Influence of Hydrocortisone on the Binding of Nitrosoureas to Nuclear Chromatin Subfractions

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ABSTRACT

The effects of steroid-induced modifications of chromatin structure on the extent and sites of chloroethylnitrosourea binding to chromatin were studied using log-phase HeLa cells. The cells were exposed to 0.1 to 2.0 μM hydrocortisone for 22 hr; this resulted in depressed DNA synthesis while transcriptional activity was stimulated. Hydrocortisone had no effect upon cellular or nuclear uptake of the two nitrosoureas under study, 0.6 mM chlorozotocin or 1-(2-chloroethyl-3-cyclohexyl-1-nitrosoureido). Both drugs were found to alkylate transcriptional chromatin preferentially, as demonstrated by DNase II and DNase I digestion. This alkylation was stimulated 2-fold by the same micromolar concentrations of hydrocortisone, 0.1 to 2.0 μM, which stimulated transcription. The extent of nuclear RNA alkylation, determined using RNase T2 as a probe, was found to contribute less than 20% of total chromatin alkylation and was unaffected by steroid pretreatment. Instead, the increased alkylation within these chromatin subfractions was attributed to a steroid-induced alteration of chromatin structure. Electron microscopic examination of HeLa nuclear morphology revealed a hydrocortisone-induced disaggregation of nuclear membrane-associated heterochromatin resulting in a more heterogeneous, less condensed distribution of chromatin. Such data are consistent with a relaxation of the supercoiled chromatin structure, resulting in increased transcription and increased accessibility of potential target sites for nitrosourea alkylolation.

INTRODUCTION

The bulk of eukaryotic chromatin is believed to be organized into nucleosomes which have a double tetrameric core of histones, around which DNA is coiled (22, 29, 35). Acidic and basic non-histone proteins and RNA are integrated into the overall chromatin array, the resultant nucleosomes being arranged in superhelical structures which are densely packed (4). The expression of the genetic material contained in this chromatin is repressed and is contingent upon the "opening up" of the supercoil allowing access to the RNA polymerase enzyme complex. Transcriptionally active and inactive regions of chromatin have structural differences which have been correlated with their differing susceptibilities to specific endonucleases. DNase II-digestible, Mg2+-soluble chromatin, extensively characterized in hybridization studies, has been found to be enriched with nascent RNA (6-10). It was concluded that this chromatin subfraction was the site of in vivo transcriptional activity. Similarly, DNase I-digested chromatin, under the conditions used in this study, has been shown to be transcriptionally active (5, 14, 32). Our previous studies have demonstrated that alkylation and carbamoylation by the chloroethylnitrosoureas, CLZ, and CCNU, occurred preferentially within transcriptional chromatin (24, 27). This finding was in concurrence with the theory that extended regions of chromatin were the preferred targets for these drugs. In addition, covalent drug binding was stimulated by pretreatment of HeLa cells with sodium butyrate (27), a fatty acid which increased transcriptional activity in this cell line (13). This, together with the fact that steroids such as estradiol have nuclear receptor sites which are preferentially localized within transcriptional chromatin (11), stimulated these studies with steroid-nitrosourea combinations.

Corticosteroids are routinely used in combination with alkylating agents in the chemotherapy of human cancers, in particular lymphoproliferative disorders, and in breast cancer (25). Although these hormones are thought to modulate gene regulation in target cells after the formation of a receptor-hormone complex and its translocation from the cytoplasm to the nucleus (31), the mechanism by which this complex interacts with chromatin to control nuclear function is not understood. The studies described herein were designed to determine (a) whether corticosteroid-induced perturbations of nuclear structure could influence the quantitative and qualitative interactions of nitrosoureas within specific regions of chromatin and (b) whether such interactions could provide a molecular rationale for steroid-alkylating agent therapeutic combinations.

