Effect of Acute Doses of 2-Acetylaminofluorene on the Capacity of Rat Liver to Repair Methylated Purines in DNA in Vivo and in Vitro

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ABSTRACT

Male Wistar rats were given various doses of 2-acetylaminofluorene (AAF) at doses of 0.74, 2.22, 6.67, or 20 mg/kg i.p. 24 hr before administration of [14C]dimethylnitrosamine (1 or 2 mg/kg i.p.). Analysis of liver DNA isolated from animals killed 5 hr later showed variations between groups treated with different amounts of AAF in the amounts of 3-methyladenine, 7-methylguanine, and O6-methylguanine (O6-mGua). However, the relative amounts of these products were unchanged by AAF pretreatment except after 20 mg/kg when the reduced O6-mGua:7-methylguanine ratio indicated enhanced O6-mGua repair. Specific enhancement of O6-mGua repair was also found 5 hr after administration of [14C]dimethylnitrosourea (11.5 mg/kg) to animals pretreated with AAF (20 mg/kg), while the amounts of O6-mGua in liver ribosomal RNA after [14C]dimethylnitrosourea were unaffected by this AAF dose. Pretreatment of rats with AAF 29 hr earlier increased the capacity of cell-free liver extracts to remove O6-mGua from [3H]dimethylnitrosourea-methylated DNA in vitro. The increase was detectable after 2.22 mg/kg and reached a maximum 3.5-fold increase after AAF, (60 mg/kg). 7-Methylguanine and 3-methyladenine-DNA glycosylase activities were also increased, but this was independent of the dose of AAF. AAF pretreatment produced a slight (3- to 4-fold) increase in incorporation of [3H]thymidine or labeled one-carbon breakdown products of [14C]dimethylnitrosamine into liver DNA which appeared to parallel in vitro O6-mGua repair enhancement, but the increased [3H]thymidine uptake was statistically significant only after the 60-mg/kg dose.

INTRODUCTION

In the investigation of the mechanism of action of chemical carcinogens, the dialkylnitrosamines have provided several useful model systems because of their ability to produce tumors in a wide variety of animal species and because of their high degree of tissue specificity (19). Furthermore, these agents are present in the environment and can be generated within the body by the reaction of primary amines with nitrite either ingested per se or produced by gut bacteria (15). They may therefore be responsible for a proportion of human malignant disease the majority of which is attributable to environmental factors (9, 45), and their mechanism of action is thus of some interest.

The biological effects of dialkylnitrosamines which also include toxicity and mutagenicity are mediated by metabolism of the administered agent. This yields short-lived reactive species which alkylate various nucleophilic sites in cellular macromolecules; other classes of chemical carcinogens such as the aromatic amide AAF3 and the mycotoxin AFB1, also require metabolic activation to produce active electrophiles. For various reasons, a critical target molecule in carcinogenesis has been considered to be DNA. Of the products of reaction of DNA with alkylating agents, one of the minor bases, O6-alkylguanine, has received particular attention because of its ability to direct the incorporation of noncomplementary nucleotides during the transcription of templates containing this product by DNA or RNA polymerases in vitro (1, 12, 18).

A variety of experimental systems have shown that in general the relative amounts of O6-alkylguanine produced in DNA in tissues or in cultured cells may be correlated with the carcinogenic or mutagenic potency of N-nitroso compounds and related alkylating agents. The occurrence of DNA synthesis on a template containing such damage would then produce a permanent change in the DNA sequence. In some instances, however, the production of O6-alkylguanine cannot itself explain the high susceptibility of certain tissues to the carcinogenic effects of alkylating agents, and in such cases 2 factors appear to be interactive, i.e., the ability of cells to repair O6-alkylguanine lesions and the frequency or rate of DNA replication (reviewed in Refs. 26, 34, 36, and 41). Thus, in the majority of experimental carcinogenesis systems which have been examined, the principal target tissue has been that in which the O6-alkylguanine repair system is least active and hence the persistence of this lesion in DNA is most prolonged. In other systems, the target organ appears to be the one which undergoes most extensive DNA replication (reviewed in Refs. 20 and 32).

