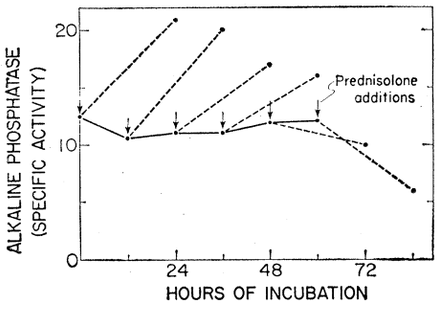

Fig. 2. Effect of preincubation time on the alkaline phosphatase inductions by prednisolone. Arrows indicate prednisolone additions at various times after plating of cells. Solid line, control; broken line, specific activity of induced alkaline phosphatase.

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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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

9–10 developed from spermatogonia.

Injection of ATP into X^{c2} yB/sc^8 $y+$ *Y Drosophila* which received no irradiation (Table 1, part i) did not significantly increase or decrease the number of XO males in the progeny. These XO's are primarily due to spontaneous loss of the ring X chromosomes which occur at any time; thus the loss may have occurred before or sometime after injection of ATP. In the series of experiments in which the ATP was injected immediately before the male was exposed to x-rays (Table 1, part ii), there was no significant change in the number of XO males induced in the first five broods. There was a significant decrease of radiation-induced XO's as a result of prior ATP treatment in broods from days 5–6, 7–8, 8–9, and 9–10. Treatment with ATP after irradiation (Table 1, part iii) resulted in a significant reduction in the number of XO males in broods from days 1–2 and 7–8. The significant differences for broods from day 1–2 probably represent just chance fluctuations in the data. Treatment with ATP after irradiation does not give the reduction in all broods that prior treatment gives. The sensitive stage in *Drosophila* spermatogenesis for the radiation-induced loss of chromosomes was close to the time of meiosis; judged by the number of F_1 offspring counted in each brood, the period of radiation-induced sterility coincides with this period of sensitivity to the loss of the chromosomes. In the case of a single male mated to three females daily, the first appearance of induced crossing-over (which must occur prior to meiosis) was in the brood from day 6–7 at 24°C. (*7*). Thus protection by ATP against loss of chromosomes was most effective on cells about to enter meiosis, or in meiosis, at the time of irradiation. The total percentage of XO's is significantly less in the groups treated with ATP both before and after radiation (Table 1), and thus the protection was not the result of a brood shift. The ATP did not afford any protection against radiation-induced nondisjunction or to the increased loss of ring X chromosome which occurred one to several cell divisions after fertilization and resulted in a "mosaic" organism.

Table 1. Effect of injection of ATP in saline and of saline alone, (i) without accompanying irradiation, (ii) before irradiation, and (iii) after irradiation, on production of XO males in *Drosophila melanogaster* (X^{c2} yB/sc^8y^+ Y × *ywf*).

Injection	(i) No irradiation			(ii) Before irradiation			(iii) After irradiation		
	Gametes (No.)	*ywf* males (No.)	XO males (%)	Gametes (No.)	*ywf* males (No.)	XO males (%)	Gametes (No.)	*ywf* males (No.)	XO males (%)
				Brood day 0–1					
ATP	2084	11	0.527	9747	211	2.164	5195	132	2.540
Saline	1530	4	0.261	10146	204	2.010	4797	104	2.168
				Brood day 1–2					
ATP	3446	22	0.638	16102	254	1.577	14517	295	2.032[a]
Saline	3703	18	0.486	14736	254	1.723	14535	370	2.545[a]
				Brood day 2–3					
ATP	2730	14	0.512	11791	272	2.333	12707	306	2.408
Saline	3765	17	0.451	11532	269	2.332	14416	345	2.393
				Brood day 3–4					
ATP	2691	13	0.483	7308	215	2.941	8119	241	2.968
Saline	2419	11	0.454	8887	237	2.666	10328	353	3.417
				Brood day 4–5					
ATP	2996	17	0.567	5127	178	3.471	4958	165	3.327
Saline	3648	12	0.328	5924	203	3.426	7241	255	3.521
				Brood day 5–6					
ATP	1940	6	0.309	3792	118	3.111[b]	2232	80	3.584
Saline	2300	11	0.478	4014	179	4.459[b]	2972	95	3.196
				Brood day 6–7					
ATP	2246	10	0.445	1929	104	5.391	1089	56	5.142
Saline	1312	5	0.381	2951	154	5.218	1581	94	5.945
				Brood day 7–8					
ATP	1269	5	0.394	1168	62	5.308[c]	877	40	4.561[d]
Saline	566	4	0.706	1226	92	7.504[c]	775	61	7.870[d]
				Brood day 8–9					
ATP	981	4	0.407	883	40	4.530[e]	400	17	4.250
Saline	744	3	0.403	648	47	7.253[e]	383	19	4.960
				Brood day 9–10					
ATP	614	4	0.651	4029	70	1.737[f]	3543	81	2.286
Saline	859	7	0.814	4995	119	2.382[f]	5384	117	2.173
				Brood day 10–11					
ATP	829	4	0.482	1134	22	1.940	4260	71	1.666
Saline	1160	5	0.431	1776	25	1.407	4799	75	1.562
				Brood day 11–12					
ATP	982	2	0.203	1679	16	0.952	3029	31	1.023
Saline	908	2	0.220	2412	20	0.829	5103	63	1.234
				Total					
ATP				64689	1562	2.414[g]	60926	1515	2.486[h]
Saline				69247	1803	2.603[g]	72314	1951	2.697[h]

[a] $x^2 = 8.561$. [b] $x^2 = 9.675$. [c] $x^2 = 4.792$. [d] $x^2 = 7.852$. [e] $x^2 = 5.246$. [f] $x^2 = 4.524$. [g] $x^2 = 4.883$. [h] $x^2 = 5.828$.

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