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Effects of Deoxyribonucleic Acid-Reactive Drugs on Ribonucleic Acid Synthesis in Leukemia L1210 Cells

HERBERT E. KANN, JR., AND KURT W. KOHN

Laboratory of Molecular Pharmacology, Office of the Associate Scientific Director for Experimental Therapeutics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

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SUMMARY

Drugs having various modes of interaction with DNA were examined for their effects on RNA synthesis in L1210 cell cultures. Two aspects of RNA synthesis were emphasized: effects on chain lengths of RNA molecules synthesized in the presence of drug, and selective inhibition of nucleolar 45 S RNA synthesis. The drugs studied appeared to fall into three groups. Group I (actinomycin, daunomycin, and cordycepin) caused moderate reduction in chain length of nucleoplasmic RNA and marked selectivity in inhibition of nucleolar 45 S RNA synthesis. Group II (anthramycin, nitrogen mustard, and camptothecin) caused marked reduction in RNA chain length in both nucleoplasm and nucleolus, and did not selectively inhibit nucleolar 45 S RNA synthesis. Camptothecin, however, differed from the first two in that RNA chains eventually approached normal lengths if sufficient time was allowed. Group III (proflavin, ethidium, and ellipticine) did not produce RNA chain shortening and had moderate selectivity in inhibition of nucleolar 45 S RNA synthesis. Some of these findings could be interpreted in terms of known modes of interaction with DNA. In particular, it is proposed that the degree of reversibility of the DNA-inhibitor complex is an important, although not exclusive, determinant of the type of RNA synthesis effects produced.

INTRODUCTION

Many chemotherapeutic agents appear to exert their effects by interaction with DNA. After physicochemical studies of such interactions, a second step would be to determine the effects on DNA function in cells. We address ourselves here to the function: RNA synthesis. In particular, two aspects of RNA synthesis are emphasized: synthesis of RNA molecules with abnormally short chain lengths, and selective inhibition of synthesis of nucleolar 45 S RNA.

Abnormally short RNA molecules, produced by premature chain termination, may have profound effects on cells. Shortened messenger RNA molecules, if translated, could generate abnormal peptide chains, as has been observed for the effect of ultraviolet light on *Escherichia coli* (1, 2). In addition, production of abnormally shortened nuclear RNA molecules, of as yet undetermined function, could have drastic consequences. We explore here the question of what types of agents may produce such shortened RNA chains.

The selective inhibition of nucleolar RNA synthesis has been a common finding with inhibitors of RNA synthesis (3–6). In a previous paper (7) we pointed out that anthramycin is exceptional in that it does not cause such selective inhibition. We suggested that this may be related to the irreversibility of the binding of anthramycin

to DNA. In the present work, we further test this idea with other drugs.

Most of the agents studied were selected on the basis of known or suspected modes of interaction with DNA.

Actinomycin, one of the most extensively studied DNA binders, appears to act through the combined action of an intercalating chromophore and a pair of cyclic polypeptide side chains which fold into the DNA minor groove (8, 9). Although the binding is not covalent, dissociation of the complex is delayed.

Daunomycin, although not chemically related to actinomycin, resembles the latter in that it has an intercalating chromophore plus a side chain that is required for tight binding. Like the binding of actinomycin, that of daunomycin is tight and noncovalent, and is not quickly reversible (10, 11). Unlike actinomycin, which is an entirely neutral molecule, daunomycin carries a positive charge on the side chain, which is essential for tight binding.

Nitrogen mustard (HN2) is one of the oldest and best studied bifunctional alkylating agents. It binds covalently to DNA and forms interstrand cross-links (12, 13). The binding is not reversible, except slowly and indirectly by depurination (13, 14) or DNA repair (15, 16).

Anthramycin, although not a classical alkylating agent, binds irreversibly to DNA, probably through the formation of a covalent bond (17). The molecule is uncharged and nonplanar, and lacks side chains, thereby differing from other DNA-binding drugs except alkylating agents.

Proflavin and ethidium are flat, positively charged molecules that bind by intercalation (18). The binding of proflavin is rapidly reversible (19). Ellipticine has a similar molecular shape and charge and produces the DNA viscosity changes expected for intercalation.[1]

Cordycepin (3′-deoxyadenosine) is activated in the cell to become an analogue of ATP which is incorporated into growing RNA chains, thereby terminating them (20).

