Actinomycin D Effects on Nucleic Acids during Tumor Regression

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SUMMARY

Early effects of sublethal doses have been compared in sensitive Ridgway osteogenic sarcoma (ROS) and resistant (DMBA) tumors [designation for a spindle cell sarcoma originally induced in AKR mice with 7,12-dimethylbenz(a)anthracene] and in small intestine in mice. Doses of actinomycin which were tumoricidal for the sensitive tumor were also toxic to intestinal crypt cells; DNA synthesis, estimated with pulses of labeled precursors, was almost completely blocked in 24 hr in ROS and intestine, while RNA synthesis was unaffected or only partially inhibited. At earlier times (2, 4, and 8 hr) RNA synthesis was also inhibited partially while DNA synthesis was unaffected. These results show that extreme inhibition of precursor incorporation into RNA by actinomycin in susceptible cells is not a necessary antecedent to cytotoxicity in vivo. RNA, DNA, and protein concentrations were measured in ROS and DMBA tumors and in intestine. The only significant change occurred in ROS which lost 30-40% of its RNA within 24 hours after treatment. Since the ROS tumor is more susceptible to damage than are other tissues, the extensive loss of RNA may be associated with actinomycin's tumoricidal action.

INTRODUCTION

For several years, actinomycin D has been subject to intensive study (9), but a causal relation between its primary biochemical inhibition of RNA synthesis (4) and its cytotoxic properties have not yet been established. In the present study we have attempted to find evidence for this relationship by comparing the effects on nucleic acids in a sensitive transplantable mouse tumor, ROS3, with those produced in a resistant tumor (DMBA) and in small intestine. ROS is the only solid tumor in experimental animals which is completely destroyed by a single dose (400-1200 μg/kg) which is sublethal to the host (2, 15, 18). A dose in this range has little effect on DMBA tumor but damages normal host proliferating cells, such as those found in the intestinal mucosa, lymphoid organs and bone marrow (8, 15). We selected the intestine for comparison with the tumors for several reasons. Its sensitivity resides in the few proliferative cells in the crypt epithelium and, although these represent only a small fraction of the total organ mass, their response is specifically measured by the extent of inhibition of thymidine incorporation into DNA (16). In contrast, the inhibition of precursor incorporation into RNA is a measure of the response of the entire intestine which is predominantly nonproliferative. Further, actinomycin stimulates the pituitary-adrenal axis and some of its effects may be mediated by adrenocorticooids (8, 13); ROS and intestine, unlike lymphoid tissue in thymus and spleen, are not destroyed by glucocorticoids (13, also see Discussion).

The present study indicates that tumoricidal doses of actinomycin inhibit RNA synthesis and produce striking losses of RNA. The depletion of RNA could result from the decreased rate of synthesis over a prolonged period of time or from activation of a degradative mechanism. In either case, the depletion may prove to be directly responsible for this tumor's unique sensitivity to the agent. In the intestine RNA synthesis is hardly affected by tumoricidal doses that severely damage the crypt epithelium, and neither intestine nor DMBA tumor lose RNA in amounts comparable to ROS tumor.

Some of the results described here have appeared in a preliminary report (14).

MATERIALS AND METHODS

Ridgway osteogenic sarcoma was carried in female AKD2F1 mice as a stock line and in AKR females (Jackson Memorial Laboratory) for experiments. DMBA tumor was obtained from Dr. Franz Schmid of this Institute and was carried only in AKR females. Tumors were transplanted aseptically every 2 or 3 weeks by s. c. trocar injection of 1-2 mm pieces into the axillary region of mice weighing 16-18 gm (15). For 4-5 days after implantation oxytetracycline (Pfizer, 7.5 gm/liter) was added to the drinking water. In experiments with doubly implanted mice, ROS and DMBA tumors were placed in contralateral axillary positions. All experiments began during the 3rd or 4th week after implantation, when tumors were established and measurable in situ. Average tumor diameters were estimated with calipers at the maxima in 2 dimensions. Mice were killed by cervical dislocation; sections taken for histopathology were fixed in Bouin's solution, cut at 7 μ, and stained with hematoxylin and eosin.

