Effect of the Vinca Alkaloids on RNA Synthesis in Human Cells in vitro

Abstract. *The* Vinca *alkaloids vinblastine sulfate and vincrystine sulfate, which are mitotic poisons, inhibit RNA synthesis in human (HEp-2) cells cultured in vitro. Analyses of RNA synthesis by cells treated with these drugs by acrylamide gel electrophoresis show that 28*s *rRNA and to a lesser extent 18*s *rRNA are preferentially inhibited. The synthesis of tRNA is affected much less than that of rRNA. The present experiments suggest that the drugs inhibit both the synthesis and processing of the nucleolar RNA precursors of rRNA. An explanation is also given for previous reports that these alkaloids preferentially inhibit the synthesis of tRNA in animal cells in vitro.*

Vinblastine and vincrystine, which are mitotic poisons (*1*), have been reported to inhibit preferentially the synthesis of transfer RNA (tRNA) in Ehrlich ascites cells grown in vitro (*2*). A preferential inhibitor of tRNA synthesis would be of great value in the study of the multiplication of viruses reported to specify their own tRNA. For this reason we have investigated the effect of these two alkaloids on RNA synthesis in human cells grown in tissue culture, using acrylamide gel electrophoresis. Our results show that vinblastine sulfate and vincrystine sulfate do inhibit RNA synthesis but the inhibition is much greater for ribosomal RNA (rRNA) than for tRNA.

We used human epidermoid carcinoma No. 2 (HEp-2) cells grown in monolayer cultures in Eagle's minimal essential medium (*3*) in our experiments. The cells were incubated at 37°C in medium containing $2 \times 10^{-4}M$ vinblastine sulfate for 30 minutes. At this time the cells were pulse-labeled for 15 minutes in the same medium with 5 μc of uridine-^{3}H per milliliter (Schwarz Bio-Research; specific activity, 21 c/mmole). The radioactive medium was removed and the cells were incubated for a further 60 minutes with medium containing $2 \times 10^{-4}M$ vinblastine sulfate and $10^{-4}M$ unlabeled uridine. The cells were harvested, then fractionated into nuclear and cytoplasmic fractions by Dounce homogenization followed by low-speed centrifugation, and the cytoplasmic RNA was deproteinized by phenol–sodium dodecyl sulfate extraction at 40°C (*4*). The deproteinized RNA was precipitated by ethanol and subjected to electrophoresis on a 10-cm polyacrylamide gel consisting of a 5-cm 2.7 percent acrylamide gel poured over a 5-cm 5.5 percent gel (*5*, *6*).

After electrophoresis the gels were scanned in a Gilford recording spectrophotometer by means of a special attachment, cut into 2-mm slices, and hydrolyzed with concentrated ammonium hydroxide (*5*). The hydrolyzed slices were assayed for radioactivity in a Packard scintillation counter (model 3375) in a solution of three parts toluene scintillator fluid and one part triton X-100 (Rohm and Haas) (*7*).

The superimposed optical density and radioactivity profiles from this experiment are shown in Fig. 1B and the results are tabulated in Table 1. The data show that $2 \times 10^{-4}M$ vinblastine sulfate reduces the amount of uridine-^{3}H incorporated into the 4*s* tRNA band to about 70 percent of that of a control culture (Fig. 1A). The incorporation of uridine-^{3}H into rRNA is much more markedly reduced; thus the specific activity of the 18*s* and 28*s* rRNA are only 40 and 20 percent, respectively, of that of the control culture. A lower concentration of the drug ($10^{-5}M$) causes a 25-percent reduction of rRNA synthesis but has no significant effect on tRNA (Fig. 1C and Table 1).

Animal cell rRNA is formed by the nonconservative cleavage of a 45*s* nucleolar precursor into first 18*s* rRNA and then 28*s* rRNA (*8*). In our experiments the pattern of RNA synthesis in cells treated with vinblastine sulfate suggests that at least two steps in the formation of rRNA are affected by the drug. The inhibition of incorporation of uridine-^{3}H into 18*s* rRNA suggests that the synthesis of 45*s* RNA is inhibited. At the same time, the even greater reduction in the incorporation of uridine-^{3}H into 28*s* rRNA also indicates an inhibition of the processing of the nucleolar RNA after the 18*s* rRNA is cleaved.