Camptothecin, although a prompt and reversible inhibitor of RNA synthesis (21, 22), does not appear to interact with DNA directly.[1] Its mechanism of action is unknown.

The findings presented show a relationship between the mode of interaction with DNA and the pattern of inhibition of RNA synthesis.

[1] K. W. Kohn, unpublished observations.

MATERIALS AND METHODS

Drugs, obtained through Drug Research and Development, National Cancer Institute, were dissolved in methanol or water and stored at −20°. Control cell cultures received solvent instead of drug solution. Anthramycin methyl ether, which is rapidly hydrolyzed to free anthramycin (23), will be referred to as anthramycin.

[2-^{14}C]Uridine (40–55 mCi/mmole) and [6-^{3}H]uridine (15–25 Ci/mmole), obtained from Schwarz BioResearch, were added to cell cultures to give final concentrations of 0.04 and 1.0 μCi/ml, respectively. The final molar concentrations were equalized by adding appropriate amounts of unlabeled uridine to the ^{3}H-labeled compound.

The experiments were carried out on L1210 cells growing exponentially in spinner flasks at 6–9 × 10^{5}/ml in RPMI 1630 medium supplemented with 20% fetal calf serum, penicillin, and streptomycin (24). Drug-treated cells were labeled (in the continued presence of drug) with [^{3}H]-uridine; control cultures were labeled with [^{14}C]uridine. After the appropriate uridine incorporation periods, cultures were rapidly cooled to 0–4° by pouring them onto frozen 0.9% NaCl solutions. Drug-treated and control cells were then combined in matched pairs, so that all further processing and analysis was done on internally controlled mixtures.

Nuclei were fractionated and RNA purified as described elsewhere (7). In brief, nuclei were isolated by gentle homogenization in phosphate-buffered NaCl containing 0.2% Triton X-100 and 2 mM $MgCl_2$, followed by centrifugation through 50% sucrose–2 mM $MgCl_2$. Nuclei were fractionated into nucleolar and nucleoplasmic fractions by a slight modification of the high-salt–DNase method of Penman *et al.* (25).

The nuclear pellet was suspended in high-salt buffer (0.8 M NaCl, 0.08 M $MgCl_2$, and 0.016 M Tris, pH 7.4) and digested for 1 min at 37° with electrophoretically purified DNase. The nuclear digest was centrifuged through a 15–30% gradient of sucrose in high-salt buffer for 15 min at 17,000 rpm. The upper portion of the gradient constituted the nucleoplasmic fraction. The nucleolar material sedimented to the bottom of the gradient, where a layer of finely ground, solid sucrose had been placed to prevent losses of nucleolar material due to adherence to the tube bottom. The nucleolar fraction was recovered by dissolving the solid sucrose in high-salt buffer. Both fractions were precipitated with ethanol and digested with Pronase. RNA was then purified from the fractions by extraction with a mixture of phenol, metacresol, and 8-hydroxyquinoline (26) at 60°. Chloroform was added to each extraction prior to removal of the phenol phase. The purified RNA was precipitated with ethanol and then dissolved in and sedimented through a gradient of 15–30% sucrose in the same solvent. Sedimentation was carried out at 22,000 rpm for 16 hr at 20° in a Spinco SW 40 rotor. Fractions were collected directly in scintillation vials and digested with "NCS" (Nuclear-Chicago).

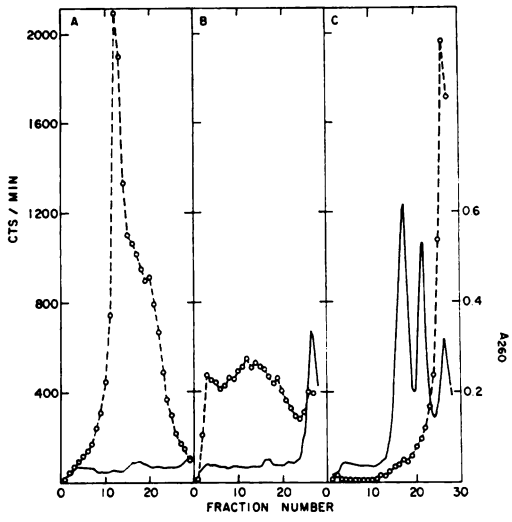

FIG. 1. *Sedimentation patterns of RNA from nucleolar (A), nucleoplasmic (B), and cytoplasmic (C) fractions after 10-min labeling with [5-³H]uridine*

Centrifugation was performed at 22,000 rpm for 16 hr at 20° in an SW 40 rotor. The direction of sedimentation is from right to left. O- - -O, radioactivity; ——, absorbance at 260 nm.