While much of this work has involved model systems in which single doses of alkylating agents are carcinogenic, the environment contains a variety of carcinogens, and exposure is generally protracted. It is therefore important to try to examine how such treatments in laboratory animals may affect the factors which have been established as important determinants in acute-dose carcinogenesis, i.e., DNA repair and replication. Experiments of this kind have shown that the capacity of rat liver to repair O6-alkylguanine in DNA can be increased by chronic administration of alkylating agents or chemically unrelated agents such as AAF or AFB1, (reviewed in Refs. 30 and 33). The mechanism of this enhanced repair phenomenon and its role if any in cancer etiology are clearly of some interest.

Earlier work in this laboratory has shown that the repair of O6-mGua produced in rat liver DNA by administration of DMN

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1 The abbreviations used are: AAF, 2-acetylaminofluorene; AFB1, aflatoxin B1; O6-mGua, O6-methylguanine; DMN, dimethylnitrosamine; MNU, methylnitrosourea; 7-mGua, 7-methylguanine; O6-mGua, O6-methylguanosine; 7-mGua, 7-methylguanine; 3-mAdo, 3-methyladenine.
was enhanced by feeding animals a diet containing AAF and that exposure to this diet for 3 weeks produced as much enhancement as did exposure for 9 weeks (3, 5). The first objective in the present experiments was to determine the minimum period required for the effect. In this paper, we have established that even a single dose of AAF results in enhanced O6-mGua repair. We have examined the effect of a range of doses of AAF and also show that the enhanced system is apparently unable to act on O6-mGua in liver RNA. The increase in repair of this base in DNA in vivo is reflected in an increase in the capacity of liver extracts to destroy O6-mGua in DNA in an in vitro assay system. Finally, in attempts to establish whether enhanced O6-mGua repair is related to DNA turnover, the effect of AAF on DNA synthesis in the liver has been determined.

**MATERIALS AND METHODS**

**Chemicals.** [14C]Dimethylamine hydrochloride (55.0 mCi/mmol), [methyl-14C]MNU (38 mCi/mmol), and [5-methyl-3H]thymidine (25.0 Ci/mmol) were from the Radiodichemical Centre, Amersham, United Kingdom. [methyl-3H]MNU (1.6 Ci/mmol) and [methyl-3H]dimethyl sulfate (4.3 Ci/mmol) were from New England Nuclear, Dreieich, West Germany. [14C]DMN was synthesized from [14C]dimethylamine hydrochloride as described in Ref. 10 and diluted with unlabeled DMN obtained from Eastman-Kodak, Ltd., Kirby, Lancashire, United Kingdom; unlabeled MNU was synthesized in these laboratories. 2-AAF was a generous gift from ICI Pharmaceuticals Division, Macclesfield, Cheshire, United Kingdom, and all enzymes were supplied by the Sigma Chemical Co., London, United Kingdom. DNA (call thymus) for use in in vitro assays was obtained from Worthington Biochemical Corp., Freehold, N. J.