The inhibition of the formation of 28*s* rRNA from the nucleolar RNA precursor after the cleavage of the 18*s* rRNA is also shown in the following experiment. HEp-2 cells were incubated for 15 minutes in the presence of uridine-^{3}H. The medium containing the labeled nucleoside was then removed and the cells were incubated for 60 minutes in the presence of $10^{-4}M$ vinblastine sulfate and $10^{-4}M$ unlabeled uridine. The amount of radioactivity in the cytoplasmic 18*s* rRNA and 28*s* rRNA of these cells was compared with that found in

Table 1. RNA synthesis in HEp-2 cells exposed to vinblastine sulfate. The amount of tRNA and rRNA recovered in each part of the experiment of Fig. 1 was computed from the recovery of optical density. The relative amount of radioactivity in the various RNA species of the vinblastine-treated cultures was calculated from the recovery of optical density and radioactivity, and the percent of RNA synthesis was computed from these figures.

Pretreatment of culture	Corrected relative recovery of radioactivity in cytoplasmic RNA (count/min)			RNA synthesis (percent of control)		
	4*s*	18*s*	28*s*	4*s*	18*s*	28*s*
None	13,500	14,700	12,650	100	100	100
$2 \times 10^{-4}M$ vinblastine	10,000	6,000	2,450	74	41	19
$10^{-5}M$ vinblastine	13,000	10,900	9,700	100	74	77

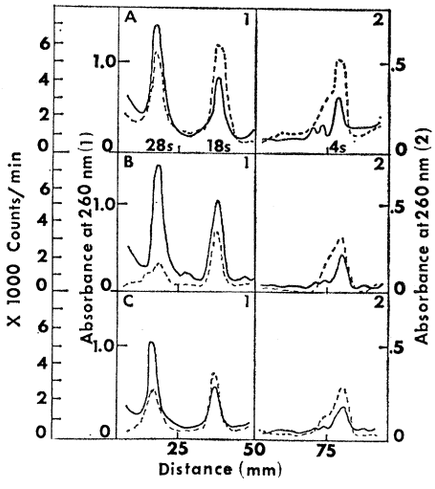

Fig. 1. The effect of vinblastine sulfate upon the incorporation of uridine-^{3}H into the cytoplasmic RNA of HEp-2 cells. (A) A monolayer culture of HEp-2 cells was incubated with 5 μc of uridine-^{3}H per milliliter for 15 minutes, followed by incubation for 60 minutes in the presence of $10^{-4}M$ unlabeled uridine. The cytoplasmic RNA from these cells was then extracted as described in the text, and the material was subjected to acrylamide gel electrophoresis at 8 volt/cm for 4 hours. The gels were then scanned at 260 nanometers (nm), and sliced, and tritium disintegrations were measured as described in the text. Solid lines, optical density; dashed lines, radioactivity. (B) The same as (A) except that the cells were exposed to $2 \times 10^{-4}M$ vinblastine sulfate 30 minutes before, during the pulse-labeling, and in the chase. (C) The same as (B) except that the concentration of vinblastine sulfate was $10^{-5}M$.

the rRNA extracted from cells of an untreated, control culture. It was found that there was little or no decrease in the amount of 18*s* rRNA in the vinblastine-treated cells but there was a 40 to 50 percent reduction in the specific activity of the 28*s* rRNA. The nature of the vinblastine-sensititve step in the processing of the nucleolar precursor into cytoplasmic 28*s* rRNA is not evident, but preliminary experiments indicate that there is no accumulation of 28*s* rRNA in the nucleus. This tends to exclude the possibility that vinblastine sulfate acts by inhibiting transport of the mature 28*s* rRNA into the cytoplasm.

It is of interest to note that incubation of untreated cells with radioactive uridine followed by immediate phenol extraction and ethanol precipitation of the cytoplasmic RNA results in the coprecipitation of a large amount of radioactive material of very low molecular weight. This material co-sediments with 4*s* tRNA in sucrose gradient centrifugation, but runs considerably faster than 4*s* tRNA upon acrylamide gel electrophoresis. As much as 30 percent of the amount of radioactivity found in the 4*s* tRNA peak in sucrose can be seen to migrate faster than 4*s* tRNA in acrylamide gel electrophoresis (*9*). In the presence of vinblastine sulfate the amount of this material of very low molecular weight is reduced by as much as 70 percent. The difficulty in separating 4*s* tRNA from smaller material in sucrose gradient may account for the earlier reports of the high inhibition of tRNA synthesis by vinblastine sulfate (*2*).