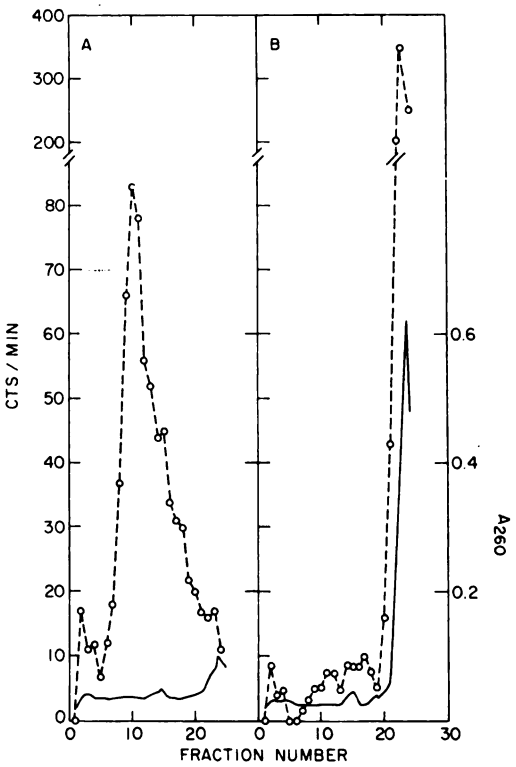

FIG. 2. *Sedimentation patterns of RNA from nucleolar (A) and nucleoplasmic (B) fractions after 10-min labeling with [methyl-¹⁴C]methionine*

RESULTS

The sedimentation patterns of pulse-labeled RNA from the nucleolar, nucleoplasmic, and cytoplasmic fractions of untreated L1210 cells (Fig. 1) are similar to what has been found in other rapidly proliferating cells (27). The nucleolar fraction shows the expected 45 S peak, with a shoulder of a more slowly sedimenting material (panel *A*). In the nucleoplasmic fraction, there is the usual broad sedimentation distribution (panel *B*). The cytoplasmic ribosomal RNA peaks are not yet labeled (panel *C*).