AKR mice were used in all experiments and received injections in the tail vein. Actinomycin D was generously provided by Merck, Sharp and Dohme Research Laboratories. Stock solutions contained 200 μg/ml and were diluted with sterile pyrogen-free saline on the day of injection so that the doses...
were given in 0.1 ml/10 gm body weight. Inorganic phosphate-
$^{32}$P (Squibb, 100 µc/ml H$_2$O) was administered free of added
carrier at a dose of 10 mc/kg body weight; results were cor-
rected for decay. Uridine-2-14C (30 µc/µmole) and thymidine-
2-14C (30 µc/µmole) were purchased (New England Nuclear
Corp.) and given in doses of 200 and 40 µc/kg respectively.

In analytic studies, tissues from at least 2 animals were
routinely pooled. Mice were killed by cervical dislocation and
pooled tissues were iced and used immediately or frozen on
solid CO$_2$. For incorporation studies, iced tissues were cleaned,
pooled, and homogenized in water; 4 volumes of 10% trichloro-
acetic acid were added as precipitant and the pellet was washed
4 times with 10% trichloroacetic acid (twice for 15 min at
90°C) for labeled thymidine studies or separated into RNA and
DNA fractions by conventional procedures (with 0.4 M NaOH
at 37°C for 18 hr to hydrolyze RNA, followed by acid pre-
cipitation of DNA) for labeled uridine studies (13). After $^{32}$P
injections, similar procedures were used except that nucleic
acids were extracted 3 times from defatted pellet with 10% NaCl
at 90°C; the soluble portions were dialyzed 4-6 hr
against cold 1 M NaCl and reprecipitated with trichloroacetic
acid (10%) and 2 volumes of cold alcohol. The pellet was then
dissolved in 0.4 M NaOH and RNA was hydrolyzed at 37°C
for 18 hr; thereafter the solution was acidified and DNA was
precipitated. Radioactivity in the separated fractions was
measured by liquid scintillation techniques with internal standards
(13).

RNA, DNA, and protein were estimated colorimetrically.
The nucleic acids were separated as described above for the
studies with labeled uridine; aliquots of the fractions dissolved
in 0.4 M NaOH were taken for protein determinations (7).
RNA was measured by the orcinol reaction, and DNA by the
diphynylamine reaction (10). All values refer to fresh weight
of tissues.

RNA from ROS tumors was isolated from both nucleic
and cytoplasmic fractions for sedimentation studies in sucrose
gradients. Methods previously described (1, 11) were modified
slightly because it was found that naphthalene disulphonate,
which is useful for preparing RNA from rat liver, was un-
necessary and difficult to remove from tumor RNA. Tumors
from 3 mice were pooled for each experimental condition. Tis-
sues were homogenized and fractionated by differential centrif-
ugation in 0.25 M sucrose, 0.003 M CaCl$_2$, and 0.03 M Tris at
pH 7.6. Cytoplasmic fractions were taken from the upper two-
thirds of the supernatant obtained from the first sedimentation
of nuclei (600 X g for 10 min). Nuclear fractions were washed
twice as above and both fractions were extracted by vigorous
shaking in ice 3 times with 90% phenol containing 1% sodium
dodecylsulphate. DNA and oligonucleotides were removed, and
RNA from both fractions was precipitated and washed as de-
scribed previously (11). Approximately 12 O.D. units (260
mµ) of isolated RNA in a volume of 1 ml (0.1 M NaCl and
0.01 M sodium acetate, pH 5.0) were layered over a 5-20%
linear sucrose gradient (27 ml, prepared in the same salt solu-
tion as the RNA). Tubes were centrifuged (Beckman/Spinco
L2) for 17 hr at 18,000 rpm (5°C) in an SW 25.1 rotor.
Twenty-drop fractions (approximately 0.75 ml) were collected
from the bottom of tubes and diluted with 0.5 ml of H$_2$O for
measurement of ultraviolet absorption at 260 mµ. Radioactiv-
ity was measured after precipitation of the RNA on Millipore
filters with 100 µg of bovine serum albumin and 5% tri-
chloroacetic acid. Sedimentation coefficients are those used by
DiGirolamo et al. (1).