MATERIALS AND METHODS

Cell Cultures

HeLa S3 cells were maintained at 37° in spinner flasks in Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) that contained 10% fetal calf serum (Grand Island Biological Co.). Cells were harvested by centrifugation and washed with spinner salts (0.68% NaCl-0.04% KCl-0.02% MgCl2-0.15% Na2HPO4-0.1% glucose). In selected experiments, HC (21-sodium succinate) (Sigma Chemical Co., St. Louis, Mo.) was added to logarithmically growing cells to final concentrations of up to 2.0 μM; the cells were then incubated for 22 hr at 37°. Nuclei were prepared from washed cell samples by the method of Sporn et al. (23).

Radioisotopes and Drug Treatment

\[\text{[Chloroethyl-}^{14}\text{C]}\text{CLZ} \quad (13.73 \text{ Ci/mol}), \quad \text{[chloroethyl-}^{14}\text{C]}\]

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2 To whom requests for reprints should be addressed.

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CCNU (10.9 Ci/mol), and 1-(2-chloroethyl)-3-{cyclohexyl-1-14C}cyclohexyl-1-nitrosourea (8.37 Ci/mol) were supplied by Drs. Harry Wood and Robert Engle, Drug Development Branch, National Cancer Institute, Bethesda, Md. CLZ was dissolved in citrate buffer (pH 4.0), and CCNU was dissolved in absolute ethanol (stored at −20°). [5-3H]Juridine (29 Ci/mmole) and [6-3H]dThd (15 Ci/mmole) were obtained from New England Nuclear (Boston, Mass.). Aqueous radioactive samples were diluted with Aquasol II (New England Nuclear) and counted on a Mark III scintillation counter (Searle Analytic, Inc., Des Plaines, Ill.), with a 40% counting efficiency for tritium.

Calculation of Cellular and Nuclear Incorporation

Following incubation of log-phase HeLa cells with radiolabeled drugs or macromolecular precursors, the percentage of acid-precipitable radioactivity incorporated into whole cells and nuclei was measured using the following information.

Percentage of Cellular Incorporation. Percentage of cellular incorporation is total radioactivity in whole cells (washed with spinner salts), divided by total radioactivity added to original cell suspension.

Percentage of Nuclear Incorporation. Percentage of nuclear incorporation is 10% trichloroacetic acid-precipitable radioactivity in whole nuclei after extraction and washing (27), divided by total radioactivity added to original cell suspension.

dThd and Uridine Pulse Label Experiments

Short-term incubation of [3H]dThd and [3H]Juridine (15 or 30 min at 37°) with cell suspensions were terminated by the addition of a 2-fold excess of iced spinner salts (to terminate incorporation), followed by centrifugation at 3000 rpm for 10 min. The resultant cell pellets were washed with ice-cold spinner salts prior to biochemical extraction procedures.

Isolation and Enzymatic Fractionation of Chromatin

DNase II. Chromatin was isolated from control and drug-treated HeLa cells by the method of Gottesfeld and Partington (10). Briefly, cells were incubated at 37° for 2 hr with radiolabeled drugs or macromolecular precursors, the percentage of acid-precipitable radioactivity incorporated into whole cells and nuclei was measured using the following information.

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RESULTS

Steroid-induced Modifications of Transcription and DNA Synthesis. Incubation of log-phase HeLa cells with μM concentrations of HC (0.1 to 2.0 μM) resulted in a hormone-mediated stimulation of transcription and inhibition of DNA synthesis, as measured by [3H]uridine or [3H]dThd incorporation (Table 1). The acid-precipitable nuclear uptake of uridine (15-min pulse) was stimulated in a hormone concentration-dependent manner, being approximately 12 times greater in cells treated with 2.0 μM HC than in control cells receiving no hormone. The nuclear uptake of [3H]dThd was inhibited by steroid treatment, with cells treated with 2.0 μM HC demonstrating one-third the incorporation of dThd when compared with control.