**Treatment of Animals.** When measuring the repair of O6-mGua in vivo, 2 animals per point were used, and the livers were combined for extraction and analysis of DNA and RNA. Analyses were performed in duplicate and averaged. In a preliminary experiment, male Wistar rats were given a single injection of AAF (20 mg/kg i.p. suspended in corn oil) 28 hr before they received [14C]DMN (2 mg/kg i.p., 4.4 or 5.4 mCi/mmol). The animals were killed 18 hr later, and the livers were removed and homogenized in 10 ml 0.2 ml 0.1 M NaCl at -80°. In subsequent experiments, male Wistar rats (200 to 260 g) were given various doses of AAF up to 60 mg/kg (0.2 ml/100 g i.p.) using 1.75% gum acacia in 0.9% NaCl solution as the vehicle. Control animals received similar volumes of 0.9% NaCl solution or vehicle. To assess the in vivo repair capacity, [14C]DMN (1 mg/kg i.p., 12.6 mCi/mmol) was given 24 hr later, and the animals were killed after a further 5 hr. To measure the incorporation of [3H]thymidine into liver DNA, the animals were treated as above except that the DMN was unlabeled and [3H]thymidine (25.0 Ci/mmol, 0.5 mCi/kg, >95% radiochemical purity) was given 30 min before death. In another experiment, animals were given AAF (20 mg/kg) suspended in gum acacia:0.9% NaCl solution 24 hr before [14C]MNU (11.5 mg/kg i.p., 5.3 mCi/mmol). The animals were killed 5 hr later, and various tissues were removed and stored at -20°. For the preparation of liver extracts to be used in in vitro assays of repair enzymes, animals were treated with various doses of AAF up to 60 mg/kg and were killed 29 hr later. The livers were removed and stored at -20°C.

**DNA and RNA Isolation and Analysis.** DNA and RNA were isolated by a phenol procedure as described previously (25, 28) and analyzed using Sephadex G-10 gel chromatography (22, 28) for the presence of methylpurines (counting efficiency, 78 to 80%). This process also allows the determination of the amount of 14C/DMN breakdown products incorporated into normal DNA purines via the one-carbon pool, thus giving an indication of the rate of DNA synthesis. In the analysis of RNA, the methylation products, 7-mGuo (which eluted together with its imidazole ring-opened form) and O6-mGuo were well separated, but only partial separation of adenosine and guanosine peaks was achieved.

**Preparation of Substrates.** [3H]MNU-methylated DNA for use in the in vitro assay for O6-mGua removal activity was prepared in the following way. Calf thymus DNA (40 mg) was dissolved in 5.0 ml 0.2 M sodium cacodylate:1 mM EDTA, pH 8.0; 250 ml methanol containing 16 μg [14C]MNU (1.6 Ci/mmol) were added; and the mixture was shaken at room temperature for 3 to 4 hr. The DNA was precipitated by the addition of 2 volumes cold ethanol and then was washed 6 times with 70% ethanol containing 2% sodium acetate. The precipitate was redisolved in 5.0 ml Buffer A (50 mM Tris:1 mM mercaptoethanol:1 mM EDTA, adjusted to pH 7.8 with HCl and dialyzed extensively against 3 2-liter batches of the same buffer, at 4°, until little radioactivity could be detected in the dialysis medium. The DNA was reprecipitated with 2 volumes cold ethanol before and washed 3 times with 70% ethanol containing 2% sodium acetate. The DNA was then dried at room temperature by washing twice in ethanol, twice in ethanol:ether (1:1, by volume), and once in ether. Finally, the DNA (which contained 1.6 to 2.0 pmol O6-methyl-mGua per mg was dried in a stream of nitrogen and stored at 4°.

The substrate for the glycosylase assay was prepared using dimethyl sulfate as follows. Calf thymus DNA (32 mg) was dissolved in 40 ml 0.2 M sodium cacodylate:1 mM EDTA, pH 7.2, and reacted with 1.16 pmol [methyl-3H]DMMS (4.3 Ci/mmol). After a stirring for 90 min at 37°, the DNA was precipitated by adding 2 volumes cold ethanol and then washed repeatedly with 70% ethanol containing 2% sodium acetate until negligible radioactivity was present in the washings. The DNA was redissolved in 7.0 ml 10 mM NaCl, dialyzed overnight against 2 liters of the same solution, and stored at -20°. The final DNA solution contained -12 pmol 7-mGua and -2.4 pmol 7-mAde in 10 μl.

