a series of monofunctional alkylating agents in inducing recessive lethal mutations was increased when the agent, eg, ethylnitrosourea, preferentially alkylated DNA at an oxygen atom; no such relationship was observed with regard to the induction of chromosome breakages [28]. One of the most direct pieces of evidence that O^6-methylguanine is the mutagenic DNA lesion derives from studies in E coli, which showed that the "adaptation" to the mutagenic activity of methyl-nitro-nitrosoguanidine [29] is linked to the increased capacity of adapted bacteria specifically to remove O^6-methylguanine from their DNA [30].

In Chinese hamster ovary (CHO) cells, the independence of stage of cell cycle from the induction of mutations by ethylnitrosourea has been attributed to the inefficiency of those cells to repair O^6-methylguanine [31,32]. Rat liver epithelial cells and C3H 10T½ mouse cells show cell cycle specificity in the induction of mutagenesis [33] or cell transformation in vitro [34] by methyl-nitro-nitrosoguanidine, the S-phase showing the highest sensitivity. These cells (C3H 10T½) can remove O^6-methylguanine from their DNA [35], and their differential sensitivity is probably due to the level of damage present in DNA at the onset of the S phase.

These in vitro studies, therefore, indicate that modifications to oxygen atoms in the various DNA bases are the molecular basis of the various adverse biological

TABLE II. Miscoding Properties of Methylated Bases*

Methylated base	Miscoding	References
7-meGua	Neg	*Ludlum*, 1970 [36]
	Neg	Hendler et al, 1970 [37]
		Abbott and Saffhill, 1979 [38]
O^6-meGua	*UMP*	*Gerchman and Ludlum*, 1973 [39]
	d-TMP	Abbott and Saffhill, 1979 [38]
3-meGua	Neg	Abbott and Saffhill, 1979 [38]
1-, 3-, 7-meAd	Neg	Abbott and Saffhill, 1977 [40]
1-meAd	*AMP, GMP, CMP*	*Kroger and Singer*, 1979 [41]
O^4-meThy(Urd)	*GMP, CMP*	*Singer et al*, 1978 [42]
	d-GMP	Abbott and Saffhill, 1977 [40]
		Saffhill and Abbott, 1978 [43]
O^2-meThy	Neg	Saffhill and Abbott, 1978 [43]
3-meUrd	*UMP*	*Kroger and Singer*, 1979 [41]
3-meCyt	*AMP, UMP, CMP*	*Ludlum*, 1971 [44]
		Ludlum and Magee, 1972 [45]
		Kroger and Singer, 1979 [41]
	Neg	Abbott and Saffhill, 1979 [38]

*The experiments indicated in italic letters consist of assays carried out using a bacterial RNA polymerase; the others are assays using bacterial DNA polymerase I.

effects of N-nitroso compounds. Although most of the data indicate that O^6-alkylguanine is the critical DNA adduct in mutagenesis and in cell transformation in vitro, few data are available to assess the possible contribution of O^4-alkylthymine [78,132].

REPAIR OF DNA ALKYLATION ADDUCTS

In prokaryotic and eukaryotic cells various mechanisms have been described for the repair of DNA damage induced by different types of agents (see [Hanawalt, these Proceedings, and 46]). Two repair processes have been described for DNA damages induced by simple alkylating agents, one involving the release of the damaged base by specific N-glycosylases, and the other involving the transfer of the alkyl group from the O^6 position of guanine without breaking the DNA strand.

Purified or partially purified enzymes that release 3-methyladenine from DNA have been found in E coli [47], Micrococcus luteus [48] and human lymphoblasts [49]. A 3-methyladenine N-glycosylase has also been purified from rat liver nuclei; this enzyme preparation also released 7-methylguanine, although to a lesser extent [50]. 3-Methylguanine N-glycosylase activity has also been suggested in M luteus [51] and in E coli [130]; and more recently, 7-methylguanine glycosylase activity has been found in cells and tissue extracts from M luteus and E coli [52], from hamster and rat liver [53], and from human lymphoblasts [54]. E coli cell extracts have been shown [52,131] to have a DNA glycosylase capable of removing 7-methylguanine residues whose imidazole rings have been opened. These DNA alkylation products, namely, 3-methyladenine, 3-methylguanine, and 7-methylguanine, in addition to the enzymic action of specific N-glycosylases, are lost to a variable extent from DNA by chemical depurination as a consequence of the instability of the N-glycosydic bond.