After chromatin fractionation, it was found that DNA synthesis was inhibited in total chromatin by 60%, a value equivalent to the reduction in precursor uptake. Approximately 30% of this radioactivity was localized in the extended chromatin (Mg2+-soluble) fraction. It is possible that the hormone-induced reduction in DNA synthesis was a function of alterations in precursor pools (28) or a direct result of HC interaction with chromatin. Cell viability remained >95% (as measured by trypan blue exclusion), although the rate of cell division was reduced, and the nuclear morphology of some cells (see Fig. 4) was abnormal. Chromatin fractionation of the uridine-labeled cells showed that transcription was stimulated approximately 6-fold by treatment with 1.0 or 2.0 μM HC (Table 1). This was emphasized further in the extended Mg2+-soluble chromatin fraction in which a 10-fold stimulation was observed with 2.0 μM HC. The preferential localization of uridine label (representing nascent RNA) in the Mg2+-soluble chromatin (ratio, 5 to 10) confirmed that this fraction represented transcriptionally active chromatin.

Acid-precipitable uridine incorporation was 8050 dpm/10^5 cells 22 hr after treatment with 1 μM HC. This value was between 17 and 32% greater than similar values measured 1, 2, 4, and 8 hr post-steroid administration. Therefore, the 22-hr point was used for subsequent drug studies with the understanding that different scheduling of these drugs may prove more effective when applied to an in vivo situation.

Nitrosourea Interaction with DNase II-sensitive Chromatin. Both CLZ and CCNU decompose at neutral pH to yield chloroethyl carbonium ions which alkylate cellular macromolecules (15). Additionally, CCNU decomposition yields a cyclohexyl isocyanate moiety which carbamoylates cellular proteins (33). The alkylation and carbamoylation potential of these compounds was confirmed by measuring covalent association of the requisite 14C label with nuclear macromolecules (Table 2). After a 2-hr incubation, the acid-precipitable cellular incorporation of the chloroethyl group of CLZ was 30 to 40% higher than that of CCNU, totaling approximately 1% of the available drug. In contrast to the data for dThd and uridine, neither the cellular nor the nuclear incorporation of the drugs was altered by HC treatment. Nuclear incorporation of the isocyanate group of CCNU was unaffected by HC but was approximately 8 times greater than that for the alkylating group.

Following isolation of whole chromatin from nuclei, the extent of alkylation by CLZ in cells without HC pretreatment was 132 pmol/A260 unit as compared with 106 pmol/A260 unit for CCNU; HC treatment suppressed this alkylation, but the reduction was not significant. Alkylation of nuclear proteins occurs at a level equivalent to 10 or 20% that of nuclear DNA when expressed as pmol/μg of the respective macromolecule (27). Nuclear protein alkylation was unaffected by HC pretreatment (data not shown). Carbamoylation of chromatin (mainly nuclear proteins; see Ref. 27) was unaffected by HC. However, the overall levels of carbamoylation by CCNU were at least 20 times greater than alkylation (2264 pmol/A260 unit in the absence of HC). This quantitative differential was maintained in the DNase II-digested, Mg2+-soluble fraction in which 816 pmol/A260 unit of the carbamoylating moiety was bound, as compared with 46 pmol/A260 unit of the alkylation moiety. Minimal proteolysis had occurred during the extraction procedure (data not shown), the presence of phenylmethylsulfonyl fluoride and sodium tetraphosphate in all solutions acting as protease inhibitors.

In contrast to whole chromatin, HC pretreatment caused a significant increase in both alkylation and carbamoylation within the Mg2+-soluble chromatin fraction. While the increased drug binding was not stoichiometric with respect to HC concentration, each value for HC-pretreated cells was significantly different from control with the exception of CCNU carbamoylation at 0.1 and 1.0 μM (Table 3, Column 4).

The Influence of Nitrosoureas on DNase II Digestion: Preferential Alkylation. Our previous findings (24, 27), as well as data from other studies (2), have shown that nitrosoureas and other classes of alkylating agents do not alter the specificity of endonuclease cleavage. In confirmation, Table 3 shows that approximately 30% of the total material which absorbed UV at 260 nm was liberated by DNase II under the conditions described. This corresponded to approximately 10% of the DNA, the difference representing RNA and protein. Neither HC nor nitrosourea treatment significantly affected these values (p < 0.1).