**Preparation of Cell-free Extracts.** Cell-free extracts were prepared essentially by the method of Pegg (39) except that Buffer A was used in the place of 0.4 M Tris and 0.2 M sucrose. The protein concentration of the extract was measured using the Bio-Rad Assay Kit (Watford, United Kingdom) with bovine serum albumin as a standard, and all extracts were stored in aliquots at -30°. Four separate liver extracts were prepared for each of the control and AAF-treated groups, and each extract was derived from the pooled livers of 2 animals.

**Assay of O6-mGua Removal Activity.** The procedure for the measurement of O6-mGua removal activity was essentially that described in Ref. 39. The [methyl-3H]MNU-methylated DNA was dissolved at a concentration of 2 mg/ml in Buffer B (50 mM Tris:1 mM mercaptoethanol:1 mM EDTA, adjusted to pH 8.3 with HCl). The assay mixture consisted of 0.5 ml substrate DNA, cell-free extract (usually 5 mg protein contained in ~120 μl), and Buffer B to a final volume of 3.0 ml. The components were thoroughly mixed before incubation at 37° for 30 min. The reaction was stopped by transferring the tubes to ice and adding 1.0 ml cold 1.0 M HClO4. After vortex mixing, the protein and DNA were allowed to precipitate for 10 to 15 min before they were collected by centrifugation at 1200 x g for 10 min at 4°. The supernatant was discarded, the pellet was dispersed in 0.2 ml 0.1 M HClO4, and O6-mGua and 7-mGua markers were added. The suspension was heated at 70° for 30 min to hydrolyze the DNA, and the mixture was centrifuged at 1200 x g for 10 min at 4°. The supernatant was retained, and the pellet was rehydrolyzed with a further 0.25 ml 0.1 M perchloric acid. After centrifugation, the supernatant was combined with the first hydrolysate and then analyzed by high-performance liquid chromatography using a 25-μm x 0.46-cm (inside diameter) column of Partisil 10-SCX (HPLC Technology, Wilmslow, Cheshire, United Kingdom). The column was eluted with 5 mM sodium acetate, pH 4.25, at 2.5 ml/min using a Gilson Model 301 pump, and samples were loaded using an Altex Sliver Valve. The absorbance at 254 nm was continuously monitored using a Gilson Model III LC detector, and 30 fractions of ~3.0 ml were collected. Adenine peak heights showed little variation (<5%) from the control values indicating that nonspecific degradation

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of the substrate was negligible (data not shown). The radioactivity in each fraction was measured by liquid scintillation counting (efficiency, 20 to 28%) thus allowing the amounts of 7-mGua and O6-mGua to be determined. Since little or no 7-mGua is released during a 0.5-hr incubation and since the ratio of O6-mGua:7-mGua in the substrate is 0.11, the initial amount of O6-mGua present in each assay can be determined, and this permits calculation of the specific activity (fmol O6-mGua lost per hr per mg protein) of each extract.

**Assay of DNA Glycosylase Activity.** The activities of the 7-mGua and 3-mAde DNA glycosylases were measured simultaneously using cell-free extracts prepared as in Ref. 27, and the assay procedure was based on that described in Ref. 27. The assay mixture consisted of 10 µl [methyl-14C]dimethyl sulfide-DNA substrate, cell-free extract (usually 10 µg contained in ~240 µl), and buffer A to a final volume of 300 µl. The mixture was incubated at 37° for 5 hr, and the reaction was terminated by cooling in ice. Carrier DNA was added (60 µl of a 2-mg/ml solution of calf thymus DNA in 0.9% NaCl solution heated at 100° for 5 min and cooled quickly on ice) along with 30 µl 2 M NaCl and 0.9 ml cold ethanol. After vortex mixing, the DNA and the protein were allowed to precipitate overnight at −10°. The alcohol supernatant containing any 7-mGua and 3-mAde released during the incubation was then analyzed by chromatography using a 25 x 1-cm column of Sephadex G-10 eluted with 50 mM ammonium formate containing 0.02% sodium azide, pH 6.75, at a flow rate of about 40 ml/hr. Thirty fractions of 10 ml were usually collected, and radioactivity in each fraction was measured by liquid scintillation counting. The amount of spontaneous depurination (about 155 fmol 7-mGuo per hr and about 145 fmol 3-mAde per hr) was measured in control assays which were incubated without the addition of protein and these values were used in the calculation of the specific activities of the glycosylase enzymes.

