Inhibition of Rat Liver RNA Polymerases by Action of the Methylating Agents DimethylNitrosamine in Vivo and Methyl Methanesulfonate in Vitro

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SUMMARY

DimethylNitrosamine maximally inhibits rat liver nuclear RNA synthesis by 50% at a dose of 40 mg/kg of body weight. The inhibition develops during the first 4 hr and persists through the 12th hr. All parenchymal cells of the liver lobule seem to be affected. The decreased RNA synthesis can be accounted for entirely by an inhibition of the RNA polymerase activities quantitatively solubilized and partially purified. A similar inhibition of the polymerase activities was demonstrated in the intact nuclei by inactivating the endogenous template with actinomycin D and assaying the polymerases with an added exogenous template, poly(deoxyadenylate-deoxythymidylate). Chromatin was prepared by two methods differing in the extent to which they remove the endogenous polymerase activity. Each preparation was transcribed with either added Escherichia coli or partially purified rat liver nucleoplasmic RNA polymerase. With either polymerase or chromatin preparation, no inhibition of the template activity of liver nuclear chromatin isolated from the DMN-treated animals was detected. A similar mechanism of inhibition of RNA synthesis was produced by the action of the methylating agent methyl methanesulfonate on whole nuclei in vitro. The dose-dependent inhibition of RNA synthesis could be accounted for by an inhibition of the RNA polymerase activities quantitatively solubilized and partially purified from the affected nuclei. Chromatin prepared from the methyl methanesulfonate-treated nuclei had a normal template capacity with either E. coli or rat liver nucleoplasmic RNA polymerase. No preferential methylation of the RNA polymerases by \(^{14}C\)methyl methanesulfonate could be demonstrated. It is concluded that the action of the two methylating agents on RNA metabolism is similar and that the inhibition of liver nuclear RNA synthesis results from inactivation of the RNA polymerases. At the same time, dimethylNitrosamine and methyl methanesulfonate leave the chromatin template intact, at least quantitatively, for the synthesis of RNA. The implications of such an effect on RNA synthesis are discussed.

INTRODUCTION

A significant portion of the research on the effects of chemical carcinogens on cells has detailed the specific interactions of these biologically active agents with a variety of the macromolecular components of the respective target cells. In many cases, the immediate functional consequences of these interactions are not known in any detail. Furthermore, the relationship between these interactions with cellular macromolecules and the inherent carcinogenicity of each particular chemical is even more obscure.

One of the most intensively studied chemical carcinogens has been the potent hepatic carcinogen DMN. DMN has been reported to methylate liver cell DNA, RNA, and protein (3-6, 17, 18, 22-25, 28, 39). At the same time, DMN is reported to produce significant functional alterations in liver cells with inhibition of RNA and protein synthesis (21, 22, 37, 41). Because of the documented interaction of DMN with cellular constituents and the reported functional effects with which these interactions are presumably associated, a study of the effects on DMN on liver cells may provide a means for determining, in one particular case, the relationship between specific chemical interactions with cellular macromolecules and the associated functional consequences.

In this study we have examined the effect of DMN on rat liver RNA synthesis. The previously reported methylation of DNA, RNA, and proteins provided several possibilities for the mechanism of inhibition of RNA synthesis. Methylation of DNA and/or chromosomal proteins could inactivate the template properties of the chromatin. Methylation of RNA could interfere with its processing. Protein methylation could interfere with the function of the RNA polymerases.

We report in this paper that DMN inhibits the activity of the solubilized and partially purified nRNA polymerases while leaving the chromatin template intact, at least quantitatively, with respect to RNA synthesis. This apparent specificity in the mechanism of inhibition of RNA synthesis by DMN is exactly reproduced by the action of the methylating agent MMS on rat liver nuclei in vitro.

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1 The abbreviations used are: DMN, dimethylNitrosamine; MMS, methyl methanesulfonate; poly(dA-dT), poly(deoxyadenylate-deoxythymidylate); TGMED, 0.05 M Tris-HCl, pH 7.9; at 4°C containing 5 mM MgCl₂, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 25% (v/v) glycerol.

