Increased cyclic AMP in lymphocytes is associated with an inhibition of phosphorylation of chromatin proteins (*13*). Whether inhibition of phosphorylation by cyclic AMP represents an effect of cyclic AMP on phosphatase activity remains to be determined.

Several recent experiments support the hypothesis that cyclic GMP and cyclic AMP exert opposing influences on the induction of lymphocyte proliferation. Results obtained with sensitized rat spleen lymphocytes indicate that cyclic GMP and cholinergic agents enhance the cytotoxic function of T (thymus-derived) lymphocytes while agents which raise intracellular concentrations of cyclic AMP are inhibitory (*14*). Both 8-bromo cyclic GMP ($10^{-6}M$ to $10^{-7}M$) and carbachol ($10^{-10}M$ to $10^{-13}M$) enhance the rate of labeled thymidine incorporation into graft-versus-host primed mouse splenocytes while theophylline and PGE_1 inhibit uptake of labeled thymidine (*14*). These findings correlate with results obtained with human peripheral blood lymphocytes indicating that cholinergic agents enhance phytohemagglutinin-stimulated uptake of labeled thymidine (*8*), while PGE_1 inhibits this stimulation of thymidine incorporation (*9*). Our results suggest that stimulation of phosphorylation of nuclear acidic proteins by cholinergic agents may be part of the mechanism by which these agents enhance the induction of lymphocyte proliferation. Experiments are necessary to determine the effects of cholinergic agents upon lymphocyte RNA synthesis. Recent data indicate that cholinergic agents can influence lymphocyte incorporation of labeled uridine. In human lymphocytes, cholinergic agents stimulate [^{14}C]uridine incorporation into RNA (*8*) while PGE_1 inhibits phytohemagglutinin-induced incorporation of uridine (*9*). These observations are in accord with the proposal (*2*) that phosphorylation of specific chromatin proteins is important in the regulation of synthesis of RNA necessary for proliferation.

Phosphorylation of a protein fraction having a molecular weight of about 52,000 is stimulated by cholinergic agents and inhibited by PGE_1 (Fig. 2). Phosphorylation of this same chromatin protein fraction is stimulated preferentially early after mitogenic stimulation of horse lymphocytes with Con A (*2*). Our data suggest that this protein plays a regulatory role in the process of induction of lymphocyte proliferation and indicate that the phosphate content of this chromatin protein is regulated by cyclic nucleotides. The evidence supports the hypothesis that control of phosphorylation of specific chromatin proteins is an important mechanism regulating early events in the induction of lymphocyte proliferation.

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Transforming Potential of the Anticancer Drug Adriamycin

Abstract. *A Fischer rat embryo cell system in vitro, which had been shown to be highly accurate in identifying chemical carcinogens and to have application in the study of chemicals having anticancer properties, was used to study the anticancer drug adriamycin. At a nontoxic dose adriamycin not only did not protect the cells from transformation by the carcinogen 3-methylcholanthrene, but was found in two separate experiments to act on its own as a transforming agent.*

We have described a sensitive in vitro Fischer rat embryo cell system (F1706) for identifying chemical carcinogens (*1*). Using a clone of this culture, which we call H43, we report here that the anticancer compound adriamycin (*2*), at a nontoxic dose, not only does not protect the cells from transformation by 3-methylcholanthrene (3MC) but was found in two separate experiments to act on its own as a transforming agent.

Using the reduction in plating efficiency and the number of cells per

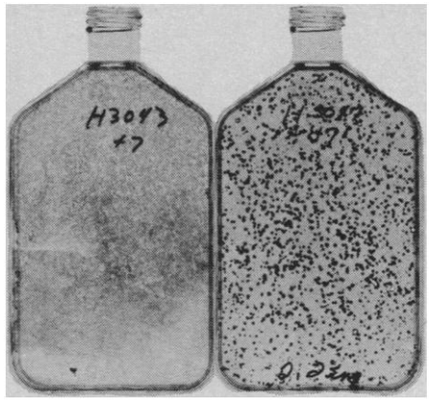

Fig. 1. Transformation of Fischer rat embryo clone H43 by adriamycin. (Left) H3043, control; (right) H3047, adriamycin treated.

colony as a test for toxicity, we found that the maximum nontoxic dose of adriamycin was 0.15 ng/ml.