**[3H]Thymidine Incorporation.** Acid-soluble DNA was extracted from individual rat livers as described in Ref. 13. The DNA content of the acid extracts was measured colorimetrically by the method of Burton (4), and radioactivity was determined by liquid scintillation counting. The results are expressed as dpm/µg DNA.

**RESULTS**

The results of a preliminary experiment to assess the effects of pretreatment of rats with a single dose of AAF (20 mg/kg) are shown in Table 1A. The levels of methylated purines in liver DNA 18 hr after administration of [3H]DMN indicate that the amounts of 3-mAde and 7-mGua were almost identical in control and pretreated animals. However, in pretreated rats, there was less than one-half the amount of O6-mGua, demonstrating that even a single dose of AAF can enhance O6-mGua repair. The amounts of methylated purine nucleosides found in the liver rRNA from these pretreated and control rats were similar (Table 1B), and the O6-mGuo:7-mGuo ratio indicates that pretreatment with AAF (20 mg/kg) has no effect on the relative levels of O6-mGuo produced by DMN.

In this and subsequent experiments, the pretreatment interval was 24 hr; however, examined using a survival time (5 hr) because it was thought that this would improve the ability to detect differences between control and pretreated groups. There was some variation in the amounts of 7-mGua and 3-mAde in the liver DNA of control and AAF-pretreated rats (Table 2), and this was probably due to several factors. AAF may affect the metabolism of DMN to an alkylating species, and this may be dose dependent; because the gua acacia controls had a higher level of methylation than did the 0.9% NaCl solution controls, there may also be a small vehicle effect on DMN metabolism (Table 2). Despite these differences in methylation levels, the 3-mAde:7-mGua ratios were similar in control and AAF-pretreated animals (Table 3). The O6-mGua:7-mGua ratios were also unaffacted by AAF pretreatment except after the highest dose (20 mg/kg) which decreased the relative amount O6-mGua to almost one-half that in control animals (Table 3).

The capacity of rat liver cell-free extracts to remove O6-mGua from [3H]MNU-methylated DNA in vitro was linearly dependent on protein concentration as far as tested (up to 20 mg protein) at which point about 84% of the O6-mGua was destroyed (Chart 1A). Control rat liver extracts had a specific activity of about 130 fmol/hr/mg which was increased by AAF pretreatment at doses of 2.22 mg/kg or higher. At the highest dose used (60 mg/kg), the specific activity was increased to about 460 fmol/hr/mg (Chart 2). The O6-mGua:7-mGua ratios in the DNA after the incubation period, which also reflects the activity of the O6-mGua removal system, are shown in Table 3.

The activities of the 7-mGua and 3-mAde DNA glycosylases were also found to be linearly dependent on protein (Chart 1B) but showed no dose-related increase in activity (Chart 2). However, the control extract showed less 7-mGua glycosylase activity than did the AAF-pretreated animals, and this is reflected in the ratio of 3-mAde to 7-mGua released during the incubation (Table 3).