Within this digested chromatin, there was preferential localization of alkylated and carbamoylated material. For CLZ, approximately 33.7% of the chloroethyl label was present in 12.5% of the DNA or 22.9% of the A260 unit. For CCNU, this ratio was 37.6% in 12.1% DNA or 24% of the A260 unit.

The preferential alkylation of Mg2+-soluble chromatin compared to total chromatin was more apparent when data were

Table 1

<table>
<thead>
<tr>
<th>Precursor</th>
<th>HC (μM)</th>
<th>% of nuclear incorporation of precursor</th>
<th>Acid-insoluble incorporation (dpm/A260 unit × 10^-5)</th>
<th>Mag2+-soluble chromatin a</th>
<th>Mag2+-soluble chromatin/Whole chromatin</th>
<th>Mag2+-soluble chromatin/Whole chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>0</td>
<td>0.4</td>
<td>0.27</td>
<td>1.57</td>
<td>5.8</td>
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<tr>
<td></td>
<td>0.1</td>
<td>1.8</td>
<td>1.31</td>
<td>9.56</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.1</td>
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<td>14.5</td>
<td>8.0</td>
<td></td>
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<td>2.0</td>
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<td>1.79</td>
<td>16.3</td>
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<tr>
<td>dThd</td>
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<td>2.26</td>
<td>0.59</td>
<td>0.26</td>
<td></td>
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<tr>
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<td>0.1</td>
<td>1.8</td>
<td>2.04</td>
<td>0.63</td>
<td>0.30</td>
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<td>1.0</td>
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<td>0.67</td>
<td>0.32</td>
<td>0.48</td>
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<td>2.0</td>
<td>0.9</td>
<td>0.75</td>
<td>0.28</td>
<td>0.37</td>
<td></td>
</tr>
</tbody>
</table>

a Mean of 3 experiments. S.E. <20% of mean.

b Mean of 4 experiments. S.E. <20% of mean.

OCTOBER 1980
Table 2

Acid-insoluble cellular, nuclear, and chromatin drug incorporation following HC

Log-phase HeLa cells (4 x 10⁶/ml) were incubated for 22 hr with HC at the concentrations shown. Cell cultures were concentrated (see "Materials and Methods") prior to drug treatment (0.6 mM for all labeled drugs). Cellular and nuclear uptake and chromatin fractionation were measured as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Drug</th>
<th>HC (µM)</th>
<th>% of cellular incorporation*</th>
<th>% of nuclear incorporation*</th>
<th>Whole chromatin</th>
<th>Mg²⁺-soluble chromatin</th>
<th>Mg²⁺-soluble ¹⁴C as % of control (0 µM HC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLZ (chloroethyl) alkylation</td>
<td>0</td>
<td>0.92</td>
<td>0.24</td>
<td>132</td>
<td>41</td>
<td>100</td>
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<tr>
<td></td>
<td>0.1</td>
<td>0.88</td>
<td>0.23</td>
<td>126</td>
<td>66</td>
<td>159</td>
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<td>1.0</td>
<td>0.14</td>
<td>114</td>
<td>85</td>
<td>206</td>
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<td>2.0</td>
<td>1.1</td>
<td>0.12</td>
<td>109</td>
<td>89</td>
<td>216</td>
</tr>
<tr>
<td>CCNU (chloroethyl) alkylation</td>
<td>0</td>
<td>0.75</td>
<td>0.36</td>
<td>106</td>
<td>46</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.86</td>
<td>0.57</td>
<td>82</td>
<td>64</td>
<td>139</td>
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<tr>
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<td>1.0</td>
<td>0.86</td>
<td>0.44</td>
<td>63</td>
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<td>129</td>
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<tr>
<td></td>
<td>2.0</td>
<td>0.90</td>
<td>0.38</td>
<td>109</td>
<td>66</td>
<td>140</td>
</tr>
<tr>
<td>CCNU (cyclohexyl) carbamoylation</td>
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<td>0.29</td>
<td>2265</td>
<td>816</td>
<td>100</td>
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<td>2.50</td>
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<td>2.0</td>
<td>2.05</td>
<td>0.23</td>
<td>2345</td>
<td>1242</td>
<td>152</td>
</tr>
</tbody>
</table>

* Mean of 3 experiments. There is no significant difference between HC-treated and untreated cells (p > 0.1).