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MATERIALS AND METHODS

DMN (Eastman Kodak Co., Rochester, N. Y.) was repurified by distillation prior to use. Material boiling between 148 and 150° was collected and stored in a brown bottle. All animals were female Wistar rats (Charles River Breeding Farms, Wilmington, Del.) weighing 150 to 170 g and fasted overnight prior to use. DMN was administered by i.p. injection of 40 mg/kg (except where indicated in the text) as a 10-mg/ml solution in 0.9% NaCl solution. Control animals received an equal volume of 0.9% NaCl solution. All animals were sacrificed 4 hr after injection.

The preparation of rat liver nuclei and assay of their in vitro rate of RNA synthesis was as previously described (10). Where indicated, the RNA synthesis assay was modified by the addition of 5 μg actinomycin D (Sigma Chemical Co., St. Louis, Mo.) and 2 A260 units of poly(dA-dT) (Miles Laboratories, Kankakee, Ill.).

Autoradiography. Four hr after animals were given either DMN (40 mg/kg) or 0.9% NaCl solution they received 300 μCi [3H]orotic acid (20 Ci/m mole; New England Nuclear, Boston, Mass.). The animals were sacrificed 30 min later and liver sections were prepared for autoradiography according to the method of Baserga and Malamud (1). The sections were exposed for 7 weeks to NTB nuclear emulsion (Eastman Kodak, Rochester, N. Y.). The number of grains per nucleus in the hepatic parenchymal cells was determined by random count of 250 nuclei.

Measurement of the Size of Nascent nRNA. At Hr 4, control and DMN-treated animals (40 mg/kg) were given 250 μCi [5-3H]orotic acid (20 Ci/mmole, New England Nuclear) and were sacrificed 0.5 hr later. RNA was extracted from isolated nuclei by the method of Soiemo and Damnell (36). The RNA was washed 3 times in and resuspended in 0.04 M Tris-HCl, pH 7.2, at 4°; 0.02 M sodium acetate; 1 mM EDTA; and 0.5% sodium dodecyl sulfate. Approximately 40,000 cpm of control and DMN RNA were centrifuged on 5 to 20% linear sucrose gradients (prepared in the same medium). The gradients were fractionated into 20 fractions by centrifugation for 2 hr at 42,000 rpm in the Ti 60 rotor of a Spinco L2-65B ultracentrifuge to remove the DNA. The RNA was washed 3 times in and resuspended in 0.3 M to 2.0 M ammonium sulfate in 0.3 M to 1.0 M ammonium sulfate at pH 7.9, at 4° containing 1.0 M sucrose, 5 mM MgCl2, and 0.5 mM dithiothreitol is referred to as “nuclear suspension” in Tables 1 and 3. “Enzyme solubilization” refers to the product following addition of 3.0 M ammonium sulfate to the nuclear suspension, sonic disruption at 50 W for 20 sec with a Branson W-135 sonic oscillator, dilution of the sonic extract with 2 volumes TGMEG, and centrifugation for 1 hr at 42,000 rpm in the Ti 60 rotor of a Spinco L2-65B ultracentrifuge to remove the DNA. This solution was diluted with 2 volumes TGMEG and loaded directly onto an A-25 DEAE-Sephadex column (0.9 x 1.2 cm) equilibrated in TGMEG containing 0.03 M ammonium sulfate. The column was washed with this buffer until no A260 material was eluted. The polymerses were eluted with a linear gradient (50 ml) of ammonium sulfate from 0.03 to 0.50 M. Fractions were collected and assayed as previously described (10).

Treatment of Nuclei with MMS. Nuclei isolated from untreated animals were suspended in TGMEG at a concentration of approximately 4 mg DNA/ml. MMS (Eastman Kodak) was added while stirring to a final concentration as indicated in the text. The nuclei were then incubated at 25° for 15 min and then assayed for RNA synthesis or used for the preparation of chromatin or the RNA polymerases as described above.

RESULTS

Inhibition of nRNA Synthesis by DMN. Administration of a single dose of DMN to rats produces inhibition of liver nRNA synthesis. Chart 1 shows the rate of in vitro incorporation of [3H]UMP into RNA by whole nuclei isolated from the livers of a series of animals treated 4 hr previously with increasing doses of DMN. The dose-dependent inhibition of nRNA synthesis reaches a maximum of 40 to 50% with 40 mg/kg of body weight, there being no further inhibition with larger doses. This inhibition of in vitro RNA synthesis is reflected in an exactly comparable level of inhibition of liver nRNA synthesis measured in the intact animal.