### Table 1

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<th>Amounts of methylated purines in rat liver DNA and RNA 18 hr after administration of [3H]DMN (2 mg/kg, 4.4 or 5.2 mCi/mmol, respectively) to animals pretreated with AAF (20 mg/kg) 24 hr earlier</th>
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<tr>
<td></td>
<td>Amounts (µmol/mol parent base)</td>
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<tr>
<td></td>
<td>3-mAde</td>
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<td>7-mGua</td>
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<td>O6-mGua</td>
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<td>3-mAde:7-mGua ratio</td>
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<td>O6-mGua:7-mGua ratio</td>
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<td>O6-mGua</td>
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<tr>
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<tr>
<td>3-mAde:7-mGua ratio</td>
<td>0.037</td>
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<tr>
<td>O6-mGua:7-mGua ratio</td>
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### Table 2

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<td></td>
<td>Amounts (µmol/mol parent base)</td>
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<td>AAF (mg/kg)</td>
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<td>2.22</td>
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<td>6.67</td>
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### Table 3

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<td>7-mGua</td>
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<tr>
<td>3-mAde:7-mGua ratio</td>
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<tr>
<td>O6-mGua:7-mGua ratio</td>
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</tbody>
</table>

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* Corrected for differences in DMN specific activity.
* Numbers in parentheses, equivalent data calculated for DNA.

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The effect of AAF pretreatment on rat liver DNA turnover is also shown in Table 3. This was investigated by measuring the incorporation of [3H]thymidine into hepatic DNA and also by measuring the incorporation, via the nucleotide pool, of 1-carbon 14C fragments from the metabolism of [14C]DMN. Both parameters show only small differences, and in the case of the [3H]thymidine incorporation only the value obtained after pretreatment with AAF (60 mg/kg) is significantly different than the control at the 95% confidence limit (Student’s t-test).

In order to investigate the extent to which the increased capacity to remove O6-mGua from DNA was restricted to those cells capable of DMN metabolism, rats were pretreated with AAF (20 mg/kg) and given a low dose (11.5 mg/kg) of [14C]MNU (5.3 mCi/mmol) 24 hr later. The amounts of methylated purines in liver and brain DNA 5 hr later are shown in Table 4. Pretreatment had no effect on the 3-mAde:7-mGua ratio in liver or brain DNA, and similarly the O6-mGua:7-mGua ratio in brain DNA was unaffected. However, the hepatic DNA of pretreated animals contained relatively less O6-mGua than did the controls. This is shown by a one-third fall in the ratio of O6-mGua to 7-mGua (Table 4) and indicates that the O6-mGua repair capacity of the liver which, as reported previously (16), is much higher than that of the brain is further increased by AAF pretreatment.

**DISCUSSION**

A number of experiments using alkylating agent carcinogenesis systems have indicated the importance of the formation of O6-alkylguanine in DNA and its subsequent repair in the tissue and species specificity of these agents (reviewed in Refs. 20, 26, 32, and 34). Of the animal O6-alkylguanine repair systems thus far examined, that of the rat liver appears to be the most active and may protect this tissue from tumor induction after single doses of DMN (31, 36). It has, however, been shown that pretreatment of rats with large single doses of various agents reduces the capacity of the liver to repair O6-mGua generated in DNA by a low dose of MNU (17). O6-mGua repair can also be inhibited by large doses of MNU (16) or DMN (37) or a series of symmetrical dialkylnitrosamines (38). These observations suggested that chronic administration of alkylating agents might produce tumors as a consequence of repair inhibition. However, there are now a number of experiments in which the efficiency of repair of O6-alkylguanine in rat liver DNA is in fact increased during chronic administrations of alkylating agents such as 3,3-dimethyl-1-phenyltriazene (8), DMN (21, 22, 28, 29, 46), diethylnitrosamine (22, 24), and 1,2-dimethylhydrazine (21) or other agents such as AAF (3, 5) or AFB (6).

It was particularly interesting in the case of AAF that an aromatic amine derivative could affect the repair of alkylated bases in DNA, and a closer examination of this phenomenon has been undertaken in this report.

