Glucocorticoid-Induced Resistance to Deoxycholate Lysis in HeLa Cells

Abstract. *HeLa cells exposed to low concentrations of prednisolone and other glucocorticoids acquired resistance to lysis with sodium deoxycholate. The effect showed considerable specificity by being confined to steroids that cause elevation of alkaline phosphatase in tissue culture and are known to have anti-inflammatory effects in vivo.*

An increase of alkaline phosphatase in several strains of mammalian cells grown in vitro in the presence of prednisolone is evidence for an effect of a derivative of an animal hormone on cell cultures. Since the original report of Cox and McLeod (*1*) many other findings have been made concerning the glucocorticoid-induced changes in animal cell lines. Increased concentrations of RNA and protein and an apparent increase in the cell volume (*2*) have been observed. The specificity of the effect was confined to steroids having hydroxyl groups at both carbon No. 11 and carbon No. 21 (*3*).

Thus far, no mechanism for the glucocorticoid-induced elevation of alkaline phosphatase in cultured cells has been proposed. The effect on cell cultures differs from the hormonal enzyme inductions in animals, since the increase in alkaline phosphatase in cell cultures becomes apparent after a fairly long exposure to the hormone (usually about 24 hours or longer), while in animals the concentrations of enzymes increase in a much shorter period (*4*). Attempts have been made to use puromycin as an inhibitor of alkaline phosphatase induction (*5*) but, so far, there is no direct evidence that glucocorticoids affect the informational pathways between the cell DNA and ribosomes.

Cox and McLeod (*1*) used lysis of cells with sodium deoxycholate to obtain cell-free preparations. During attempts to repeat this procedure, I noticed that cells exposed to prednisolone were less susceptible to deoxycholate lysis than untreated cells. This indicated that the steroid, even at a concentration of 0.1 μg per milliliter, might have some specific effect on the cell membrane. To test this under controlled conditions, HeLa cells were grown in prescription bottles in Eagle's minimal essential medium supplemented with 10 percent calf serum (MEM 10). After 16 hours of incubation at 36.5°C, the medium was replaced by serum-free MEM containing various steroids at concentrations ranging from 0.1 to 1.0 μg per milliliter, and the cell cultures were re-incubated for varying periods of time. Sodium deoxycholate was then added to induce cell lysis. Twenty-four hours later the protein content of cells that adhered to glass was determined. Cells exposed to prednisolone were considerably more resistant to the lytic effect of deoxycholate than control cells (Fig. 1), and specificity of the protective effect resembled alkaline phosphatase induction. Table 1 shows the results obtained with a limited number of steroids, of which three were active in stimulating alkaline phosphatase induction and three were inactive; all were used at a concentration of 0.1 μg per milliliter. Two replicate cultures from each group were used to determine the basal level of protein before addition of deoxycholate. Total amount of protein in cells adherent to glass was taken as an index of resistance to lysis, and the results were expressed in percentage of the initial protein content before treatment with deoxycholate. In control cultures only about one-third of the initial cell protein remained, but the steroid-treated cells were protected to varying degrees, depending upon the steroid used. There was a remarkable correlation between ability of the steroid to induce an increase in alkaline phosphatase and its protective effect,