Chart 2 shows the time course of the inhibition of nRNA synthesis that is produced by 40 mg DMN per kg. RNA synthesis decreases during the 1st 4 hr and remains inhibited through the 12th hr.

A preferential breakdown of the newly synthesized nRNA cannot explain the decreased rate of accumulation of radioactive RNA. Liver nRNA in control and DMN-treated animals...
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was labeled in vivo with [\(^{14}\)C]orotic acid. Nuclei from these animals were isolated and incubated in vitro under the conditions used to measure RNA synthesis. No difference in the rate of loss of acid-precipitable radioactivity from the DMN nuclei was detected. No evidence of a soluble inhibitor could be obtained by mixing equal aliquots of liver nuclei from control or DMN-treated animals. RNA synthesis was inhibited to the same extent in the nuclei isolated from DMN-treated animals when either [\(^{3}\)H]ATP, [\(^{3}\)H]GTP, or [\(^{3}\)H]CTP was substituted for [\(^{3}\)H]UTP in the assay. The size range of the newly synthesized RNA molecules was similar in nuclei of DMN-treated animals to that of control RNA, as shown by analysis of in vivo synthesized nRNA on linear sucrose gradients.

Two features distinguish the inhibition of RNA synthesis from the liver cell necrosis that also results from the acute administration of DMN. The degree of liver cell necrosis continues to increase with doses of DMN greater than 40 mg/kg. This in contrast to the absence of any further decrease in the rate of RNA synthesis with doses of DMN greater than 40 mg/kg. In addition, the lobular distribution of the 2 effects is different. The liver cell necrosis induced by 40 mg DMN per kg is very reproducibly found in the centrolobular zone of the hepatic lobule. The lobular distribution of the effect of the same dose of DMN on RNA synthesis was determined by autoradiography. Animals treated with either 0.9% NaCl solution or DMN for 4 hr were given 300 \(\mu\)Ci [\(^{3}\)H]orotic acid and sacrificed 30 min later. Chart 3 shows the distribution of nuclear grains in randomly counted nuclei from control and DMN-treated animals. In the control, there is a normal distribution of the number of grains per nucleus. There is no portion of the lobule that has a preferential increase or decrease in the rate of incorporation of the [\(^{3}\)H]orotic acid. A similar normal distribution that has been shifted to much lower numbers of grains per nucleus resulted from the DMN treatment. There is no evi-
Chart 3. Distribution of nuclear grain counts in autoradiographs from control and DMN-treated animals. Control animals (C) received 0.9% NaCl solution i.p. and the DMN-treated animals (A) received 40 mg/kg. Four hr later, all animals received 300 μCi [3H]orotic acid (20 Ci/mM; New England Nuclear) and were sacrificed 30 min later. Liver slices were prepared for autoradiography as described in "Materials and Methods." Slides were exposed for 7 weeks. Counts of the number of grains/nucleus were made in 250 random nuclei.

Chart 4. Template activity of chromatin prepared from nuclei isolated from control (O) or animals treated with 40 mg DMN per kg for 4 hr (A). Chromatin was prepared by the method of Reeder (30). The polymerase was E. coli RNA polymerase (20 μg). Inset, the endogenous polymerase of the same control (C) and DMN chromatin (A).

this chromatin, when determined with an added E. coli RNA polymerase, is the same as that of control chromatin. Chart 5 shows that a similar result is obtained with the DMN-chromatin, which is transcribed with added rat liver nucleoplasmic RNA polymerase. With the rat liver polymerase, there is no inhibition of the template capacity of the chromatin prepared from the DMN-treated animals. At the same time, there is inhibition of the activity of the endogenous polymerase activity.

The 2nd method of chromatin preparation exposes the nuclei to a higher salt concentration and to EDTA (7). With this method, more proteins are removed from the nucleo-protein gel. In particular, the endogenous polymerase activity is considerably reduced to the point where it is barely detectable with the same assay used to measure the endogenous activity in the previously described experiments. Chart 6 shows that the template activity of chromatin prepared by this method from the nuclei of DMN-treated animals is indistinguishable from the control chromatin with added rat liver nucleoplasmic RNA polymerase. A similar result was obtained with E. coli RNA polymerase.