Initially, and to simplify subsequent experiments, it was necessary to determine whether the effect could be produced after a single dose of AAF or whether, like the alkylating and other DNA-damaging agents (see above), such treatment would inhibit the repair of O6-mGua produced by administration of DMN. Rats were given AAF (20 mg/kg) and, after allowing 24 hr for metabolism, a low dose of [14C]DMN (2 mg/kg). In control animals, the absolute and hence relative amounts of methylated purines in liver DNA 18 hr later were close to those reported previously for this dose of DMN (11). In AAF-pretreated animals, the amount of O6-mGua was less than one-half the control value (Table 1). Since the levels of 7-mGua and 3-mAde were unchanged, it is unlikely that AAF pretreatment had any marked effect on the metabolism of DMN to an alkylating species or on the activity of the glycosylase enzymes acting on these products. Furthermore, since the alkylating species from DMN produces an initial O6-mGua ratio of 0.11 (i.e., in the absence of repair reactions), these results indicate that the activity of the hepatic O6-mGua repair system which is already able to reduce the ratio to 0.071 by 18 hr after DMN administration is further increased by AAF pretreatment. The extent of enhancement was very close to that produced by pretreatment of rats with DMN on weekdays for 3 weeks (29). In these experiments, further treatment with DMN caused no additional enhancement, but shorter periods of treatment were less effective. To determine the minimum dose of AAF required for enhanced O6-mGua repair, animals were given a lower dose of DMN (1 mg/kg) and were killed 5 hr later in order to increase the ability to detect enhancement. Under these conditions, pretreatment with AAF (20 mg/kg) again resulted in an increase in the capacity to repair O6-mGua, but lower doses of AAF did not affect the post-DMN methylation levels in liver DNA in comparison with 0.9% NaCl solution or vehicle-treated controls.

It is conceivable that the decrease in O6-mGua levels may...
AAF-induced Enhancement of O6-MGua Repair

have been produced by factors other than an increase in repair activity such as an alteration in chromatin structure or redistribution of methylation damage within DNA or into cells which have an inherently greater repair capacity. Therefore, we next examined the ability of cell-free extracts from the livers of control and AAF-pretreated rats to remove O6-mGua from methylated DNA in an in vitro assay system. Pretreatment with AAF at doses of 2.22 mg/kg or above increased this activity, and the extracts from animals given AAF (60 mg/kg) were able to remove ~70% of the O6-mGua from the substrate during the

incubation period.

The activity of extracts from animals given AAF at 20 mg/kg was lower than after 6.67 or 60 mg/kg, and this may have been due either to biological variation or to some unknown dose-dependent effects. Grouping the in vitro data with those obtained in vivo (Table 3), it is evident that the in vitro assay is more sensitive than the in vivo assay in detecting small changes in the activity of a DNA repair system. In agreement with the in vivo data (Table 2), a different in vitro assay system indicated that AAF pretreatment had only a slight effect on the activity of 3-mAde-DNA glycosylase (Chart 2). This is in broad agreement with other results which show an increase in the activity of this enzyme in extracts of nuclei prepared from the livers of rats given AAF either in the diet or as a single dose. The activity of 7-mGua-DNA glycosylase was apparently increased by AAF pretreatment (Chart 2), but this was not dose dependent.

The changes in the amounts of O6-mGua and the O6-mGua:7-mGua ratios in AAF-pretreated animals are therefore principally a consequence of an increase in the activity of the system responsible for the removal of O6-mGua from DNA. The effects of a single dose of AAF thus parallel those of chronic administration of DMN (28, 29). Having demonstrated that single doses of AAF induce the repair of O6-mGua, we attempted to answer some questions about the limitations and the mechanisms of the effect. These were (a) did the enhanced system act on O6-mGua in RNA; (b) could AAF enhance the repair of similar damage produced in liver DNA after administration of the direct acting agent MNU; and (c) was there any evidence that enhanced repair might be related to the induction of DNA synthesis as a consequence of the hepatotoxic effects of AAF? RNA is also a target for alkylation after administration of