The sedimentation patterns of these fractions show that there is no appreciable

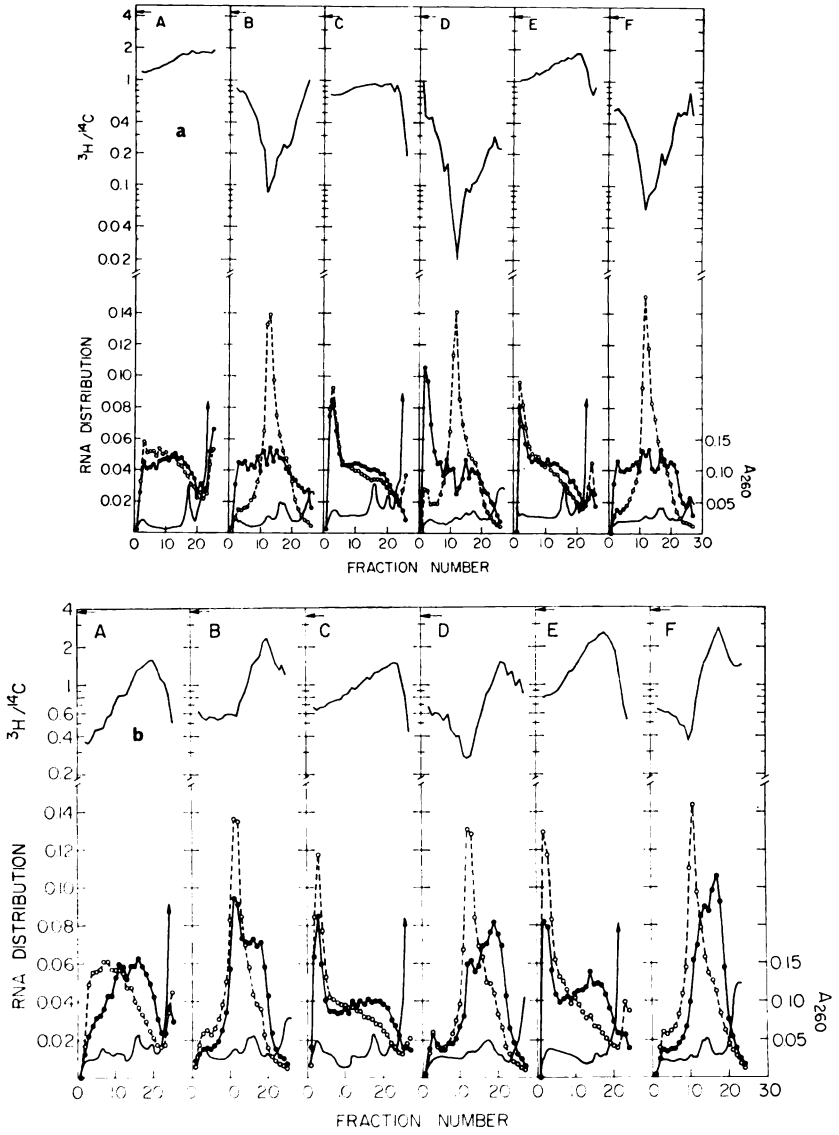

FIG. 3. *Effects of drugs on synthesis patterns of nuclear RNA*

Cells were exposed to drug for 30 min (90 min. in the case of anthramycin) and then allowed to incorporate [^{3}H]uridine for 10 min. in the presence of drug. Control cells were labeled with [^{14}C]uridine for 10 min. The ratio of ^{3}H to ^{14}C radioactivity added to the cell cultures is indicated by an arrow in the upper left of each panel; the solid line in the upper portion of each panel represents the ^{3}H:^{14}C ratio measured for each fraction from the sucrose gradient. The sedimentation distributions of labeled RNA are normalized to unit area under each curve. ●——●, ^{3}H; O---O, ^{14}C;——, A_{260}. Panels *A*, *C*, and *E* represent nucleoplasmic fractions; panels *B*, *D*, and *F*, nucleolar fractions. Drug treatments (numbers in parentheses are the extents of inhibition of radioactive uridine incorporation into the nucleoplasmic and nucleolar fractions, respectively) were carried out as follows. a. Group I: panels *A* and *B*, actinomycin D, 0.188 μM (65%, 94%); panels *C* and *D*, daunomycin, 4.8 μg/ml (81%, 97%); panels *E* and *F*, cordycepin, 100 μg/ml (71%, 96%). b. Group II: panels *A* and *B*, anthramycin, 0.32 μM (79%, 78%); panels *C* and *D*, HN2, 20 μM (75%, 84%); panels *E* and *F*, camptothecin, 12 μM (72%, 74%). c. Group III. panels *A* and *B*, proflavin, 7 μg/ml (65%, 91%); panels *C* and *D*, ethidium, 50 μg/ml (65%, 93%); panels *E* and *F*, ellipticine, 20 μM (75%, 90%).

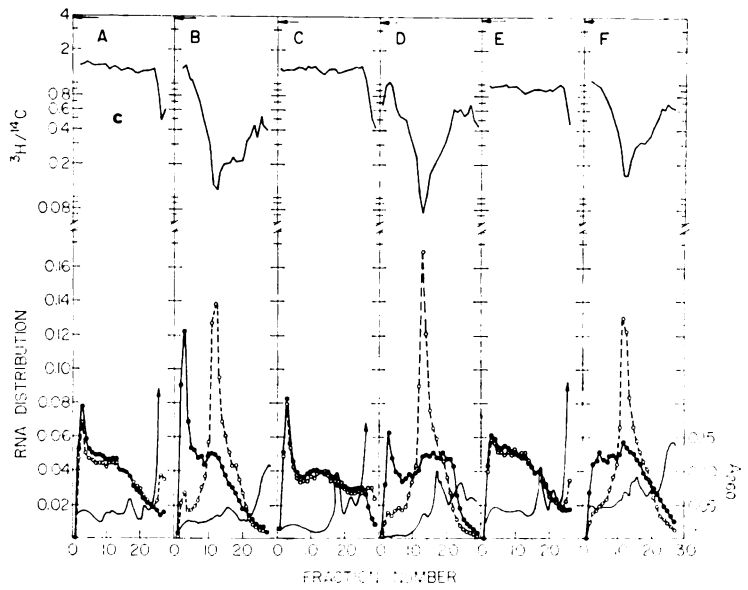

FIG. 3c

cross-contamination. The lack of significant contamination of the nucleolar fraction by nucleoplasmic RNA is shown by the low level of radioactivity in the bottom third of the gradient (panel *A*). The lack of the converse contamination is shown by the absence of a significant 45 S peak in panel *B*. The lack of cytoplasmic contamination of both nuclear fractions is shown by the absence of the rRNA optical density peaks seen in the cytoplasmic fraction. These criteria are also a guide to detecting possible drug effects on the fractionation. No such effects were evident, although in some experiments detectable cytoplasmic rRNA peaks were present in the nuclear fractions.