The results of this series of experiments imply that the inhibition of RNA synthesis by DMN does not seem to be the consequence of the inactivation of the template properties of chromatin for the synthesis of RNA.

Inhibition of the Activity of the Solubilized RNA Polymerases. RNA polymerase activity was solubilized from a whole nuclear homogenate and fractionated on DEAE-Sephadex. Table 1 shows the recovery of RNA polymerase activity from nuclei prepared from control and DMN-treated animals. The data show the recovery at the end of the solubilization steps and following DEAE-Sephadex chromatography. In this experiment, the respective RNA polymerases were prepared from the same total amount of DNA as whole nuclei. Chart 7 shows the fractionation on DEAE-Sephadex of the RNA polymerase activity solubilized from the control and DMN-
Inhibition of RNA Polymerases by Methylating Agents

There is a decrease in the activity of both major polymerase species solubilized from the nuclei of DMN-treated animals. From Table 1 it can be seen that the total polymerase activity recovered from the DEAE-Sephadex chromatography of the DMN polymerases is 56% reduced with respect to the total activity recovered from control nuclei. There was 57% less polymerase activity in the original nuclear homogenate from which the DMN polymerase activity was prepared. The data in Table 1 also show that the reduced activity recovered from the DEAE-Sephadex column chromatography cannot be explained by a preferential loss during either solubilization and/or fractionation of the DMN polymerase activity. There is virtually quantitative recovery of the polymerase activity after its solubilization from the nuclei and its fractionation on DEAE-Sephadex.

In order to confirm the conclusion that there is inhibition of the activity of the RNA polymerases independent of any effect of the chromatin template on the activity of the polymerases, we measured the activity of the RNA polymerases in the intact nuclei by a procedure that inactivates the template properties of the endogenous template (42). Actinomycin D is added to whole nuclei at such a concentration as to virtually completely inhibit the function of the endogenous template. An artificial template, poly(dA-dT), to which actinomycin will not bind and yet which can be utilized by the RNA polymerases, is then added. RNA synthesis in the presence of the actinomycin D and poly(dA-dT) should reflect the activity of the RNA polymerases independent of their endogenous templates. We have previously shown that there is a strict correlation between the activity of the polymerases when measured in the whole nuclei in the presence of actinomycin and poly(dA-dT) and when measured by solubilization and ion-exchange chromatography (14, 29).

Table 2 shows the effect of actinomycin and poly(dA-dT) on the rate of incorporation of $[^3H]$UMP into RNA by liver cell nuclei isolated from control and DMN-treated animals. Actinomycin inhibits the RNA synthetic capacity of both nuclear preparations to the same extent. Addition of poly(dA-dT) and actinomycin increased the rate of incorporation of $[^3H]$UMP by both the control and DMN-treated nuclei. However, the DMN-treated nuclei were still inhibited

![Chart 6. Template activity of chromatin prepared from nuclei isolated from control animals (○) and animals treated with 40 mg DMN per kg (●). Chromatin was prepared by the method of de Pomerai (7). The added polymerase was rat liver nucleoplasmic polymerase (10,000 cpm of $[^3H]$UMP incorporated into RNA with calf thymus DNA at 50 μM UTP and with 1 μCi $[^3H]$UTP).](chart6)

![Chart 7. DEAE-Sephadex column chromatography of the RNA polymerases solubilized from the nuclei isolated from control animals (○) or animals treated with 40 mg DMN per kg for 4 hr (●). In each case, the polymerases were prepared from the same amount of whole nuclei (50 mg DNA).](chart7)

Table 1

<table>
<thead>
<tr>
<th>Purification step</th>
<th>$[^3H]$UMP incorporated into RNA (cpm)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DMN treated</td>
</tr>
<tr>
<td>Nuclear homogenate</td>
<td>2,105,040</td>
<td>920,305</td>
</tr>
<tr>
<td>Enzyme solubilization</td>
<td>2,060,330 (99)*</td>
<td>886,500 (96)</td>
</tr>
<tr>
<td>DEAE-Sephadex chromatography</td>
<td>1,888,430 (88)</td>
<td>796,800 (86)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of the total activity in the nuclear homogenate recovered at each subsequent step.
to the same extent as when RNA synthesis was measured without any additions to the assay. The data from these experiments support the conclusion that the polymerase activities themselves are inactivated by DMN.