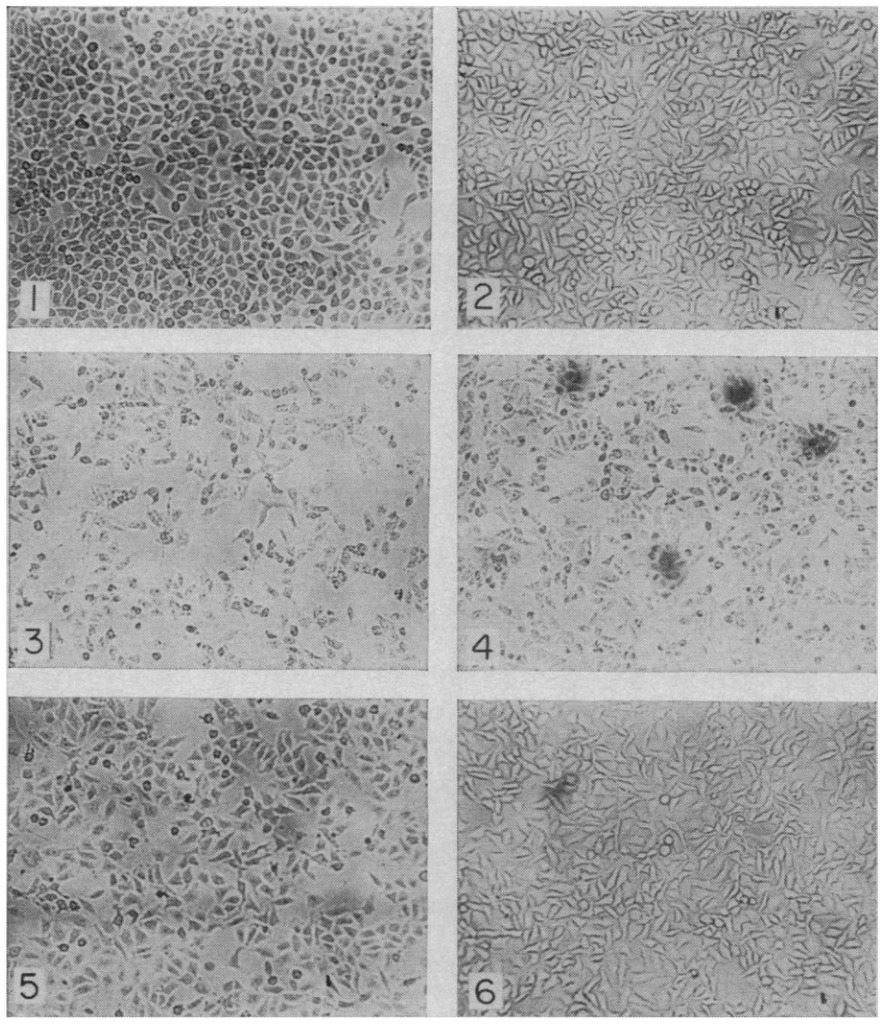

Fig. 1. Prevention of lysis by deoxycholate in HeLa cells pretreated with prednisolone. Cultures 1, 3, and 5, no pretreatment; cultures 2, 4, and 6, incubated with prednisolone (0.5 μg per milliliter). Deoxycholate lysis: cultures 1 and 2, no deoxycholate; cultures 3 and 4, 100 μg of sodium deoxycholate per milliliter; cultures 5 and 6, 50 μg of sodium deoxycholate per milliliter (× 200; fresh unstained culture).

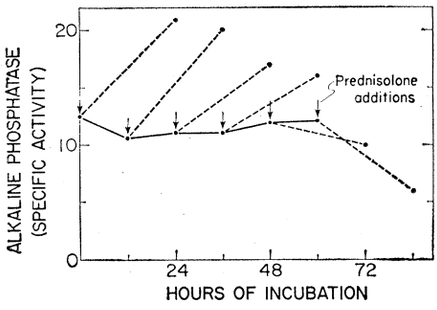

Fig. 2. Effect of preincubation time on the alkaline phosphatase inductions by prednisolone. Arrows indicate prednisolone additions at various times after plating of cells. Solid line, control; broken line, specific activity of induced alkaline phosphatase.

since no decrease, and perhaps even a slight increase, in total protein was evident in cells pretreated with prednisolone, hydrocortisone, 9 α-fluoroprednisolone, and hydrocortisone hemisuccinate. The remaining three steroids, which did not bring about elevation of alkaline phosphatase, were much less effective in protecting cells from lysis by deoxycholate. Increase in the intracellular enzyme did not result from prevention of the leakage of alkaline phosphatase from cells by the steroid known to protect against lysis by deoxycholate, since all assays for alkaline phosphatase activity in supernatant media from both steroid-treated and control cultures were negative.