The repair of O^6-methylguanine is not catalyzed by a specific N-glycosylase. Olsson and Lindahl [55] found that in E coli the methyl group of O^6-methylguanine is transferred to a protein cysteine residue; and there is some evidence [56] that the

methyltransferase inactivates itself during the process of transferring the methyl group. These results are corroborated by experiments using a synthetic DNA substrate containing ring-labeled O^6-methylguanine [57]. This newly observed mechanism of DNA repair is an inducible process which occurs in E coli treated with low doses of alkylating agent and is called the "adaptive response" [29] (see also below).

There is some preliminary evidence from experiments with liver extracts in vitro of the existence in mammalian cells of an inducible repair process in which O^6-methylguanine is removed from alkylated DNA by transfer of the methyl group [53,58–61]. Recently, it has been reported [62,63] that the activity responsible for the release of O^6-ethylguanine in rat liver is localized mainly in chromatin; the process is carried out by an enzyme with a low turnover rate and does not result in DNA breakage. There is possibly more than one mechanism to repair O^6-alkylguanine.

The elimination of O^6-methylguanine from DNA alkylated in vitro has also been observed in experiments with human liver extracts [64].

CARCINOGENICITY OF N-NITROSO COMPOUNDS, ALKYLATION, AND DNA REPAIR

One of the critical factors in determining the probability that an organ will develop a tumor after being exposed to nitrosamine is the persistence of promutagenic base(s), eg, O^6-alkylguanine, in the DNA of the target organ or cells. Such persistence results from the reduced capacity of the target organ to repair these promutagenic bases. Subsequent to the initial observation by Goth and Rajewsky [65], substantial evidence of such a correlation has been provided by various carcinogenicity experiments in vivo following a single or multiple exposure to an alkylating agent, as reviewed recently by O'Connor [66].

However, this factor cannot be considered without taking into account the importance of cell proliferation; what appears to be more critical is the amount of this promutagenic base that is present at the time of DNA replication. The *initial* level of O^6-alkylguanine in the DNA of target and nontarget organs appears of less relevance in this regard; in fact, the amount of alkylation detected soon after administration of ethylnitrosourea, a N-nitroso compound not requiring metabolic activation, in the DNA of brain (target) and liver (nontarget) is quite similar. This is exemplified also by a series of experiments in which hamsters or rats were exposed to a single dose of DMN, that is metabolized equally well by the liver of these two species. The Syrian golden hamster is sensitive to the induction of liver tumors by single doses of DMN, whereas the rat is not [16]; a single dose of DMN produces a 30% incidence of liver tumors in the former species [67]. Figure 1A shows the amounts (mol/10^6 mol of guanine) of 7-methylguanine and O^6-methylguanine in hamster liver DNA 24 hr after administration of various doses of DMN (0.01–25 mg/kg body weight). The amounts of 7-methylguanine were directly proportional to the dose of DMN; in the case of O^6-methylguanine, proportionality with the dose of DMN was observed with 0.5-25 mg/kg body weight and a ratio of O^6- to N-7-methylguanine of around 0.185, whereas with 0.25 mg/kg body weight or less the ratios were 0.027 (0.25 mg/kg body weight), 0.018 (0.1 mg/kg body weight), and 0.005 (0.01 mg/kg body weight). Since there is no reason to believe that the initial ratio of alkylated bases produced in DNA would be different with different doses of DMN, these results indicate that removal of O^6-methylguanine from ham-