RESULTS

Labeled Precursor Studies. It seemed reasonable to expect
that tumoridial doses of actinomycin (400-1200 µg/kg) would
result in early and marked inhibition of RNA synthesis. In-
stead, there were only moderate inhibitions of $^{32}$P incor-
poration into RNA at 2, 4, or 8 hr after treatment (Table 1). Even
at 24 hr, a time when incorporation of $^{32}$P into DNA was
almost totally blocked, the inhibition was incomplete. Similar
results were obtained when we studied AKD2F$_1$ mice bearing
ROS and treated with the same doses administered i.p. (data
not shown).

Small intestine taken from the same animals indicated that
actinomycin had inconsistent effects on $^{32}$P incorporation into
RNA. As in ROS, however, intestinal DNA synthesis at 24 hr
was consistently and significantly blocked. The DNA inhibition
increased with increasing doses and, similarly, crypt damage
was proportional to the amount of actinomycin administered.

In the experiments described in Table 1, the nucleic acids
of both ROS and intestine were determined colorimetrically.
When the study was completed, we found significant losses
(i.e., deviating more than 2 S.D. from control average) of RNA
from tumors of every pair of mice sacrificed 24 hr after treat-
ment. In addition, pairs killed 4 hr after 800 µg/kg and 8 hr

<table>
<thead>
<tr>
<th>Dose of actinomycin D</th>
<th>Time of sacrifice (hr)</th>
<th>ROS</th>
<th>Intestine</th>
</tr>
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<tr>
<td></td>
<td>Incorporation (dpm/mg tissue)</td>
<td>RNA</td>
<td>DNA</td>
</tr>
<tr>
<td>Controls</td>
<td>1070± 440</td>
<td>130± 46</td>
<td>840± 350</td>
</tr>
<tr>
<td>400</td>
<td>2</td>
<td>81</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>4</td>
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<td>56</td>
<td>81</td>
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<tr>
<td></td>
<td>4</td>
<td>54</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>1200</td>
<td>2</td>
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<td>110</td>
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<tr>
<td></td>
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<td>32</td>
<td>4</td>
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</table>

Incorporation of $^{32}$P into ROS tumor and small intestine of
AKR mice. Each value was obtained from pooled tissue of 2
mice. Controls received saline and were concurrently treated with
experiments (zero time); all received isotope 1 hr prior to
sacrifice.

* Average values for all controls ±1 S.D.; the values include
results of analysis of 10 pools of ROS and intestine.

* Experimental value differing from control average by more than
2 S.D.
Actinomycin D and Tumor Regression

Table 2

<table>
<thead>
<tr>
<th>Dose of actinomycin D (µg/kg body wt.)</th>
<th>Time of sacrifice (hr)</th>
<th>Incorporation (dpm/mg tissue)</th>
<th>Colorimetric µg/mg tissue</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>RNA</td>
<td>DNA</td>
</tr>
<tr>
<td>Tumor</td>
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<tr>
<td>1200</td>
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<tr>
<td>Intestine</td>
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<td>1200</td>
<td>4</td>
<td>31</td>
<td>1.2</td>
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<td>24</td>
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<td>0.3</td>
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<td>4</td>
<td>72</td>
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<td></td>
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Incorporation of uridine-2-¹⁴C into ROS tumor and small intestine. See Table 1 for conditions; except that isotope was given 20 min prior to sacrifice.