Pulse-labeling with [*methyl*-^{14}C]methionine was used as a further check on the fractionation procedure for L1210 cells (Fig. 2). Most of the labeled material in the nucleolar fraction (panel *A*) is either in the 45 S peak or in more slowly sedimenting material. The nucleoplasmic fraction (panel *B*) is free of appreciable contamination by methylated high molecular weight RNA.

This fractionation and sedimentation procedure was used to study the effects of drugs on RNA synthesis patterns. All the drugs used are inhibitors of RNA synthesis and were tested over a range of concentrations. The time of exposure of drug was usually 30 min, followed by a 10-min uridine incorporation period in the continued presence of drug. Examples of the effects produced by various drugs, shown in Fig. 3, were selected to give a comparable set of data. The examples have comparable extents of inhibition of uridine incorporation into nucleoplasmic RNA. Drug exposure times were 30 min in all cases, except for anthramycin. For anthramycin, a 90-min exposure time was chosen for comparison, because of the apparent delay in the full development of the anthramycin effect (7); however, the observations to be presented were verified also with 30-min treatment time.

The sedimentation patterns of newly synthesized RNA shown in Fig. 3 are normalized to the unit area under each curve to facilitate comparison between drug-treated (●) and control (○) curves. The over-all extents of inhibition of radioactive uridine incorporation are given in the legend. The ratio of incorporation of ^3H (treated cells) to ^{14}C (control cells) is plotted in the upper half of each panel; the ^3H:^{14}C ratio expected for uninhibited cells is indicated by an arrow at the upper left of each panel.

Three major drug effects are seen: (a) selective inhibition of nucleolar 45 S RNA

synthesis, (b) production of shortened RNA chains in the nucleolar fraction, and (c) decreased sedimentation of nucleoplasmic RNA.

Selective inhibition of nucleolar RNA synthesis is typified by actinomycin (Fig. 3a, panel *B*). The 45 S peak in the sedimentation pattern is obliterated, and the ratio shows a sharp dip at 45 S. The over-all inhibition of RNA synthesis is much greater in the nucleolar fraction than in the nucleoplasmic fraction. The selectivity of the inhibition of nucleolar RNA synthesis is more striking at drug concentrations lower than those of Fig. 3 (which were selected to be high enough to cause extensive inhibition of nucleoplasmic RNA also). The selectivity over a range of drug concentrations is illustrated in Fig. 4. The greatest selectivity is found with actinomycin, daunomycin, and cordycepin (panel *A*). There is no selectivity with anthramycin, HN2, or camptothecin (panel *B*), and intermediate selectivity with proflavin, ethidium, and ellipticine (panel *C*).

Shortened nucleolar RNA chains were produced by cells treated with anthramycin, HN2, and camptothecin (Fig. 3b, panels *B*, *D*, and *F*, respectively). Although HN2 and camptothecin show a dip at 45 S in the ratio plots, there is no over-all selective inhibition of nucleolar RNA synthesis. Rather, the sedimentation of most of the labeled nucleolar RNA is shifted below 45 S.

Decreased sedimentation of newly synthesized, nucleoplasmic RNA is typified by anthramycin (Fig. 3b, panel *A*). The shift to reduced sedimentation is reflected in the slope of the $^{3}H:^{14}C$ ratio plot, which shows the relative lack of rapidly sedimenting RNA produced in the drug-treated cells. This effect is also seen with nitrogen mustard and camptothecin (Fig. 3b, panels *C* and *E*) and, less prominently, with actinomycin, duanomycin, and cordycepin (Fig. 3a, panels *A*, *C*, and *E*). (The drop in $^{3}H:^{14}C$ ratio near the top of these gradients is partially due to the presence of label in degraded DNA.)