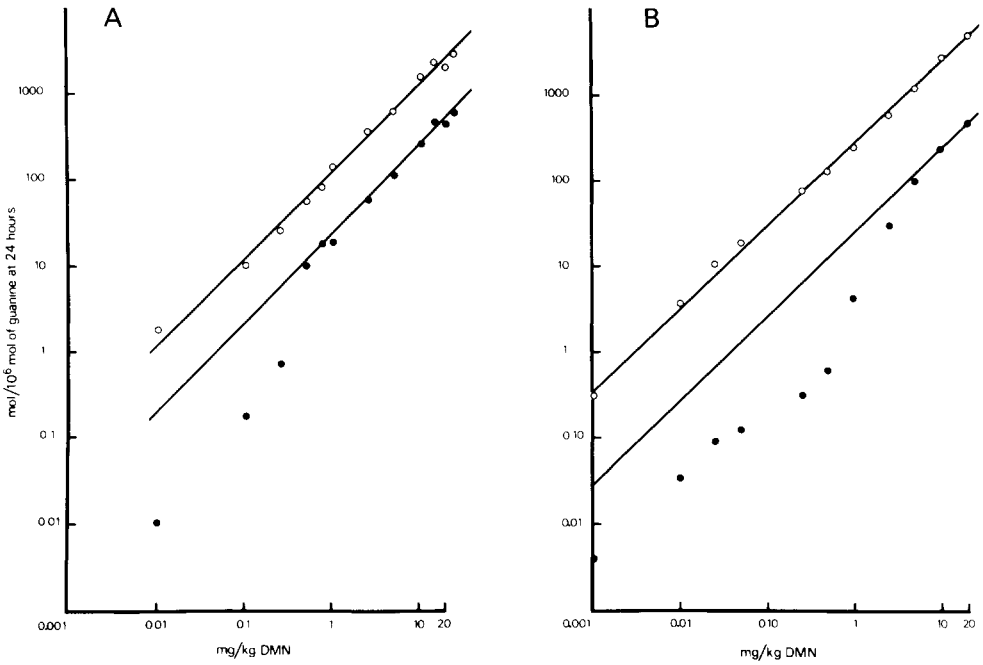

Fig. 1. Methylated guanine adducts in Syrian golden hamster (A) and rat (B) liver DNA 24 hr after injection of various doses of DMN: (○) 7-methylguanine, (●) O^6-methylguanine. Data from Stumpf et al [69] and Pegg and Hui [70]. Figure reproduced from Montesano et al [80].

ster liver DNA takes place after exposure to low doses of DMN, but that when higher doses (0.5 mg/kg body weight or more) are given the system responsible for the removal is much less effective. In fact, little or no O^6-methylguanine was removed from hamster liver DNA after alkylation produced by doses of 0.5 mg/kg body weight DMN or more [68,69].

These results differ from those obtained in rat liver after administration of similar doses of DMN [70]. Removal of O^6-methylguanine from DNA was much more efficient after exposure to low doses of DMN, and removal still occurred after exposure to higher doses (Fig. 1B). Thus, in hamster liver the amount of O^6-methylguanine produced by administration of 0.25 mg/kg body weight DMN was reduced by 50% within 24 hr, but in rat liver the O^6-methylguanine produced by a dose 10-fold higher (2.5 mg/kg body weight) was removed to the same extent. The reduced ability of hamster liver to remove this promutagenic base could be responsible for its much greater sensitivity to tumor induction by a single dose of DMN. This is also apparent from Table III which shows the half-lives of various DNA purine adducts in the livers of Syrian golden and Chinese hamster and of rat and the carcinogenic responses after a single dose of DMN.

A significant incidence of liver tumors is induced in rats if the single dose of DMN is given 24 hr after partial hepatectomy, ie, at the time of maximal DNA synthesis [71].

TABLE III. Liver Carcinogenicity and Persistence of Alkylation After a Single Dose of DMN (Data From [68,81])

Species	Dose (mg/kg body weight)	No. of tumor-bearing animals	Half-live (hr) of DNA adducts		
			O^6-meG	7-meG	3-meA
Syrian golden hamster	21	10/28	Stable	24	5
Chinese hamster	20	14/17	Stable	~ 24	12
Rat	30	None	20	70	6
In vitro (37°C, pH 7.0)	—	—	Stable	~ 140	18

More recently [72], accumulation of O^6-methylguanine was observed in non-parenchymal cells of the liver, but not in hepatocytes, of rats treated with 1,2-dimethylhydrazine, a methylating agent which induces hemangiosarcomas but not hepatocellular tumors. These findings show the high degree of cell specificity in the DNA repair capacity of an organ.

