The chelating property of these nucleotides rather than their high free energy, as usually presumed, may also explain their inhibitory action in other *in vitro* hemolytic systems.

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Dactinomycin: Relative Resistance of Green Monkey Kidney Cell Cultures to its Action.* (32014)

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Evidence has accumulated that suppression of DNA dependent RNA synthesis by dactinomycin (U.S. Pharmacopeia official name and also referred to as actinomycin D, actinomycin C_1 and actinomycin IV) begins at about 0.01 μg/ml and maximum inhibition is reached at about 1-5 μg/ml(1-5). In the present study it was unexpectedly observed that while near maximum inhibition of yield of vaccinia virus occurred in HeLa and mouse embryo cells (ME) beginning at 2 μg dactinomycin/ml, in primary green monkey kidney cells (GMK) it was necessary to use a concentration of 200 μg/ml to achieve similar inhibition. The experiments reported here were performed to analyze this phenomenon. Results demonstrated the requirement for unusually high doses of dactinomycin for inhibition of RNA synthesis in GMK cells. A probable explanation for the findings is that GMK cells take up much less dactinomycin than do HeLa cells.

Materials and methods. Cell cultures. HeLa cells, ME, and GMK cell cultures were obtained from Microbiological Associates, Inc., and maintained on Eagle's minimum essential medium in Earle's balanced salt solution (EMEM) containing 2% heated (56°C for 30 minutes) Agamma calf serum (Hyland), 4 mM of glutamine, 100 units of penicillin, 100 μg of streptomycin, 10 μg of kanamycin and 20 μg of polymyxin B per milliliter.

Virus. Vaccinia virus obtained through the courtesy of Dr. Klaus Schell, Microbiological Associates, Inc., Bethesda, Md., was propagated in monolayers of GMK cells and titers of 10^7 plaque forming units (PFU) per ml were regularly obtained. A liquid overlay vaccinia plaque system(6) was used to determine titers. Monolayer cultures of 4×10^5 cells in roller tubes were infected at a multiplicity of 5-10 pfu/cell in medium with or without dactinomycin. After one hour adsorption at 37°C, the medium was removed, the cells were washed 5 times with Earle's balanced salt solution (EBSS), and 1 ml of fresh maintenance medium was added. After 24 hours of incubation at 37°C, the cell-associated virus from each treated group was harvested and pooled after 3 cycles of freezing and thawing.

RNA synthesis. The effect of dactinomycin on uptake of ^{3}H-uridine into nucleic acids of HeLa and GMK cultures was determined.

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TABLE I. Effect of Various Concentrations of Dactinomycin on RNA Synthesis and Growth of Vaccinia Virus in Various Cell Cultures.

Dactinomycin (μg/ml)	% Inhibition of ^{3}H uridine incorporation		% Inhibition of virus*			Cytotoxicity after 6 hr			Color of cell pellet	
	HeLa	GMK	HeLa	ME	GMK	HeLa	ME	GMK	HeLa	GMK
.02	49.3	—	—	—	—	—	—	—	—	—
.2	77.0	37.0	75.0	87.5	—	0	+	0	White	—
2.0	98.0	63.0	97.5	95.0	37.0	+	+	0	Slight yellow	White
20.0	99.0	87.0	—	—	75.0	—	—	0	Yellow	"
64.0	—	—	—	—	87.5	—	—	0	—	—
200.0	—	92.0	—	—	96.5	—	—	0	—	Yellow

* Virus titers determined 24 hr after infection.

Roller tubes were incubated in maintenance medium containing 0.5 ml volumes of the various concentrations of dactinomycin (7 roller tubes per group), for 2 hours at 37°C. After addition of ^{3}H-uridine (0.1 μc/0.2 ml/roller tube) for 45 minutes, the cultures were chilled, washed 3 times with cold EBSS, and harvested by scraping into 1 ml of EBSS. The cells from each group were pooled and centrifuged for 10 minutes in an International PR2 centrifuge at 1,000 *g*. Cell pellets were solubilized with 0.4 ml of 0.5 N NaOH, and then 1.6 ml of distilled water was added. The mixture was allowed to stand for 5 minutes and samples of 0.3 ml were precipitated with 2 ml of 10% trichloroacetic acid (TCA). Acid precipitable material was then collected on 27 mm membrane filters, washed once with 0.1% TCA, dried in an oven for 10 minutes and radioactivity determined in a Packard scintillation counter. Dactinomycin (NSC 3053 Lot No. L554651-0-10, Merck Sharp & Dohme) was kindly supplied by Dr. I. Kline, Microbiological Associates, Inc., Bethesda, Md. A stock solution of the drug was made in maintenance medium at a concentration of 500 μg/ml and stored at 4°C in the dark. Appropriate dilutions were made in maintenance medium before use in the experiments.