Interpretation of the effects on the nucleoplasmic patterns is complicated by the variability of these sedimentation patterns, especially near the bottom of the gradient (compare, for example, control patterns in Fig. 3a, panels *A* and *C*). This is probably caused by aggregation effects (28). Despite this variability, we find (perhaps surprisingly) that when nucleoplasmic RNA from treated and control cells, labeled with ^{3}H and ^{14}C, respectively, are sedimented together, the slope of the $^{3}H:^{14}C$ ratio gives consistent results. Nevertheless, the question must be raised whether this change in sedimentation produced by

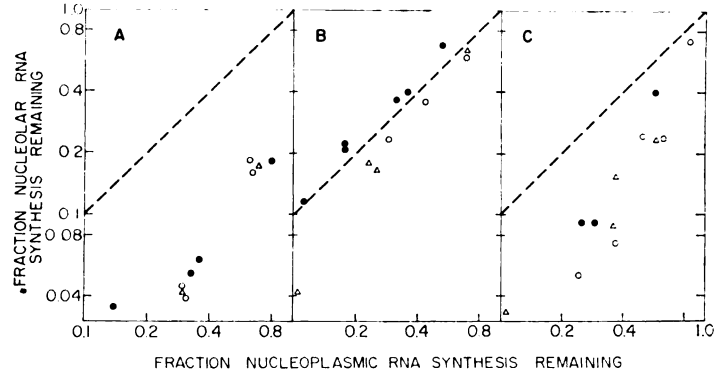

FIG. 4. *Relative extents of inhibition of radioactive uridine incorporation into nucleolar and nucleoplasmic RNA, determined by experiments similar to those shown in Fig. 3.*

Panel *A*, group I drugs: ●, actinomycin (0.03–0.188 μM); ○, daunomycin (1–5.1 μg/ml); △, cordycepin (87–100 μg/ml). Panel *B*, group II drugs: ●, anthramycin (0.08–0.84 μM); ○, camptothecin (0.5–12 μM); △, nitrogen mustard (4–50 μM). Panel *C*, group III drugs: ●, ellipticine (2.8–20 μM); ○, ethidium (5–50 μg/ml); △, proflavin (3.5–14 μg/ml).

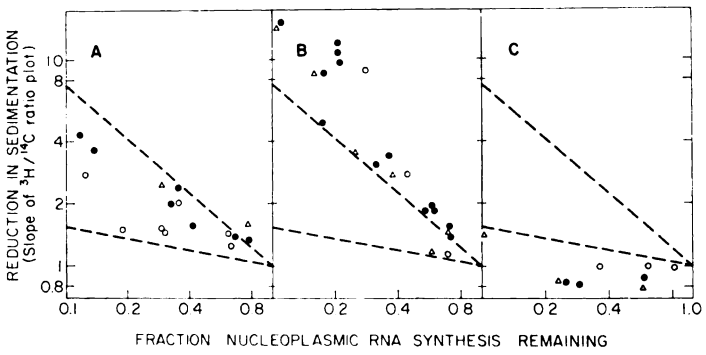

FIG. 5. *Reduction in sedimentation of nucleoplasmic RNA as a function of extent of inhibition of RNA synthesis.*

Every experiment in which drug effects on nucleoplasmic RNA were assessed is represented; each point is from a single experiment. The broken lines are placed to give the best possible separation of results with drugs from each of the three groups. Panel *A*, group I drugs: ●, actinomycin; ○, daunomycin; △, cordycepin. Panel *B*, group II drugs: ●, anthramycin; ○, camptothecin; △, nitrogen mustard. Panel *C*, group III drugs: ●, ellipticine; ○, ethidium; △, proflavin.

drug treatment is due to (a) reduction in length of the RNA chains synthesized (which could be caused by selective inhibition of synthesis of longer chains, premature termination of growing chains, or chain scission), (b) reduction in the extent of aggregation, or (c) change in RNA conformation. In work to be reported subsequently, we have found that the reduced sedimentation persists upon reaction of the RNA with formaldehyde, indicating that the length of the chains is reduced.