The amount of drug bound to chromatin from cells without HC pretreatment was standardized at 100% and each of the values for HC-treated cells was expressed as a function of this control. All values for HC-treated cells differ significantly from control (p < 0.05), except CCNU carbamoylation with HC concentrations of 0.1 and 1.0 µM.

Table 3

Proportional interaction of nitrosoureas with chromatin following HC treatment and DNase II digestion

Experimental details are as for Table 2. Values for DNase II digestion of chromatin from cells receiving neither HC nor nitrosoureas: percentage of DNA, 10.5 ± 2.6; percentage of A₂₆₀ unit, 34 ± 5.8. By analysis of variance, these values do not differ significantly from those from cells receiving HC or nitrosourea pretreatment. Data are expressed as Mg²⁺-soluble chromatin/total chromatin x 100.

<table>
<thead>
<tr>
<th>Drug</th>
<th>HC (µM)</th>
<th>% of DNA</th>
<th>% of A₂₆₀ unit/ml</th>
<th>% of ¹⁴C</th>
<th>pmol/mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLZ alkylation</td>
<td>0</td>
<td>12.5</td>
<td>22.9</td>
<td>33.7</td>
<td>112²</td>
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<tr>
<td></td>
<td>0.1</td>
<td>10.6</td>
<td>24.0</td>
<td>42.2</td>
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<td></td>
<td>1.0</td>
<td>12.1</td>
<td>23.6</td>
<td>43.4</td>
<td>173</td>
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<tr>
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<td>2.0</td>
<td>12.5</td>
<td>25.1</td>
<td>36.4</td>
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</tr>
<tr>
<td>CCNU alkylation</td>
<td>0</td>
<td>12.1</td>
<td>24.0</td>
<td>37.6</td>
<td>123⁴</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
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<td>25.8</td>
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<td>2.0</td>
<td>11.9</td>
<td>24.8</td>
<td>41.6</td>
<td>152</td>
</tr>
</tbody>
</table>

² Taken as a group, values of drug incorporation in HC-treated cells differ significantly from those of control (p < 0.05).

nRNA Alkylation. RNase T₂ released approximately 90% of the tritium incorporated from a [³H]uridine pulse, confirming that most of the chromatin-associated RNA was released under the digestion conditions used (Table 4). Ten % of [³H]dTthd was also released, suggesting either that a proportion of the labeled DNA was being digested or that the label was metabolized to a form which could be incorporated into RNA (26). Treatment with HC caused no significant alteration in the release of uridine or dThd. In whole chromatin, less than 20% of the total nitrosourea alkylation was in RNA; however, exact quantitation is impossible, since approximately 10% of the nRNA remained
Steroid Nitrosourea Combinations

Chart 1. DNase I digestion of HeLa cell chromatin after treatment with CLZ (A) or CCNU (B). Drug treatment was for 2 hr at 0.6 μM. Ten units of DNase I per A260 unit were added at time 0, and samples were taken at the various times shown. Ordinate, ratio of the percentage of digested alkylated nucleotides as a function of bulk DMA digestion. A.S., acid soluble; •, no hormone pretreatment; O, 0.1 μM HC for 22 hr; A, 2.0 μM HC for 22 hr.