The sequence of treatment of the high-passaged Fischer rat embryo cells with 3MC and adriamycin is shown in Table 1. The growth and transfer medium consisted of Eagle's minimal essential medium in Earle's salts (EMEM) supplemented with 10 percent fetal bovine serum, 2 m*M* L-glutamine, and 100 μg of gentamycin. The 3MC was diluted in acetone to 1 mg/ml, and further diluted in growth medium to 0.1 μg of 3MC per milliliter. Adriamycin (supplied by the Division of Cancer Control, National Cancer Institute) was diluted to 10 μg/ml in deionized water, and further diluted to a final dilution of 0.15 ng/ml in the growth medium. After treatment the cultures were coded, and the results were read blind.

At each subculture, one set of cultures was set aside to be held for 4 weeks (the medium was changed twice each week) and the other was divided into halves weekly to provide two new sets of cultures, one for the holding series and one for subdivision (vertical series) (*3*).

In each of two separate experiments, adriamycin at the maximal nontoxic dose, as well as the 0.1 μg of 3MC by itself and in combination with adriamycin, transformed the test cells (as judged by production of progressively growing foci of cells lacking contact inhibition and orientation) (Fig. 1). In each case, 1×10^6 transformed cells from the eighth vertical subculture produced progressively growing undifferentiated fibrosarcomas when inoculated subcutaneously into newborn Fischer rats (F344/f Mai) (Table 2).

Prior to transformation the cells are negative for rat leukemia virus (RaLV) expression. However, the information to code for the virus is present because eight to ten subcultures after the cells are transformed the RaLV gs-1 (species specific) expression is detectable (*4*).

Both the normal and transformed cells can be readily induced by 5′-iododeoxyuridine (IdU) to "turn on" virus (*5*). This induction of the endogenous RaLV by IdU is monitored by the viral RNA instructed DNA polymerase (reverse transcriptase) assay (*6*) and by banding of [^{3}H]uridine-labeled virus in isopycnic (20 to 50 percent) sucrose gradients. At the concentration used, adriamycin neither increased nor decreased this induced virus expression. Using this H43 system, we recently demonstrated that, at nontoxic doses, streptonigrin (*7*) and cordycepin (*8*)—two compounds that had been shown to inhibit type C RNA viruses and to have application in the treatment of cancer—inhibited the induction by IdU (*9*) of the endogenous, rat type C RNA virus and protected the cells from transformation by 3MC.

Table 1. Sequence of treatment of Fischer rat embryo cells. Abbreviations 3MC, 3-methylcholanthrene.

Day No.	Treatment
1	Inoculate cultures at 150,000 cells per milliliter
2	Replace medium with growth medium containing adriamycin (0 or 0.15 ng/ml)
3	Divide controls and adriamycin cultures each into two groups: Control A, medium replaced with fresh medium containing acetone 1:1000 Control B, medium replaced with fresh medium containing 0.1 μg of 3MC Adriamycin A, medium replaced with fresh medium containing adriamycin (0.15 ng/ml) Adriamycin B, medium replaced with fresh medium containing adriamycin (0.15 ng/ml) and 3MC (0.1 μg)
5	Transfer half the cells in their respective media to new flasks
7	Refeed with respective media
9	Refeed but omit 3MC from medium (3MC and acetone are no longer incorporated into medium)
10	Subdivide 1:2 and subculture weekly. Where called for, incorporate adriamycin into the medium at each medium change

Table 2. Results of treatment. Abbreviations: 3MC, 3-methylcholanthrene; Ad, adriamycin.

Treatment	Transformation*	Tumors†
	Experiment 1	
3MC alone	+(5)	1/ 9
Ad alone	+(4)	3/10
3MC and Ad	+(4)	10/10
Control	—	0/10
	Experiment 2	
3MC alone	+(6)	1/ 9
Ad alone	+(5)	4/10
3MC and Ad	+(5)	4/10
Control	—	0/10

* Numbers in parentheses represent subculture level when transformed foci were first noted. In each experiment, the cultures were carried for eight vertical subcultures and held for 4 weeks at each subculture. † Ratio of the number of newborn syngeneic Fischer rats with tumors to the number inoculated.

We know from double-blind studies that chemicals which transform the Fischer rat embryo cells are also carcinogenic in mice and rats. Chemicals which do not transform the cells are likewise noncarcinogenic in mice and rats (*1*). If we can extrapolate from the transformation of rat embryonic cells in vitro to tumor induction in humans in vivo, the possibility exists that the reemergence of cancer cells several years after chemotherapy may in some cases be due to induction by the treatment.

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