A possible mechanism of the steroid effect was indicated when alkaline phosphatase induction by prednisolone was measured as a function of the time of preincubation of cell cultures before addition of the hormone. In one such experiment, cells were plated out in the serum-supplemented medium, and prednisolone was added to replicate cultures at different times after plating. Results indicated that induction of alkaline phosphatase had fallen off rapidly in cells preincubated for more than 24 hours (Fig. 2). Thus, it could be possible that leakage of some precursors required for formation of alkaline phosphatase might be prevented by prednisolone. Additional support for this speculation may be found in the observation that in cell cultures originating from dense inoculums the specific activity of alkaline phosphatase is usually higher than in thinly inoculated cultures, and that frequent changes of medium prevent enzyme induction (*6*).

Much accumulated evidence indicates that in animals hormonal induction takes place through effects on the formation of messenger RNA (*7*). Whether glucocorticoid induction of alkaline phosphatase in cell cultures will fit this proposed mechanism remains to be determined. There is, however, a possibility, raised by my observations, that the effects of prednisolone and related steroids on tissue culture cells involve primarily the cell surface. Other effects, such as increase in total cellular RNA and cell protein and in alkaline phosphatase, may be brought about indirectly through regulation of the intracellular concentrations of compounds that control enzyme activities through feedback inhibition mechanisms.

Table 1. Relation between the ability of different steroids to induce alkaline phosphatase and to prevent lysis by deoxycholate. Growth conditions and treatment same as in Fig. 1.

Steroid (0.1 μg/ml)	Change in protein conc. per culture after deoxycholate treatment (%)	Alkaline phosphatase induction (*3*)
Prednisolone	+32	+
Hydrocortisone	+ 9	+
9 α-Fluoroprednisolone	+ 9	+
Hydrocortisone hemisuccinate	+ 4	+
Cortisone	−21	−
Prednisone	−37	−
Reichstein's substance S	−47	−
Control	−67	−

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References and Notes

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8. I thank Carole Bishop for technical assistance. Supported in part by research grant No. CA-08315-01 from the National Cancer Institute.

21 January 1966

Adenosine Triphosphate: Protection against Radiation-Induced Chromosome Loss in Drosophila

Abstract. *Injection of 5 milligrams of adenosine triphosphate per milliliter into adult* Drosophila melanogaster X^{c2} yB/sc^8 y^+ *4 to 8 hours old either immediately before or after administration of 2000 roentgens of x-rays protected those cells in spermatogenesis which were in or near meiosis from the loss of the ring X or the* y^+ *portion of Y chromosome. The loss of the chromosomes was determined by appearance of exceptional XO males in the offspring.*

Evidence accumulated in the last decade indicates that interference with the recovery process of chromosomes injured by radiation results in increased damage (*1*). A possible explanation (*2, 3*) is that inhibition of the oxidation systems necessary for production of the adenosine triphosphate (ATP) needed to repair injured chromosomes may account for the increased genetic and chromosomal aberrations. Prior treatment with ATP was reported to protect chromosomes of *Vicia faba* from x-ray radiation (*3*) and to aid in recovery of radiation-injured chromosomes in the first microspore division of *Tradescantia* (*4*).

To test whether extraneous ATP can aid in preventing the loss of radiation-injured chromosomes in *Drosophila*, the XO method, in which appearance of exceptional males indicates a loss of the X or y^+ portion of the Y chromosomes, was used. Adult male *Drosophila* X^{c2} *yB*/sc^8 y^+ *Y*, aged 2 to 16 hours, were injected by means of a micropipet (*5*) with 5 mg of ATP per milliliter of 0.85-percent NaCl; 0.1 μl of fluid was introduced near the testis of each fly. The *Drosophila* were injected with ATP or saline just before or immediately after a dose of 2000 r of x-rays from a Mattern unit with a Thermax tube (100 kv, 5 ma, 1/2-mm Al filter, 11 cm distance, 320 r/min). The males were mated daily for 12 days to a new group of *ywf* females at a ratio of one male to three females. By this brood method, the effect of the treatment and radiation on the various stages of spermatogenesis could be ascertained (*6*). The broods from days 0–1 and 1–2 developed from mature and almost mature spermatozoa, broods from days 6–7 and 7–8 developed from cells in or near meiosis at the time of treatment, and broods from day