Table 4

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole chromatin</td>
<td>0</td>
<td>87.9 ± 2.8</td>
<td>18.7 ± 4.3</td>
<td>15.0 ± 3.0</td>
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<tr>
<td>0.1</td>
<td>86.5 ± 4.0</td>
<td>9.1 ± 2.0</td>
<td>15.7 ± 4.6</td>
<td>16.9 ± 4.3</td>
</tr>
<tr>
<td>1.0</td>
<td>86.0 ± 4.2</td>
<td>9.3 ± 2.1</td>
<td>16.4 ± 4.2</td>
<td>15.1 ± 3.2</td>
</tr>
<tr>
<td>2.0</td>
<td>87.9 ± 3.6</td>
<td>10.4 ± 2.1</td>
<td>16.2 ± 4.2</td>
<td>16.8 ± 3.9</td>
</tr>
<tr>
<td>Mg²⁺-soluble chromatin</td>
<td>0</td>
<td>86.3 ± 2.6</td>
<td>15.4 ± 3.9</td>
<td>21.0 ± 4.6</td>
</tr>
<tr>
<td>0.1</td>
<td>85.2 ± 3.3</td>
<td>13.6 ± 1.9</td>
<td>20.5 ± 5.6</td>
<td>10.0 ± 2.8</td>
</tr>
<tr>
<td>1.0</td>
<td>86.8 ± 4.7</td>
<td>15.0 ± 4.2</td>
<td>20.3 ± 5.2</td>
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<tr>
<td>2.0</td>
<td>84.9 ± 5.0</td>
<td>12.1 ± 2.9</td>
<td>20.3 ± 6.1</td>
<td>8.8 ± 2.0</td>
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</tbody>
</table>

*Mean ± S.E. for 3 experiments.*

Although the chemistry of DNA alkylation is well understood, the importance of the molecular configurations of chromatin to this reaction requires clarification. Such considerations are complicated by the variety of nuclear metabolic functions which result, at any one time, in the destabilization of the chromatin superstructure. Briefly, we have considered how such config-

undigested and approximately 10% of the DNA was digested. There were no differences in RNA alkylation between CLZ and CCNU, and HC did not affect the percentage of solubilized 14C. In the Mg²⁺-soluble fraction, alkylation of nRNA by CLZ was approximately twice that of CCNU, but neither was significantly altered by HC.

Nuclear Morphology. The electron micrographs shown in Figs. 1 to 4 illustrate the alterations in nuclear-structural morphology of interphase HeLa cells induced by HC treatment. Normal HeLa cell nuclear morphology (Fig. 1) had diffuse regions of dispersed, lightly stained chromatin in the central part of the nucleus (euchromatin) with more densely stained heterochromatin at the periphery, associated with the nuclear membrane and nucleolus. Progressive disaggregation of the heterochromatin was found in nuclei of cells treated with HC (Figs. 2 and 3). Chromatin appeared less condensed at the periphery and, although the nucleolus was still identifiable, it too was less heavily stained. A proportion of the cells elicited the morphology shown in Fig. 4. The nuclear membrane-chromatin associations have broken down, causing a separation of the membrane lamellae. The chromatin, although generally dispersed (as in Fig. 3), had reaggregated in seemingly random arrays. The granular structure of the nucleolus was maintained. Such nuclear structure is presumably a prelude to the break-

such nuclear integrity and eventual cell death, suggesting that HC per se exerted a cytotoxic effect towards HeLa cells.

DISCUSSION

Although the chemistry of DNA alkylation is well understood, the importance of the molecular configurations of chromatin to this reaction requires clarification. Such considerations are complicated by the variety of nuclear metabolic functions which result, at any one time, in the destabilization of the chromatin superstructure. Briefly, we have considered how such config-

Fig. 1. Untreated HeLa cells. Lightly stained euchromatin is surrounded by more densely stained, membrane-associated heterochromatin (large arrow). Small arrow, nuclear membrane showing a nuclear pore and both inner and outer lamellae. The nucleolus is the dark-staining area juxtaposed to the membrane on the right center of the nucleus. × 15,000.

Fig. 2. HeLa cells pretreated for 22 hr with 1 μM HC. The nuclear membrane (large arrow) is less clearly defined, and the membrane-associated heterochromatin (large arrow) is less densely stained. × 15,000.
Fig. 3. HeLa cells pretreated for 22 hr with 1 μM HC. Details are as for Fig. 2. x 15,000.

Fig. 4. HeLa cells pretreated for 22 hr with 1 μM HC. The breakdown of nuclear structural integrity is apparent. The inner and outer membrane lamellae have become separated (2 opposed large arrowheads) with the inner being pulled inwards with the chromatin, which has formed irregular clumps (2 small arrowheads). The nucleolus (small arrowhead) has begun to break down and is located between chromatin aggregates. x 15,000.