In order to make the comparison of the chain-shortening effects of various drugs more quantitative, we related the slopes of H^3:C^{14} ratio plots to the extent of inhibition of uridine incorporation into nucleoplasmic RNA (Fig. 5). [The slopes given in Fig. 5 5 were read from plots of log (H^3:C^{14}) with respect to chain length. Since these plots generally had a slight curvature, the estimated slopes are only approximate. Alternative methods of estimating slopes from ratio plots, however, produced the same general pattern seen in Fig. 5.] By viewing the data in this way, it is possible to place the drug effects into three groups. The largest chain-shortening effects were produced by anthramycin, nitrogen mustard, and camptothecin (group II). These drugs give points that fall above the upper dashed line in Fig. 5 (panel *B*). (Two points, at low extents of inhibition, where the error is greatest, fall below this line.) At the opposite extreme were proflavin, ethidium, and ellipticine (group III), which produced no chain shortening at all (Fig. 5, panel *C*). Intermediate between these were actinomycin, daunomycin, and cordycepin (group I); these gave points between the dashed lines in Fig. 5 (panel *A*).

The question may be raised whether the decreased RNA chain lengths are due to reduced chain elongation rates, so that 10-min uridine incorporation periods do not allow enough time for the chains to grow to their full lengths. Longer incorporation periods present the complication of processing of nucleolar 45 S RNA, which generates lower molecular weight species in both nucleolus and nucleoplasm. In the case of group I agents, however, nucleolar 45 S RNA synthesis is completely inhibited, and we were able to show directly that the sedimentation patterns of nucleoplasmic RNA synthesized in the presence of actinomycin are unchanged by an increase in uridine incorporation time to 30 min (7). For group II drugs the complication of nucleolar 45 S RNA processing was circumvented by the use of toyocamycin, an adenosine analogue that becomes incorporated into nucleolar RNA and prevents its processing (29). Toyocamycin at a concentration of 0.1 μg/ml prevented nucleolar 45 S RNA processing in L1210

cells while inhibiting uridine incorporation into RNA by approximately 50%. When cells were treated for 30 min with toyocamycin together with anthramycin or HN2 and then labeled with uridine for as long as 60 min, the marked reductions in sedimentation of nucleoplasmic and nucleolar RNA were still seen. The increase in labeling time from 10 to 60 min did not significantly affect the sedimentation patterns. It is concluded that the chain-shortening effects of these two agents are not due simply to slowing of chain elongation rates. Furthermore, the presence of toyocamycin in these experiments argues against the possibility that the slowly sedimenting material is derived from rapidly processed 45 S ribosomal precursor RNA. Camptothecin differed from anthramycin and nitrogen mustard in that the marked reduction in sedimentation of nucleoplasmic RNA completely disappeared when the labeling time was extended to 60 min. This result is compatible with a reduction in the chain elongation rate as the primary action of camptothecin on nucleoplasmic RNA synthesis. In the case of nucleolar RNA, however, the longer labeling time increased the sedimentation only slightly. These findings were of particular interest because, although the effects of camptothecin on RNA synthesis were in some ways similar to those produced by the irreversible DNA binders, we could detect no change in its absorption spectrum to support the possibility of interaction with DNA. Since camptothecin reportedly causes fragmentation of DNA (21), it had seemed possible that fragmentation of DNA or RNA might be responsible for the observed shifts to slower sedimentation of the nucleoplasmic RNA. This possibility is now excluded by the finding that nucleoplasmic RNA molecules of normal size are synthesized when the labeling time is extended sufficiently to offset a reduced RNA chain elongation rate. Furthermore, we find that RNA synthesized after removal of camptothecin has normal sedimentation patterns.

DISCUSSION

The nine drugs studied can be tentatively classified into three groups, based on their effects on RNA synthesis (summarized in Figs. 3–5 and Table 1).

Group I (actinomycin, daunomycin, and cordycepin; Fig. 3a, and panels *A* of Figs. 4 and 5) is characterized by selective inhibition of nucleolar 45 S RNA synthesis and moderate reduction in sedimentation of nucleoplasmic RNA.

Group II (anthramycin, HN2, and camptothecin; Fig. 3b, and panels *B* of Figs. 4 and 5) is characterized by approxi-

TABLE 1

Tentative classification of drugs on basis of effects on RNA synthesis

Group	Drugs	Selective inhibition of nucleolar RNA synthesis	Production of shortened nucleolar RNA chains	Reduced sedimentation of nucleoplasmic RNA	Inhibition of nucleolar 45 S RNA processing[a]
I	Actinomycin	++	−	+	−
	Daunomycin	++	−	+	−
	Cordycepin	++	−	+	N.D.[b]
II	Anthramycin	−	++	++	−
	HN2	−	++	++	−
	Camptothecin	−	++	++	−
III	Proflavin	+	−	−	++
	Ethidium	+	−	−	+
	Ellipticine	+	−	−	++

[a] Taken from ref. 33.