The inhibition patterns reported here for vinblastine sulfate are also seen with vincrystine sulfate. Vincrystine sulfate is only about 50 percent as effective as vinblastine sulfate at the same concentration in inhibiting the incorporation of radioactivity into cytoplasmic RNA of HEp-2 cells; this result is in agreement with those reported for Ehrlich ascites cells in vitro (*2*).

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6. The use of such a double gel allows the rRNA of high molecular weight to enter and band in the 2.7 percent gel, whereas the tRNA is concentrated at the interface between the gels resulting in a much sharper band in the 5.5 percent gel than that which would be found in a gel consisting entirely of 2.7 percent acrylamide.
7. The composition of the toluene scintillation fluid is 7.4 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]-benzene in 1 liter of toluene.
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9. This has also been found by D. Bernhardt and J. E. Darnell (personal communication).
10. Supported by grants from the American Cancer Society (E 314D and PRA-36), NSF (GB 4555), and PHS (CA 08494-03). One of us (E.K.W.) is a Helen Hay Whitney Foundation postdoctoral fellow.

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Hurler and Hunter Syndromes: Mutual Correction of the Defect in Cultured Fibroblasts

Abstract. *The biochemical defect of cultured skin fibroblasts from Hurler or Hunter patients (faulty degradation of sulfated mucopolysaccharide, resulting in excessive intracellular accumulation) may be corrected if cells of these two genotypes are mixed with each other or with normal cells. The effect is mediated by substances released into the medium.*

The Hurler and Hunter syndromes are genetically transmitted disorders, characterized by retarded growth and mentation, skeletal abnormalities, excretion of chondroitin sulfate B and heparitin sulfate in urine, and deposition of these two mucopolysaccharides (MPS) in various tissues (*1*). The biochemical abnormality, accumulation of mucopolysaccharide, is preserved in fibroblasts cultured from the skin of affected individuals (*2*). We have previously shown this accumulation to be the result of inefficient degradation of intracellular MPS, rather than of excessive synthesis or reduced secretion (*3*).

Table 1. Effect of preincubated medium on accumulation of labeled intracellular mucopolysaccharide. Hurler or Hunter fibroblasts were incubated with unlabeled medium for 2 days, followed by 2 days in medium supplemented with $^{35}SO_4$, 20 × 10^6 count/min per milliliter. In each case, 7 ml of medium was used, of which 2 ml was fresh while 5 ml had been exposed to cells of the genotype indicated.

Cells	Medium previously exposed to	MPS accumulation (count/min per mg of protein)
Hurler	Hurler	258,000
	Hunter	132,000
Hunter	Hunter	238,000
	Hurler	110,000

Although the two diseases have similar clinical and biochemical manifestations, they are genetically distinct; Hurler's is transmitted as an autosomal recessive, while Hunter's, also recessive, is X-linked (*1*). The biochemical lesion must therefore occur at different points in the pathway of MPS degradation. One would expect that hybrid cells, containing a normal allele for the Hunter gene (derived from the Hurler parent) and a normal allele for the Hurler gene (derived from the Hunter parent), might be able to degrade MPS adequately. Surprisingly, we found that Hurler and Hunter cells can compensate for each other's defect when simply mixed in culture, or even when cells of one genotype are exposed to medium preincubated with the other.

Fibroblasts were obtained from skin explants from one adult Hunter patient, four Hurler patients under the age of 6, one 5-year-old SanFilippo patient, two normal infants, and one normal adult. The cells were cultured in Eagle's minimal essential medium (with $MgCl_2$ substituted for $MgSO_4$), supplemented with nonessential amino acids, 10 percent fetal calf serum, penicillin, streptomycin, and nystatin, in an atmosphere of 5 percent CO_2. The growth rate of the cell lines was sufficiently similar to preclude overgrowth of one line by another during the experiments.

The incorporation of $^{35}SO_4$ into intracellular MPS of normal, Hurler, and Hunter cells begins at the same rate; but whereas the labeled MPS reaches a steady state in normal cells within 24 to 48 hours, it continues to increase in linear fashion in the Hurler and Hunter cells for the duration of the experiment (*3*). However, mixtures of these two genotypes with each other display almost normal kinetics, as do mixtures