Table 3

<table>
<thead>
<tr>
<th>Dose of actinomycin D (µg/kg body wt.)</th>
<th>Time of sacrifice (hr)</th>
<th>Incorporation (dpm/mg tissue)</th>
<th>µg/mg tissue</th>
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<tr>
<td></td>
<td></td>
<td>RNA</td>
<td>DNA</td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>4</td>
<td>32</td>
<td>8.3</td>
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<td>58</td>
<td>7.0</td>
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<tr>
<td></td>
<td>24</td>
<td>3</td>
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</tr>
<tr>
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<td>&lt;1</td>
<td>3</td>
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<tr>
<td></td>
<td>24</td>
<td>31</td>
<td>8.6</td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>35</td>
<td>9.4</td>
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<tr>
<td>Small intestine</td>
<td></td>
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<tr>
<td>1200</td>
<td>4</td>
<td>30</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>54</td>
<td>4.6</td>
</tr>
<tr>
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<td>4</td>
<td>43</td>
<td>7.5</td>
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<tr>
<td></td>
<td>35</td>
<td>46</td>
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<td>6.7</td>
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<tr>
<td></td>
<td>42</td>
<td>6.2</td>
<td>5.7</td>
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</table>

Incorporation of thymidine-2-¹⁴C into ROS tumor and small intestine. See Table 1 for conditions, except that isotope was given 20 min prior to sacrifice.

after 1200 µg/kg also had significantly less RNA than controls. The content of RNA in tumors of these experimental animals was 4.6-6.7 µg/mg tissue. RNA values in the remaining experimental tumors were within ±1 S.D. of the control average (8.6 ± 0.9 µg/mg). There was no significant change in the DNA content of the tumors, or in the RNA and DNA of the intestine. Similar selective losses of RNA were obtained in ROS at 24 hr after 400, 800, and 1200 µg/kg in the experiments with AKD₃F₁, mice mentioned above.

Tables 2 and 3 show that incorporation studies with labeled uridine and thymidine confirmed the effects seen with ³²P. Incorporation into tumor RNA was unaffected at 4 hr and only partially inhibited at 24 hr when total RNA content was markedly lowered and DNA synthesis was almost completely blocked. In the intestine, incorporation of uridine into RNA at 4 hr and at 24 hr was partially inhibited. Similar results have been obtained with uridine in other experiments which are now in progress. Incorporation of uridine and thymidine into intestinal DNA was slightly inhibited at 4 hr and almost completely blocked at 24 hr.

Nucleic Acids and Protein in ROS and Intestine. Results described in the preceding section indicated that tumoricidal doses of actinomycin caused a marked loss of tumor RNA. This was also found in the additional experiments described in Table 4 in which RNA, but not DNA, of tumors was changed significantly at 24 hr after treatment. By 48 hr the RNA in the tumor decreased still further to 23% of control values; at this time there was also a measurable loss of DNA. Small changes in protein content were not significant even at 48 hr, and there were no significant changes in the intestine.

Growth of DMBA Tumors. We have previously shown that single doses of actinomycin of 800 or 1200 µg/kg cause complete destruction of ROS tumors. Chart 1 shows that a similar dose of the agent did not produce regression of established DMBA tumors in AKR mice. This experiment was replicated 3 times with similar results at doses of both 800 µg/kg and 1200 µg/kg.

The injection of actinomycin into mice bearing both ROS and DMBA tumors caused regression of ROS while growth of DMBA tumors continued (3 experiments). In the experiment described in Chart 2, all treated ROS regressed at least partially and all died with growing DMBA tumors. Three of the ROS that regressed initially subsequently started growing again, suggesting that the 800 µg/kg dose may be somewhat less effective in mice bearing both tumors than in those bearing only ROS. All controls died with large, growing ROS and DMBA tumors.

Nucleic Acids and Protein in Tumors of Doubly Implanted Mice. In mice bearing both ROS and DMBA tumors, there was a loss of about 30% of the RNA from ROS 24 hours after treatment (Table 5). This depletion exceeded the DNA loss but was slightly less than that observed in ROS of singly implanted mice (see Table 4). However, as mentioned above, actinomycin seemed somewhat less potent against ROS in doubly implanted mice. The small loss of RNA from DMBA tumor indicates that the agent was active in the tumor (see Discussion) even though cytotoxicity was not apparent.