An interesting point made evident by Figure 1 is that O^6-methylguanine (but not 7-methylguanine) is removed with greater efficiency after low level of DNA damage; this observation may be relevant to the carcinogenic and mutagenic effects of alkylating agents, as has been discussed recently by Medcalf and Lawley [73].

In contrast to the above data (see also [66]) some experiments have been interpreted as indicating that the formation and persistence of O^6-alkylguanine in DNA is not a biologically relevant lesion in the carcinogenicity or mutagenicity of N-nitroso compounds; however, such evidence has limitations. Hodgson et al [74] reported that there was no evidence of removal of O^6-methylguanine from liver or kidney of Xenopus laevis treated with MNU, but no tumors were observed in these organs after injection of MNU or DMN. Khudoley and Picard [75] reported a significant incidence of liver and kidney tumors in Xenopus borealis when the carcinogen was administered directly into the water. In another experiment the brain of Mongolian gerbils was shown not to remove O^6-methylguanine from DNA efficiently [76], but no brain tumors have been observed in this species after MNU treatment [77]; this could be due to the low doses of MNU used and to the short survival time of the animals, which died from tumors that developed at the site of injection.

Data on the formation and persistence of other oxygen alkylation products in the DNA of various tissues in vivo are also very limited. It was reported recently [78] that the relative persistence of O^4-ethylthymine in brain DNA of rats could contribute to the carcinogenic effect of ethylnitrosourea in that organ.

Most of the above observations were made in experimental systems in which tumors were induced by a single dose of nitrosamine. The exposure of human beings and the induction of tumors in experimental animals occur, however, mainly by chronic exposure to one or many carcinogens: it was thus considered of interest to examine the behavior of various DNA alkylation products during continuous treatment with a carcinogen. In the more complex situation encountered when multiple doses of nitrosamines or nitrosamides are administered, an accumulation of

O^6-alkylguanine is also observed in the DNA of target tissues [66]; however, it is reasonable to assume that during chronic treatment with a carcinogen the rate of DNA synthesis would be as or more relevant than the persistence in the DNA of promutagenic bases [79]. Recent studies in vivo have shown that the removal from liver DNA of O^6-alkylguanine can be inhibited or increased depending on the schedule and dose of carcinogen administration. It is thus of interest to assess the relevance of these observations to carcinogenesis dose–response with nitrosamines.

MODULATION OF REPAIR OF DNA ALKYLATION ADDUCTS

Repeated administration to rats of low doses of DMN results in a greater removal of O^6-methylguanine from liver DNA than in rats treated with a single dose [82]. This effect appears to be specific for O^6-methylguanine, since it was not observed for other DNA adducts, such as 7-methylguanine or 3-methyladenine. The effect is mediated by an induced enzymic process, since liver extracts from pretreated rats specifically remove O^6-methylguanine from DNA alkylated in vitro than do extracts from control rats [83]. This increased removal of O^6-methylguanine was dependent on the dose with which the animals were pretreated; the effect occurred within 2 weeks of daily pretreatment with 2 mg/kg body weight of DMN (see Fig. 2).

Under these experimental conditions it was also found [84,85] that the increased removal can be detected rapidly (10 min) after administration of the challenging dose (2 mg/kg body weight) of ^{14}C-DMN, after which the liver again has a limited capacity to remove O^6-methylguanine. Following the rapid removal, the rate of loss of this DNA adduct does not appear to be significantly different in pretreated and control rats and may indicate the presence of two processes for the removal of this DNA adduct. Similar results have been obtained in another strain of rats by Swann and Mace [86].

These results are in marked contrast to the finding that inhibition of O^6-methylguanine removal is produced by large single doses of alkylating agents [70,87–89].