Inhibition of RNA Synthesis by the In Vitro Methylation of Nuclei. Dimethyl nitrosamine reportedly methylates liver cell DNA, RNA, and protein (3-5, 17, 18, 23-25, 26, 37). Our results seem to indicate that, in spite of this apparent variety of substrates for methylation, there is some degree of functional specificity in the effect on nRNA synthesis. While inactivating the function of the RNA polymerases, the chromatin template is intact, at least quantitatively, for the synthesis of RNA. In the next series of experiments, we attempted to determine whether this specificity could be reproduced by the action of a methylating agent, in this case MMS, directly on normal liver cell nuclei in vitro.

Nuclei were prepared from untreated rats and incubated at 25° with increasing concentrations of MMS for 15 min. The nuclei were then assayed for RNA synthesis. Chart 8 shows that MMS produced a dose-dependent inhibition of RNA synthesis.

Preliminary experiments to rule out an accelerated breakdown of newly synthesized RNA and/or the production of a soluble inhibitor to explain the inhibition of RNA synthesis by MMS were negative. We then proceeded to evaluate the effect on the chromatin template and the RNA polymerases.

Chromatin was prepared from control and MMS-treated nuclei by the 2 methods described above, and the template capacity of the isolated chromatin was determined with E. coli and rat liver nucleoplasmic RNA polymerase in each case. Chart 9 shows that the template capacity of chromatin prepared from MMS-treated nuclei by lysis in a low ionic strength buffer and determined with the rat liver RNA polymerase is identical to that of the control, untreated chromatin. At the same time, the endogenous RNA synthetic capacity of the MMS chromatin is inhibited with respect to the control. A similar result was obtained when the chromatin was transcribed with the E. coli polymerase. Chart 10 shows that the template capacity with added rat liver RNA polymerase of chromatin prepared from MMS-treated nuclei by exposure to higher ionic strength and to EDTA is also indistinguishable from the template capacity of control chromatin. A similar result was also obtained if the same chromatin preparations were transcribed with added E. coli polymerase.

The effect of the in vitro treatment of whole nuclei with MMS on the activity of the solubilized and fractionated RNA polymerases was then examined. Chart 11 shows the fractionation on DEAE-Sephadex of the RNA polymerase activity solubilized from control nuclei and nuclei treated with MMS. There is a decrease in the activity of both major polymerase species solubilized from the MMS-treated nuclei. Table 3 shows that there is no loss of activity during preparation of either the control or MMS-treated polymer-
ases at each step of the procedure. There was 30% of the control activity present in the nuclear homogenate from the MMS-treated nuclei and 30% of the control activity was recovered from the DEAE-Sephadex column of the RNA polymerase activity.

Table 4 shows that this inhibition of the RNA polymerases can be demonstrated in the intact nuclei-treated MMS. Using the same conditions as with the nuclei from animals treated with DMN, actinomycin produces virtually complete inhibition of RNA synthesis in control and MMS-treated nuclei. Addition of poly(dA-dT) to the actinomycin-inhibited nuclei allows incorporation of \(^{3}H\)UMP. The MMS nuclei are still inhibited to the same extent as when assayed in the absence of actinomycin and poly(dA-dT).

In the final experiment, we looked at the pattern of nuclear protein methylation by MMS in an attempt to see if there is preferential methylation of the RNA polymerases. Nuclei from untreated animals were incubated with \(^{14}C\)MMS and the RNA polymerases were solubilized and chromatographed on DEAE-Sephadex.

Chart 12 shows that the incorporation of \(^{14}C\)methyl groups is proportional to the absorbing content of each fraction across the entire column without any relationship to the peaks of RNA polymerase activity.