Sucrose Gradient Profiles of RNA from ROS Tumors. We attempted to define the RNA which was lost following treatment. In the experiments shown in Chart 3, uridine-2-¹⁴C was given to prelabel both nuclear and cytoplasmic RNA in ROS tumors. After 3 hr a set of animals was killed; the results are shown in Chart 3A (upper panels are nuclear RNA) and D (lower panels are cytoplasmic). After the same labeling period

4 Dr. Stephen S. Sternberg examined slides prepared from these animals and found no drug-induced cytotoxicity in DMBA tumors at 24, 48, and 72 hr after treatment. Intestinal epithelium was damaged as previously reported, but damage to ROS in doubly implanted mice was less extensive than expected from previous studies with this dose (14).
Table 4

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment and hr after injection</th>
<th>µg/mg tissue ± S.D.</th>
<th>RNA</th>
<th>DNA</th>
<th>Protein</th>
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<tbody>
<tr>
<td>Tumor</td>
<td>Act. D, 24</td>
<td>3.9 ± 0.44</td>
<td>6.6 ± 0.43</td>
<td>43 ± 4.2</td>
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<tr>
<td></td>
<td>Saline, 24</td>
<td>6.7 ± 0.36</td>
<td>7.0 ± 0.53</td>
<td>48 ± 4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% Act. D/saline</td>
<td>58±</td>
<td>94</td>
<td>90</td>
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<tr>
<td></td>
<td>Act. D, 48</td>
<td>1.6 ± 0.51</td>
<td>3.7 ± 0.51</td>
<td>41 ± 6.2</td>
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<tr>
<td></td>
<td>Saline, 48</td>
<td>7.1 ± 1.40</td>
<td>6.7 ± 1.07</td>
<td>51 ± 10.5</td>
<td></td>
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<tr>
<td></td>
<td>% Act. D/saline</td>
<td>23±</td>
<td>55±</td>
<td>80</td>
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<tr>
<td>Small intestine</td>
<td>Act. D, 24</td>
<td>4.1 ± 0.59</td>
<td>3.2 ± 0.21</td>
<td>42 ± 10.2</td>
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<td>Saline, 24</td>
<td>4.6 ± 0.78</td>
<td>3.2 ± 0.24</td>
<td>40 ± 9.5</td>
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<td>% Act. D/saline</td>
<td>89</td>
<td>100</td>
<td>105</td>
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<td></td>
<td>Act. D, 48</td>
<td>5.2 ± 0.74</td>
<td>3.1 ± 0.57</td>
<td>50 ± 6.2</td>
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<td>Saline, 48</td>
<td>5.5 ± 0.45</td>
<td>3.4 ± 0.51</td>
<td>45 ± 3.3</td>
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<tr>
<td></td>
<td>% Act. D/saline</td>
<td>95</td>
<td>91</td>
<td>111</td>
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</table>

Nucleic acids and proteins in ROS tumor and small intestine after one injection of actinomycin D (800 µg/kg) or saline. Each set of determinations was replicated 4 or 5 times with pooled tissue from 2 mice. Act. D, actinomycin D.

* P < 0.01; for all others, P > 0.05.

Chart 1. Growth of DMBA tumor after a single i.v. dose of actinomycin D (ACT. D). Drug (800 µg/kg) or saline was given on Day 0 to groups of 7 mice in this experiment. Treated animals died on Days 17, 17, 19, 21, 37, 60, 60; controls on Days 17, 17, 18, 35, 35, 60, 60.

(3 hr) another set was given actinomycin (panels B and E) or saline (panels C and F) and killed 18 hr later. At 3 hr when actinomycin was given, the control set had considerably higher specific activities in the nuclear RNA (Chart 3A) than in the cytoplasm (Chart 3B), although the latter had well-defined peaks that were labeled at 28 S (tubes 12-20), 18 S (tubes 22-27), and 4-10 S (tubes 28-35). Eighteen hr after actinomycin, the label in the cytoplasm (Chart 3E) was still well-defined; the relative amount of label also increased from that of the 3-hr control (Chart 3B) although it was less at 28 S and 18 S than in the 18-hr saline control (Chart 3F). In the nuclear fraction from treated mice (Chart 3B), the activity at 28 S and 18 S was lower than in either control (Chart 3A and C). At 4 S, however, it was lower than the 3-hr control and higher than the 18-hr control. These results indicate a decrease in the amount of newly labeled nuclear RNA from the amounts re-