Long-term carcinogenicity studies (R. Peto, personal communication) with a wide range of doses of DMN indicate that, with doses of DMN above a certain level, the risk for liver cancer in rats increases rapidly resulting in a more than 1000-fold increase in tumor indicence with a 10-fold increase in daily dose rate. This type of response is consistent with the kinetics of repair of O^6-methylguanine in liver DNA during continuous treatment with various doses of DMN. This repair process(es) may thus be further activated by exposure to low levels of alkylating agents, but high doses of such agents may overtax the capacity of the constitutive and even of the induced DNA repair process(es), thus substantially increasing the risk of cancer in animals that receive large doses. Further studies are required, however, to substantiate such a conclusion. The results of our studies and the changed efficiency of removal of O^6-methylguanine after high and low degrees of DNA damage (see Fig. 2) suggest that liver cells may have more than one repair system for specifically removing O^6-methylguanine lesions from DNA.

Table IV lists the various animal systems in which the effect of pretreatment with various carcinogens on the removal from liver DNA of O^6-methyl- or O^6-ethylguanine was so far examined. The limited data available from studies of extrahepatic tissues do not permit a proper evaluation.

Increased removal of O^6-methylguanine from rat liver DNA has also been observed after pretreatment with 1,2-dimethylhydrazine, diethylnitrosamine, and ace-

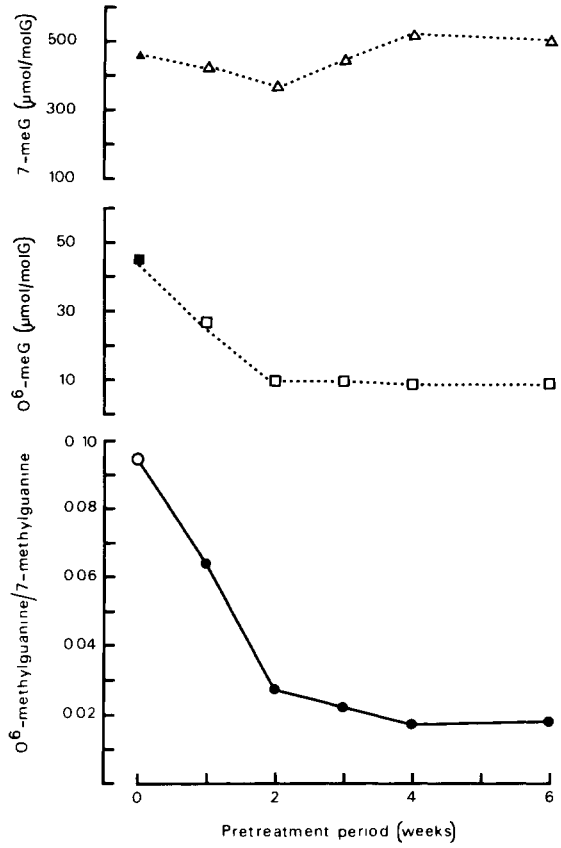

Fig. 2. Alkylation in liver DNA of BDIV rats after pretreatment for 1 or several weeks (5 days/week) with unlabeled DMN (2 mg/kg body weight) and one administration of 2 mg/kg body weight ^{14}C-DMN. Control rats (▲,■,○), received ^{14}C-DMN only. The control values are means of assays carried out at the beginning and at the end (6 weeks) of the experimental period. G=guanine. From Montesano et al [83].

tylaminofluorene. It has been suggested [90] that the increased capacity of rat liver to remove O^6-methylguanine after pretreatment with acetylaminofluorene is associated with augmented cell proliferation; in fact, Pegg et al [61] have found that the enzyme that catalyzes the removal of O^6-methylguanine from methylated DNA was more active during liver regeneration following partial hepatectomy. This observation is consistent with the results of Rabes et al [91] who found in a synchronized liver system in vivo that the O^6-/7-methylguanine ratio decreased during the S and G_2-M phases as compared with the G_1 and G_0 phases. In the case of pretreatment with acetylaminofluorene, it is possible that the increased removal of O^6-methylguanine results from increased cell proliferation; however, the increased rate of DNA synthesis appears of a lower order of magnitude than that observed after partial hepatectomy [92]. In the case of pretreatment with DMN, it appears less likely that the increased activity could be attributed to increased cell proliferation, since the