**DISCUSSION**

The inhibition of rat liver mRNA synthesis by the *in vivo* action of DMN and the *in vitro* action of MMS seems to be due to a decreased activity of the RNA polymerases themselves and not to a decreased availability of the chromatin template. This conclusion is based on the results of 3 kinds of experiments: solubilization of the polymerases, measurement of the polymerase activity in intact nuclei independent of the endogenous template, and determination of the template capacity of chromatin. We have not shown that the inhibition of RNA synthesis results from methylation of the RNA polymerase molecules or, for that matter, that methyla-

![Chart 10. Template activity of chromatin prepared from control (○) and nuclei treated with MMS (■)]. Chromatin was prepared by the method of de Pomerai (7). The added polymerase was rat liver nucleoplasmic polymerase (10,000 cpn of \(^{3}H\)UMP incorporated into RNA with calf thymus DNA at 50 μM UTP and with 1 μCi \(^{3}H\)UTP).

![Chart 11. DEAE-Sephadex column chromatography of the RNA polymerases solubilized from MMS-treated nuclei. Ten mg DNA as whole nuclei were treated with 1.23 M MMS for 15 min at 25°. The RNA polymerases were then solubilized and chromatographed on DEAE-Sephadex as described in Materials and Methods. The control polymerases were prepared from 10 mg DNA as whole nuclei incubated at 25° without any additions. ○, MMS; ●, control.]

**Table 3**

*Recovery of total activity during preparation of rat liver mRNA polymerases from control and MMS-treated nuclei*

<table>
<thead>
<tr>
<th>Purification step</th>
<th>[^{3}H]UMP incorporated into RNA (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Nuclear homogenate</td>
<td>265,000</td>
</tr>
<tr>
<td>Enzyme solubilization</td>
<td>253,264 (95)*</td>
</tr>
<tr>
<td>DEAE-Sephadex chromatography</td>
<td>233,000 (88)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of the total activity in the nuclear homogenate recovered at each subsequent step.
tion per se is related directly or indirectly to the inhibition. Our results show that these 2 agents produce a similar functional consequence.

A reduced total RNA polymerase activity was recovered virtually quantitatively from both the nuclei isolated from animals treated with DMN and from nuclei treated in vitro with MMS. In each case the decrease in RNA polymerase activity recovered agreed closely with the extent of inhibition of RNA synthesis in the whole nuclei. The nucleoplasmic and nucleolar polymerases seem to be affected, but in both cases there is a proportionately greater reduction of the nucleoplasmic polymerase (20) to the nuclear assays in the presence of actinomycin-poly(dA-dT) gave the same degree of inhibition of [3H]UMP incorporation as seen with the nuclei in the absence of actinomycin-poly(dA-dT) (14, 29). This indicates that polymerase II is responsible for the same fraction of the total RNA synthesis under both conditions. At least with respect to polymerase II, our results with the DMN and MMS nuclei indicate that this enzyme is inhibited when assayed independent of the endogenous chromatin template in the intact nucleus.

In contrast to the effects on the activity of the RNA polymerases, we were unable to demonstrate an effect of either DMN or MMS on the quantitative template activity of isolated chromatin. The similar template capacities of the control and DMN- and MMS-treated chromatin preparations under all 4 conditions of estimation would seem to rule out any very significant effect of either of these agents on the template capacity of chromatin in the intact nucleus. The results taken together, then, of the enzyme solubilization studies, the experiments with actinomycin-poly(dA-dT) and the chromatin template determinations seem to imply that decreases in the activity of the RNA polymerases themselves are the most significant mechanisms whereby DMN and MMS inhibit the rate of RNA synthesis.

This apparent specificity in the mechanism of inhibition of RNA synthesis by both DMN and MMS contrasts with the reported variety of the macromolecular targets of these 2 alkylating agents. We have shown incorporation of [14C]methyl groups from MMS into nuclear proteins that chromatograph with all of the proteins that bind to DEAE-Sephadex. DMN has been shown to methylate proteins, RNA, and DNA under the conditions of our use of this agent. Some caution is needed, however, in ascribing the effects of DMN to methylation. It has been pointed out that DMN and other nitroso carcinogens are potentially capable of yielding other metabolites as well as alkylating intermediates (13). Among such possible decomposition products are nitrous acid, formaldehyde, and derivatives of hydroxylamine and hydrazine. Although it has not been possible to detect any of these compounds other than formaldehyde in rats treated with DMN (6, 13), the possibility exists that trace
amounts might be formed which react with available chemical groups in the cell and are destroyed themselves in the process.