* V. M. Craddock, personal communication.
DMN, and earlier reports indicated that there was no removal of specific chemically methylated bases from rRNA (25). While the simplified analytical procedure used in the present experiments would not allow detection of a slight effect, the results suggest that the O6-mGua repair system induced by AAF pretreatment acts only on the lesion in DNA. Chronic administration of a series of symmetrical dialkynitrosamines all of which enhanced the repair of O6-mGua in DNA similarly had no effect on this base in rRNA (22). The absence of activity on RNA is important in view of the mechanism of the O6-mGua repair system which appears to act by transfer of the methyl group to an amino acid residue in the repair protein (2, 35). A priori, there seems to be no reason why the system should not act on RNA; however, there may be a specificity either for a double-stranded structure or for deoxyribopolynucleotides or both.5

It has been reported previously that chronic pretreatment of rats with DMN does not enhance the repair of O6-mGua produced by a single dose of [14C]MNU (23). It was interesting therefore to observe that, in contrast, AAF pretreatment did enhance the repair of such damage in the liver, while there was no effect on the damage produced in brain DNA (Table 4). This suggests that AAF has a greater effect than DMN on those cell populations within the liver in which MNU produces DNA damage and is thus far the only parameter in which AAF and DMN pretreatments have differed.

There are now several reports that chronic pretreatment of rats with low doses of DNA-damaging agents increases the capacity of the liver to remove O6-alkylguanine from DNA (5, 7, 8, 21, 22, 24, 29, 33, 44). Because the agents which have been used are hepatotoxic to varying degrees, it is possible that enhanced repair activity is related to liver cell proliferation. This suggestion is supported by the observation that chronic administration of enhancing doses of DMN to rats does increase the incorporation of [3H]thymidine into liver DNA (28). Furthermore, it has been shown recently that partial hepatectomy of rats increases the capacity of liver extracts to repair O6-mGua in DNA in vitro (40). However, in the present experiments, AAF pretreatment produced only slight (and dose-independent) increase in the levels of [3H]thymidine uptake into liver DNA, these being small (3- to 4-fold) in comparison with the increases which have been reported after partial hepatectomy (100-fold) (14). In contrast, the increase in O6-mGua repair activity of liver extracts (3- to 4-fold) was of an order similar to that produced by partial hepatectomy (5-fold) (see Ref. 40). This could argue against a simple relationship between the extents of prereplicative DNA synthesis and O6-mGua repair induction. However, the inducible O6-mGua repair capacity might be restricted to a small cell population, and increased proliferation within this compartment, either as a general response to partial hepatectomy or perhaps more selectively to AAF, may lead to enhancement of O6-mGua repair. On the other hand, inasmuch as the enhancement of O6-mGua repair produced by low doses of AAF, DMN, and diethylnitrosamine are not necessarily associated with substantial increases in DNA turnover, it is not practical to exclude the possibility that this repair system may, by analogy with the "adaptation" phenomenon in Escherichia coli (42, 43), respond to nontoxic levels of DNA damage.

In vitro studies by Gombar et al. (14) have shown that 3-mAde-N-glycosylase activity is increased after partial hepatectomy, and there is some evidence for this after administration of AAF (see above). On the other hand, in vivo experiments have consistently failed to detect these small changes (present data; Refs. 28 and 29). It would appear from these results that different factors can control the expression of DNA repair enzymes.

The chronic administration schedules in which enhancement takes place are generally hepatocarcinogenic (33), but to what extent the induction of DNA repair may play a causative role or provide additional protection against tumor initiation under such circumstances is not clear. The demonstration that single doses of AAF enhance O6-mGua repair will be most useful in further experiments which should establish the kinetics of formation and loss of the induced system and its capacity to deal with alkylation damage in DNA.

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Effect of Acute Doses of 2-Acetylaminofluorene on the Capacity of Rat Liver to Repair Methylated Purines in DNA \textit{in Vivo} and \textit{in Vitro}

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