TABLE IV. Effect of Chronic Administration of Various Carcinogens on the Removal of O^6-Alkylguanine From Rat Liver DNA

Pretreatment[a]	Challenge[a]	Species	Effect[b]	References
DMN	DMN	BDIV rats	+	Montesano et al, 1979, 1980 [82,83]
		Sprague-Dawley rats		Pegg, 1980 [60]
		Wistar rats	+	Swann and Mace, 1980 [86]
1,2-DMH	DMN	Sprague-Dawley rats	+	Pegg, 1981 [103]
		Wistar rats	+	Margison, 1981 [104]
DEN	DMN	Wistar rats	+	Margison et al, 1979 [105]
		Sprague-Dawley rats	+	Pegg, 1981 [103]
AAF	DMN	Wistar rats	+	Buckley et al, 1979 [106]
				Charleworth et al, 1981 [92]
MNU	DMN	Wistar rats	–	O'Connor and Margison, 1981 [107]
MMS	DMN	Wistar rats	–	O'Connor and Margison, 1981 [107]
DMN	MNU	BDIV rats	–	Margison et al, 1976 [108]
MNU	MNU	Wistar rats	–	Margison, 1981 [104]
MMS	MNU	Wistar rats	–	Margison, 1981 [104]
DMN	DMN	Chinese hamsters	–	Margison et al, 1979 [109]
DMN	DMN	Syrian hamsters	–	Smith and Margison, 1981 [110]
DEN	DEN	Syrian hamsters	–	Smith and Margison, 1981 [110]
DEN	DEN	Wistar rats	+	Margison et al, 1979 [111]
DMPT	DMPT	BDIX rats	+	Cooper et al, 1978 [112]

[a]DMN, dimethylnitrosamine; 1,2-DMH, 1,2-dimethylhydrazine; DEN, diethylnitrosamine; AAF, N-acetylaminofluorene; MNU, methylnitrosourea; MMS, methylmethanesulfonate; DMPT, 3,3-dimethyl-1-phenyltriazene.

[b](+) Indicates that increased removal from DNA of O^6-alkylguanine, but not of 7-alkylguanine or 3-alkyladenine, was observed in pretreated as compared with control rats.

maximal increase in repair of O^6-methylation is obtained after a few weeks of DMN treatment and does not increase further with continuation of treatment (see Fig. 2).

The fact that no effect is observed when MNU is used for pretreatment and DMN for the challenging dose, or vice versa (see Table IV) could be due to the fact that these two carcinogens affect different cell populations (periportal and centrolobular areas) in the liver. The inability of pretreatment with methylmethanesulfonate to increase removal of O^6-methylguanine formed by a challenging dose of MNU or DMN could be attributed to the fact that only a low yield of O^6-methylguanine is formed by this compound. Further information is required, however, on the kinetics of formation and removal of O^6-methylguanine and on restorative hyperplasia in various cell populations of the liver in order to understand the mechanism(s) of induction of this DNA repair process. Two points appear to be relatively clear, namely, that the increased removal is specific for O^6-methylguanine and that it cannot be induced in tissues (like the livers of Chinese or Syrian golden hamsters) that have a very low constitutive capacity to remove this DNA adduct.

In CHO cells and in human skin fibroblast cell lines, chronic treatment with nontoxic doses of N-methyl-N′-nitro-N-nitrosoguanidine render the cell resistant to the toxic effect of the chemical and to the induction of sister chromatid exchange by further alkylation damage [93]. Preliminary results (Drevon, Piccoli, Montesano, unpublished data) obtained in IAR epithelial liver cells under experimental conditions similar to those utilized by Samson and Schwartz [93] show that increased sur-

vival and reduced mutagenicity are observed after a challenging dose of MNU.