The inability to demonstrate an effect of either DMN or MMS on the template capacity of nuclear chromatin is of interest in light of the well-documented ability of these agents to interact with DNA. Although the major products in DNA are the same (7-methylguanine) (16, 17), there are differences between the minor methylation products formed by the 2 drugs, and it may be argued that it is not completely valid to compare the functional effects of DMN and MMS in light of these differences. Very little guanine methylated at 0 to 6 is found with MMS (19, 28), while approximately 6% of the methylation of DNA by DMN is at this position (3, 28). It is conceivable that an agent that more exactly reproduces the pattern of DNA methylation produced by DMN would have a different effect than that of MMS on the chromatin template. A recent report by Grab et al. (12) would seem to make this only a very remote possibility. Using nuclei isolated from methylazoxymethanol acetate-treated rats, it was found that the chromatin template activity from RNA synthesis was essentially unimpaired with either E. coli or rat liver RNA polymerase. There was marked inhibition of UTP incorporation into RNA, however, when "aggregate" enzyme (equivalent to the endogenous polymerase activity of chromatin prepared by our 1st method) was the source of both template and RNA polymerase (12). A similar pattern of methylation of DNA by methylazoxymethanol acetate and DMN was observed in the intact rat (35). It would seem, therefore, that it has now been documented, with 3 separate agents the biological activity of which is presumably based on their ability to methylate cellular macromolecules, that there is no restriction of the template capacity of chromatin in the face of significant inhibition of RNA synthesis, which can in the cases of DMN and MMS be attributed to inhibition of the RNA polymerase activities. Our data do not exclude the possibility of there being more subtle effects of DMN and MMS on the function of the chromatin template. There could be changes in the qualitative template properties of the chromatin that would not be reflected in our quantitative assays.

From the results of previous work (12) and the present study, a picture is emerging of the functional consequences with respect to RNA synthesis of the exposure of animal cells to the action of methylating agents. Of interest is the similarity in the functional effects of DMN demonstrated in the present study and the recently reported effects of several other hepatocarcinogens on RNA metabolism. Ethionine, N-hydroxyacetilaminofluorene and aflatoxin B1 are, in addition to DMN, potent inhibitors of liver RNA synthesis. In each of these cases, inhibition of the solubilized and partially purified RNA polymerase activity has been demonstrated (10, 14, 27, 32). With the possible exception of aflatoxin, this inhibition of the polymerase activity does not seem to be accompanied by an inactivation of the template properties of the nuclear chromatin. Information is not available on the effects of more chronic exposure of liver cells to these agents at lower doses. Therefore, how these disturbances in RNA metabolism may be related to carcinogenesis can only be conjectured.

The development of cancer in many organs and tissues, including the liver, is preceded by the appearance of 1 or more new cell populations that differ from the original target cells and that are believed to be involved in the cellular evolution to malignant neoplasia (8, 11, 34). With respect to the mechanisms underlying chemical carcinogenesis, the information necessary for the altered phenotypes of these cells must obviously derive from the interaction of the chemical carcinogen and its target cell. At the present time, it is widely held that this interaction interferes with the chemical integrity of the DNA or with those mechanisms responsible for the differential utilization of the genetic information contained in this DNA. It is conceivable that inhibition of the transcriptional activity of the nRNA polymerases, if it occurs during the exposure of liver cells to chemical carcinogens under those circumstances known to induce malignant neoplasia, could provide a mechanism for altering the differential expression of the genome. Inhibition of the polymerase function without an apparent effect, at least quantitatively, on the chromatin template would reduce the level of genetic transcription without interfering with the integrity of this information. This could result in fewer repressors being made and for some genes to become derepressed. The significance of such a presumptive hypothesis is 2-fold. It suggests a mechanism for the variations in the control of gene action that may play a major role in the new patterns of cell behavior seen during the cellular evolution to cancer. What is more important, it suggests areas of study that would seem relevant to this aspect of the carcinogenic process.

REFERENCES

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