Chart 2. Growth of DMBA and ROS in doubly implanted mice after a single dose of actinomycin D (ACT. D). Each mouse had one of each tumor. Drug (800 µg/kg) or saline given on Day 0 to groups of 9 mice. The split dashed lines indicate the regrowth of 3 ROS and the continued regression of the others. This experiment was concurrent with the one described in Chart 1.
Table 5

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Treatment</th>
<th>μg/mg tissue</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DNA</td>
</tr>
<tr>
<td>ROS</td>
<td>Act. D</td>
<td>46,4</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>64,6</td>
</tr>
<tr>
<td></td>
<td>% Act. D/saline</td>
<td>71</td>
</tr>
<tr>
<td>DMBA</td>
<td>Act. D</td>
<td>69,7</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>84,8</td>
</tr>
<tr>
<td></td>
<td>% Act. D/saline</td>
<td>85</td>
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Nucleic acid and protein in ROS and DMBA tumors 24 hr after a single injection of actinomycin D (800 μg/kg) or saline. Pooled tissue from 5 mice was used for each determination. Act. D, actinomycin D.

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ported in the 3-hr control; since there was also less activity in the cytoplasmic fraction, there must have been a loss of 18 S and 28 S RNA, presumably by degradation to soluble polynucleotides. This is also consistent with the relative increase in optical density peaks at 4-10 S in both nuclei and cytoplasm of the treated set. Since our control nuclear fractions contain relatively small amounts of soluble RNA, the increase in this nuclear fraction after treatment is particularly significant. Moreover, the increase also occurs in the cytoplasm (Chart 3E). The 28 S and 18 S peaks appear to be slightly blunted in the nuclear fraction, as if loss of more rapidly sedimenting forms had resulted in increased amounts of soluble RNA.

Chart 3. Sedimentation profiles of phenol-extracted RNA in 5-20% linear sucrose gradients. RNA from ROS was prepared from nuclear (A, B, C) and cytoplasmic (D, E, F) fractions. One group was labeled with uridine-2-14C for 3 hr and killed (A, D). Others were similarly labeled, then given 800 μg actinomycin D/kg (B, E) or saline (C, F) and killed 18 hr later.
DISCUSSION

We are presently concerned with those actions of actinomycin which are responsible for cell death in susceptible tissues. After administration of tumoricidal doses, drug concentrations in ROS approach maximal values by 3 hours and are retained for at least 48 hours (12). Thus it seems reasonable to expect that sufficient drug is present in the tumor by 3 or 4 hr to initiate the events that lead to cell death. Despite the known mechanism of action of the agent, it is clear that the doses used in this study, while sufficient to destroy tumor cells, allow at least some incorporation of \(^{32}\)P or uridine into RNA at these early times. Subsequent cell death, then, does not result from an immediate and total blockade of RNA synthesis; it is likely that other mechanisms must account for cell death. It is also true that in some tissues (e.g., rat liver) where actinomycin may produce profound effects on RNA synthesis, cell death is not a notable sequel to the inhibition (13, 17).

It is not possible from the data presented here to define the mechanism which finally results in cell lethality. For example, actinomycin may limit production of a critical species of RNA (e.g., messenger RNA) while other fractions continue to be synthesized. Alternatively, cell death could result from intolerable losses of RNA. As much as 40% of the total RNA in ROS was lost by 24 hours and it seems reasonable to expect that the degradative action starts soon after administration, perhaps with the loss of the same critical species mentioned above. With either alternative, the continuing presence of the agent in the tumor would permit the lethal action to develop in surviving cells. Taking liver again as a converse example, the drug is present for relatively short periods of times (as compared to ROS); thus the inhibition of RNA synthesis is short-lived and the loss of RNA here is apparently confined to the nuclear fraction (11-13). Further, newly synthesized nuclear RNA, such as ribosomal precursors, appears to be more susceptible to degradation than mature cytoplasmic forms. It seems possible from observations such as these that inhibition of RNA synthesis would lead to intolerable depletion of RNA and subsequent cell death if permitted to continue.