Transcriptional mobility affects the interaction of nitrosoureas with regions of transcriptionally active chromatin.

The relative merits of techniques used to isolate transcriptionally active chromatin have been reviewed elsewhere (9). The DNase II technique has the advantage of producing small chromatin fragments which have minimal disruption of nucleoproteins and few of the artifacts produced by physical shearing. Quantitatively, the amount of DNA recovered in the Mg²⁺-soluble fraction has been shown to vary between tissues but to correlate with the transcriptional activity of the cell type under investigation (1). Similarly, DNA undergoing transcription has been shown to be highly sensitive to digestion by DNase I compared to bulk chromatin DNA (5, 14, 32). The present data show that both DNase I- and DNase II-digested chromatin regions are preferentially alkylated by CLZ and CCNU. This is in agreement with our previous findings using a column chromatography technique (27) and adds evidence to the theory that transcriptional chromatin, possibly because of its less constrained structure, acts as a preferential target for these alkylating agents.

This theory gains further credence from the finding that sodium butyrate causes new genomic expression in Friend erythroleukemic cells (16). A destabilization of the transcriptionally inactive chromatin may result in a quantitative increase in the proportion of the genome which undergoes transcription. This could account for both the novel gene expression and the observed stimulation of chromatin alkylation by both CLZ and CCNU (27). Steroids characteristically increase transcriptional activity in target cells, such being the case for HeLa cells. The observed increase in alkylation within the nuclease-sensitive chromatin regions could be a function of the steroid-induced alteration of chromatin structure. Mechanistically, this would involve interaction between the steroid-receptor complex and chromatin. In HeLa cells, HC receptors are both cytoplasmic and nuclear, but at 37° the nuclear receptor system predominates (21). In chick oviduct cells, progesterone has been shown to cause DNA unwinding (19, 20). In prostatic chromatin, androgen receptor binding required intact link DNA (17), and there is evidence that all DNA functions originate in this internucleosomal chromatin (18). This is consistent with our preliminary data which show that HC treatment of HeLa cells alters the chromatin-digestion pattern produced by micrococcal nuclease (26). Potentially, such steroid interactions can alter chromatin structure to yield the observed increase in alkylation. Both CLZ and CCNU preferentially alkylate DNA which is not susceptible to micrococcal nuclease (24, 27); it is possible that HC alters the structural characteristics of this internucleosomal DNA and in so doing confers a susceptibility to both the RNA polymerase system and to increased alkylation.

The relevance of the increased alkylation within DNase I- and DNase II-sensitive regions of chromatin may relate to increased cytotoxicity. It is known that the correct scheduling of prednisolone and chlorambucil is capable of killing rat carcinoma cells which are resistant to the alkylating agent alone (34). These studies have shown by electron microscopy that the combination causes gross alterations of nuclear morphology. Our microscopic observation of HC-treated cells showed details of chromatin rearrangement which were consistent with an increased availability of disaggregated transcriptional chromatin for drug interaction. The fact that alkylation of bulk nuclear chromatin was unaltered by HC pretreatment may have been a function of the proportional increase in the number of dying cells (cf. Fig. 4) within the cell population. The steroid-induced decondensation of chromatin architecture did not occur in all of the cells; this served to create a subpopulation of cells, the chromatin of which could act as a preferential target for alkylation. It remains to be shown whether the 1.5- to 2-fold increased alkylation within that portion of the genome which is actively transcribing is more important to cytotoxicity than is the drug binding which occurs within “functionally inert” or redundant chromatin. Such a hypothesis would not appear unreasonable. These studies have generated preliminary data.
which are pertinent to the molecular aspects of steroid-alkylating agent combinations and their interaction with chromatin. Since the concentrations of both nitrosoureas and HC are consistent with those found in some cancer patients, such data may have potential biological and clinical relevance.

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REFERENCES


Steroid Nitrosourea Combinations

Influence of Hydrocortisone on the Binding of Nitrosoureas to Nuclear Chromatin Subfractions


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