[b] Not determined.

mately equal inhibition of nucleolar and nucleoplasmic RNA synthesis and marked reduction in sedimentation of RNA from both these fractions.

Group III (proflavin, ethidium, and ellipticine; Fig. 3c, and panels *C* of Figs. 4 and 5) is characterized by moderate selectivity in inhibition of nucleolar RNA synthesis and little or no change in sedimentation of nucleoplasmic RNA labeled in a 10-min uridine incorporation period. Longer incorporation periods, however. show a shift to an *increase* in sedimentation. A trace of this effect, which will be the subject of a separate report, can be seen in Fig. 3c, panel *A*.

Some of these findings can be interpreted in terms of the nature of the interactions of these drugs with DNA (or related sites). Anthramycin and HN2 are known to bind almost irreversibly to DNA. This could be responsible for the reduced average length of the nucleoplasmic RNA synthesized, either by selective inhibition of the longer transcription segments or by premature termination of growing RNA chains (1). The reduction in sedimentation observed in the nucleolar RNA, however, can be explained only by the second possibility (since 45 S nucleolar RNA sediments as a homogeneous species).

Actinomycin and daunomycin bind tightly to DNA, but the binding is slowly reversible. This slow reversibility may explain the lesser degree of reduction in sedimentation of nucleoplasmic RNA. The selective inhibition of nucleolar RNA synthesis might be due to possible differences between the nucleolar and nucleoplasmic RNA polymerases (30, 31) in their ability to displace these drugs from the template. Drugs which bind irreversibly would not be displaced by either enzyme, thus perhaps accounting for the lack of selectivity in the case of anthramycin and HN2.

The effects of camptothecin, although similar to anthramycin and HN2 in regard to the factors considered here (Table 1), must be due to a different mechanism. Camptothecin differs from anthramycin and HN2 in that (a) nucleoplasmic RNA chains eventually approach normal lengths after long incorporation times, compatible with a slowing of chain elongation rate, (b) RNA synthesis is rapidly reversible upon removal of the drug (21, 22), and (c) we have not been able to detect any interaction with DNA *in vitro*. Camptothecin has been reported to produce breaks in DNA (21); we do not see at this time how to relate this to the effects on RNA synthesis.

The effects of cordycepin (3′-deoxyadenosine), although similar to those of actinomycin and daunomycin, appear to originate from a different mechanism. Cordycepin becomes incorporated into RNA and prevents further chain growth. This readily explains the reduced sedimentation of RNA. The selective inhibition of nucleolar RNA synthesis might be explained by relative inability of the nucleolar RNA polymerase to displace the prematurely terminated RNA chain from the template. The selective inhibition of nucleolar RNA by cordycepin has been reported previously in HeLa cells (5). The reduction in sedimentation of nucleoplasmic RNA, however, was not detected in HeLa cells (32).

The compounds in group III (Table 1) are intercalating agents. This type of interaction with DNA has been studied extensively with proflavin and ethidium (9, 18). The complex of proflavin with DNA has been shown to be rapidly reversible, with a relaxation time of a few milli-sec onds (19). This rapid reversibility might explain the absence of RNA chain-shortening effects. An additional effect is involved, however, since longer uridine incorporation times disclose a chain-lengthening effect.[2] Correlating with this effect is inhibition of the processing of nucleolar 45 S RNA (33) (Table 1).

The classification proposed in Table 1 has the following limitations. (a) Studies have so far been carried out mainly with L1210 cells; we do not know whether the same generalizations will apply to other cell types. (b) A limited number of drugs have been studied; the validity or usefulness of the generalizations for other drugs re-

[2] H. E. Kann, Jr., A. L. Snyder, and K. W. Kohn, unpublished observations.

mains to be determined. (c) Drugs with totally different mechanisms of action may fall into a common group. This was seen in the case of camptothecin. The question whether the physiological consequences of the inhibited RNA synthesis depend on the types of effects categorized in Table 1 remains open.

Wu, Kumar, and Warner (34) recently reported that in HeLa cells camptothecin selectively inhibits uridine incorporation into the nucleolar fraction and produces a peak of radioactivity in the nucleoplasmic fraction at about 25 S. These observations could be brought into line with our findings if it were supposed that some of the radioactivity of the 25 S peak actually represents nucleolar RNA chains, which in this case were released into the nucleoplasmic fraction.

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