The results described here are consistent with the possibility that cytotoxicity in ROS was due to an intolerable depletion of RNA. Reich and Goldberg (9) also noted that loss of RNA after actinomycin is a frequent concomitant of intoxication, and attributed it to a secondary effect of RNA inhibition. However, unpublished studies by R. Leeper indicate that changes in ribonuclease inhibitor in ROS may be directly responsible for RNA depletion. Decreasing inhibitor results in increased activity of free ribonuclease in ROS 0.5, 4, and 24 hr after treatment; in contrast, neither inhibitor nor enzyme levels are affected in DMBA tumor. There are two likely explanations for a loss of inhibitor activity in ROS. Actinomycin may bind and so inactivate the inhibitor. Alternatively, the inhibitor may be unstable in situ and, therefore, more rapidly depleted than ribonuclease when RNA synthesis is blocked. In either case, the net result would be increased ribonuclease activity and subsequent depletion of RNA.

With respect to those proliferating cells which are sensitive to actinomycin, it seems reasonable to suggest that RNA depletion may prevent synthesis of enzymes necessary for DNA replication (3), resulting in the delayed inhibition of DNA synthesis in ROS and in intestine. Delayed inhibition of DNA synthesis after actinomycin has also been observed in rat tissues: intestine, thymus, bone marrow, and regenerating liver (13, 16). In rat intestine, inhibition of DNA synthesis precedes cytopathology and both occur after RNA synthesis has recovered from a moderate inhibition.

It is possible that glucocorticoids mediate some of the actions attributed to actinomycin. Besides stimulating the pituitary-adrenal axis, actinomycin has direct lymphoctic actions similar to those of the adrenocorticoids (8, 13). In mice bearing ROS, cortisone slowed tumor growth when treatment was initiated 24 hr after implantation, but even then the tumor was not destroyed (18). Currently, we find that repeated treatment with cortisone (20 mg/kg/day for 5 days) does not alter the growth rate of well-established tumors, such as those used here (unpublished observations). Insofar as is presently known, proliferation in intestinal epithelium also appears not to respond to adrenal stimulation, so that both ROS and intestine are at least comparable in this respect. Nevertheless, we cannot yet rule out the possibility that losses of RNA in ROS are a response to circulating adrenal corticoids rather than to the tumoricidal actions of actinomycin itself. Thus the question remains for future investigation though it is known, for example, that drug-stimulated degradation of RNA occurs in liver of rats which had been adrenalectomized (11).

It is reasonable to presume that factors other than inhibition of RNA synthesis ultimately determine the susceptibility of various cells to intoxication by actinomycin. The mechanism which results in extensive depletion of RNA in the ROS may be absent from the DMBA tumor. It is also conceivable that the mechanism is present in both tumors, but its activation may require higher concentrations of drug than those that cumulate in the DMBA tumor after sublethal doses. It is known that the DMBA tumor concentrates significantly less actinomycin than does ROS (13). Moreover, such sensitive tissues as ROS, thymus, and spleen retain the drug for long periods after injection, whereas liver, intestine, and DMBA lose the drug relatively soon after the initial uptake. Similar initial concentrations of drug are found in ROS and in intestine; these levels are maintained in ROS at least for 48 hr, but in the intestine for only 6 hr or less (12). Since the time of the mitotic cycle in mouse intestinal mucosa is about 12 hr, retention of even relatively high concentrations of drug for only 6 hr might permit a fraction of the proliferating crypt cells to escape lethal intoxication (6, 12). The marginal survival of bone marrow and lymphoid tissue may be due to a stem cell reserve which is activated after cells in the proliferative compartments of these tissues have been destroyed (5, 15). It seems unlikely that ROS has a similar reserve.

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REFERENCES

Actinomycin D Effects on Nucleic Acids during Tumor Regression

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