These findings in mammalian cells parallel very closely the phenomenon of "adaptation" described originally by Samson and Cairns [29], who described the induction in E coli of an error-free repair process during exposure to low doses of the alkylating agent, N-methyl-N′-nitro-N-nitrosoguanidine. E coli exposed to sublethal concentrations of such alkylating agents develop resistance to the mutagenic and killing effects of the agents appear to be independent [29,94]. This resistance was shown [94] to be due to an inducible, error-free repair process that is distinct from the SOS repair process [95]. It could not be induced by other DNA-damaging agents, like ultraviolet irradiation or 4-nitroquinoline 1-oxide. It has also been found [30,96] that the kinetics of mutagenesis in bacteria pretreated with N-methyl-N′-nitro-N-nitrosoguanidine, parallel the appearance of O^6-methylguanine in DNA; the bacteria develop an increased ability to remove this DNA adduct from their DNA. No such parallel was observed for other DNA adducts, such as 7-methylguanine and 3-methyladenine.

A characteristic of this repair process in adapted bacteria is that the removal of O^6-methylguanine is very rapid, with a half-life of less than 1 sec [97]; and the reactant molecules that are responsible for the removal are consumed during the reaction [56,98]. Another characteristic is that the induced repair process is very effective for low degrees of DNA damage, but with greater damage accumulation of O^6-methylguanine and mutations occur at the same rate as in nonadapted bacteria [30,96]. Although the nature of the factor that triggers the induction process is not yet clear, the phenomenon of "adaptation" is much better understood in bacteria than in mammalian cells [99].

Recently, cell cycle dependent and inducible DNA repair processes distinct from the "SOS" type of repair have been reported in various other systems [100–102].

CONCLUSIONS

The studies discussed in the previous sections indicates that (a) N-nitroso compounds induce various types of DNA damages which are repaired by specific DNA repair processes; (b) only some DNA modifications (eg, O^6-alkylguanine) appear biologically relevant; (c) the efficiency of repair varies for the various DNA adducts and it depends on the amount of DNA damage; (d) continuous treatment with N-nitroso compounds modulates the removal of O^6-alkylguanine, but not of other DNA adducts, (e) the persistence of the promutagenic base O^6-alkylguanine in the DNA correlates with the high probability of that organ to develop tumors; and (f) no such correlation is observed with other DNA adducts like 7-alkylguanine or in the case of the various modifications detected in the RNA (see also [11,113]). The majority of the findings were made in experimental systems in which the various alkylating products are measured in total DNA extracted from all the organs; to examine the different degree of formation and persistence of the various DNA adducts among the various cell populations of a target organ would probably greatly contribute to a better understanding of nitrosamine carcinogenesis.

Another aspect that has been investigated only partially is the formation and the rate of repair of the various DNA modifications induced by these alkylating agents within the different fractions of chromatin. The findings so far have apparently been contradictory [114–119] but this could be due to the limited refinement of

the techniques used for such studies. The recent development of highly specific and sensitive radio-immunological methods for the detection of such DNA modifications (see [120–123]) is a great advance for the study of the distribution of these DNA adducts within cells and within various DNA fractions.

The alkylating intermediates formed from N-nitroso compounds are known to react covalently, not only with DNA but also with various other nucleophilic cellular constitutents, like proteins and RNA [11]. Although there is substantial evidence that the initiation of the carcinogenesis process and the mutagenic effect of these compounds are due to the formation of specific DNA alkylation products, recent evidence obtained in eukaryotic cells also shows that these carcinogens [124] as well as others [125–127] inhibit normal enzymatic DNA methylation, a process for which there is good evidence of its involvement in gene regulation and differentiation [128,129]. Future studies will assess the contribution of such alterations of gene expression to the multistage process of carcinogenesis.

ACKNOWLEDGMENTS

I would like to thank Drs. A. Likhachev, J. Laval, G.P. Margison, P.J. O'Connor, and L. Tomatis for the critical reading of this manuscript. The investigations of the author were partially supported by Contract No 1-CP-5560 awarded by the National Cancer Institute, United States Department of Health, Education, and Welfare. The secretarial assistance of Miss P. Collard is greatly appreciated.

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