Results and discussion. Table I depicts the inhibitory effects of dactinomycin on cellular RNA synthesis and on multiplication of a DNA virus (vaccinia virus) in the different cell cultures. The results are expressed as the percentage of the values obtained with control cultures infected similarly but incubated without the antibiotic. Approximately 10- to 100-fold higher concentrations of dactinomycin were required in GMK cells *vs* HeLa and ME cells in order to achieve comparable inhibition. More than 95% inhibition of vaccinia virus multiplication was caused by 200 μg/ml dactinomycin in GMK cells as compared to 2 μg/ml dactinomycin in HeLa cells. Similarly, a 10- to 100-fold higher concentration was required to achieve equivalent inhibition of RNA synthesis in GMK cells.

There were visible signs of cytotoxicity after 6 hours of incubation of HeLa cells in the presence of 2 μg/ml dactinomycin (Table I). However, no signs of cytotoxicity appeared after 24 hours of incubation of GMK in the presence of 200 μg/ml dactinomycin.

The explanation for the difference between GMK and HeLa sensitivity may be related to the data presented in the last column of Table I. Dactinomycin stained HeLa cells a yellow color at a concentration of 2-20 μg/ml, whereas a concentration of 200 μg/ml was required to stain GMK cells. A more quantitative determination of the uptake of dactinomycin by cell cultures was done as follows.

Determination of the uptake of dactinomycin by cell cultures. Monolayer cultures of 10^7 cells in 32 fluid ounce bottles of HeLa and GMK cells were each treated with 20 ml of 0, 0.1, 1 or 10 μg/bottle of dactinomycin in maintenance medium. After 2 hours' incubation at 37°C, the fluid was removed and the cells were thoroughly washed 3 times with EBSS, and they were then scraped into 10 ml of EBSS for dactinomycin extraction. Each sample of dactinomycin-treated cells was sonicated for 20 seconds and 3×10^{-3} molar of final concentration of magnesium was added. They

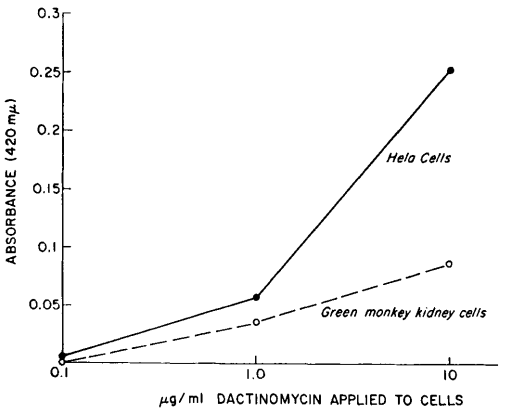

FIG. 1. Dactinomycin uptake by HeLa and GMK cells.

were further incubated for 30 minutes at 37°C in the presence of 50 μg/ml of DNAase. The preparations were brought to pH 9.0 by adding 0.2 ml of 0.5 N NaOH before the extraction of dactinomycin by xylene. Each sample was thoroughly mixed with 2 ml xylene and the resultant emulsion separated by centrifugation at 10,000 rpm in a Servall centrifuge. The absorption at 420 mμ was used as a measure of dactinomycin concentration. The results of such an experiment are illustrated in Fig. 1. It may be seen that the uptake of dactinomycin by GMK cells for a given external concentration of dactinomycin is less than that for HeLa cells and that the difference increases with increasing concentration of dactinomycin. This finding suggests that GMK cells may be substantially less permeable to dactinomycin than are most cells in cultures. It is also possible that the dactinomycin enters most cells in culture. It is also possible that the dactinomycin enters both cell types equally well but there is more rapid breakdown or less irreversible binding in GMK with the consequent removal of drug during washing. For example, differences in nuclear membrane permeability rather than cell membrane permeability could account for the observations. The present finding and the previously reported observations on drug resistant lines of HeLa cells(7) do not permit a choice between these two possibilities.

The unusually high dose of dactinomycin needed to inhibit DNA dependent RNA synthesis in GMK may help explain the unexpected findings that the generally used doses of dactinomycin (1 μg/ml) resulted in only partial inhibition of DNA virus-specified proteins(8,9).

Also, it is of interest to note that GMK cells are relatively resistant to the action of puromycin(10). Two μg puromycin/ml substantially inhibited poliovirus synthesis in HeLa cells, but at least 100 μg/ml of the drug was required to achieve comparable inhibition in GMK cells. It would be of interest to determine whether the high dosages of dactinomycin and puromycin necessary for their action in GMK are due to relative impermeability to these inhibitors. A related but unanswered question is whether all cell types within an animal species have similar sensitivity to dactinomycin.

Summary. The action of dactinomycin on cellular RNA synthesis and vaccinia virus multiplication in HeLa, mouse embryo (ME), African green monkey kidney (GMK) cells in culture were compared. Approximately a 10-100 fold higher concentration of dactinomycin was required in GMK cells as compared with HeLa cells and ME to equivalently suppress the cellular RNA synthesis and vaccinia virus multiplication. Also, dactinomycin was approximately 100-fold less toxic for GMK. The higher concentrations of dactinomycin required to inhibit GMK as compared with HeLa cells is probably due to a lower uptake of the antibiotic by the GMK cells.

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