bility of the chorion to enzymatic action, a comparison was made between non-diapause embryos at stage 33 and embryos at this stage which had been induced to undergo diapause. The data in figure 2 illustrate that the chorion of diapause II embryos were more resistant to pronase digestion. The values determined for embryos at diapause II for 4 and 28 weeks were virtually identical, indicating that longer duration in diapause does not enhance the resistance of the chorion to pronase.

The use of pronase was first demonstrated by Smithberg[7] in the chorion of medaka. In this species, which does not exhibit any instance of diapause, differences in the duration of chorion digestion were not observed between embryos of different stages. The stage specific variation in chorion susceptibility to pronase in annual fish embryos may represent an evolutionary response to the erratic climatic conditions to which these fishes are normally exposed. *N. guentheri* is native to the isolated ponds and mudholes along the coastal lowlands of Kenya and Tanzania[16]. During the dry season all the adults die due to evaporative waterloss[17]. The survival of the population becomes dependent entirely upon embryos which undergo diapause at specific stages of their normal development. The evolution of a resistant egg envelope is an essential requirement for survival since these embryos are normally exposed to extremes in temperature, water chemistry, and desiccation.

The role of the chorion in the survival of annual species and the mechanism by which the embryo modifies its chorion are speculative at this time. The present study demonstrates that the susceptibility of the annual fish chorion to external chemical damage changes with the stage of development and upon entry into diapause.

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Differential lethality following treatment with ionizing radiations of various energies in *Drosophila*

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Summary. Male and female gametes of *Drosophila* were treated with various doses of ionizing radiations: X-rays at different energy, and gamma-rays from 2 sources given singly and in 2 temporal sequences. The induced lethality was assessed in successive developmental stages by scoring the number of eggs, larvae and adults. The results clearly show that the effects of various radiations appear in terms of difference among developmental stages and/or between treated sexes/genotypes. It is suggested that the various energies affect different gene functions which are not completely independent, as supported by the non-additive effects of the two temporal sequences.

The results obtained using UV in yeast[2-4] suggest that mutagenic repair is not a single process, but rather comprises a number of partially independent processes, each of which gives rise to a different category of mutation.

The comparison between UV, gamma and chemicals mutagenesis[5,6] supports the model of mutagenic repair in which partially independent sets of gene functions are required for the production of mutations of different kinds, for their formation at different sites and for their induction by different mutagens. Further results[7] indicated that both the genetic and the developmental state of the organism influence the induction and the repair of the X-irradiation damage and that many of the same processes which are utilized in normal cell growth and division are involved in the repair mechanism.

In previous work[8] we found different mutagenic responses by irradiation of selection lines genetically related and we suggested that the different mutation frequencies observed could result from some kind of gene activity control; this view was supported by further results[9] showing that when the two sexes are separately irradiated there is a different relationship between developmental stage and response to the treatment.

Since the various mutagens may involve distinctive patterns of genetic control and, on the other hand, the developmental patterns may affect the expression of induced mutations, it seemed interesting to study the interaction between mutagens and developmental stages. For this purpose we used the biological system tested in our previous work[9], on which we checked whether lethality expression is modified during development by ionizing radiations of various energies.

Table 1. Regression coefficients (b ± SE) estimating the relationship between the dose applied and the survival at different developmental stages after treatment with X-rays at 30 and 97 KeV

		X-rays 30 KeV b ± SE	d.f.	X-rays 97 KeV b ± SE	d.f.
L/E	Cross A	−1.82 ± 0.545***	12	−0.30 ± 0.494	12
	Cross B	−2.80 ± 0.280***	12	−2.12 ± 0.850**	12
F/L	Cross A	−1.31 ± 0.722*	12	−1.37 ± 0.697*	12
	Cross B	−1.67 ± 0.510***	12	−0.79 ± 0.772	12
1-(L-F)/E	Cross A	−0.16 ± 0.417	12	−0.71 ± 0.499	12
	Cross B	0.42 ± 0.276	12	0.59 ± 0.448	12
F/E	Cross A	−1.97 ± 0.698***	12	−1.29 ± 0.514**	12
	Cross B	−2.70 ± 0.329***	12	−1.80 ± 0.800**	12

* $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$.

Table 2. Regression coefficients (b ± SE) estimating the relationship between the dose applied and the survival at different developmental stages after treatment with gamma-rays from ^{137}Cs and ^{60}Co, singly and in combination

		γ-rays ^{137}Cs b ± SE	γ-rays ^{60}Co b ± SE	d.f.	γ-rays $^{137}Cs + ^{60}Co$ b ± SE	γ-rays $^{60}Co + ^{137}Cs$ b ± SE	d.f.
L/E	Cross A	−1.08 ± 0.758	−1.80 ± 0.631**	12	−1.68 ± 0.712**	−1.60 ± 0.387***	10
	Cross B	−1.93 ± 0.708**	−1.69 ± 0.532***	12	−2.36 ± 0.662***	−2.31 ± 0.389***	10
F/L	Cross A	−1.78 ± 0.723**	−0.73 ± 0.703	12	−1.84 ± 0.717**	−2.30 ± 0.587**	10
	Cross B	−1.11 ± 0.790	−0.39 ± 0.697	12	−2.17 ± 1.258	−0.61 ± 1.120	10
1-(L-F)/E	Cross A	−0.94 ± 0.406**	0.23 ± 0.495	12	−0.26 ± 0.403	−0.78 ± 0.382*	10
	Cross B	0.03 ± 0.302	0.40 ± 0.399	12	0.12 ± 0.467	1.20 ± 0.434**	10
F/E	Cross A	−1.78 ± 0.842**	−1.61 ± 0.705**	12	−2.10 ± 0.785**	−2.32 ± 0.505***	10
	Cross B	−1.95 ± 0.858**	−1.42 ± 0.664**	12	−2.62 ± 1.001**	−1.57 ± 0.823*	10

* $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$.

Materials and methods. The effects of the following radiations of various energies were studied: X-rays at 30 effective KeV (60 kV, 4 mA, 173.9 rad/min); X-rays at 97 effective KeV (220 kV, 11 mA, 1 mmCu, 150 rad/min); γ-rays from ^{137}Cs at 662 effective KeV (36.5 rad/min); γ-rays from ^{60}Co at 1250 effective KeV (91.8 rad/min). Furthermore the gamma-rays were combined in 2 temporal sequences ($^{60}Co + ^{137}Cs$ and $^{137}Cs + ^{60}Co$).

Flies from a selected line K described in previous work[9] were treated with 7 doses of single radiations (from 1 to 4 Krad) and 6 of combined radiations (from 1.5 to 4 Krad).

The following experimental design was realized:

cross A treated vg/vg ♀ × vg/vg$^+$ ♂

cross B vg/vg ♀ × treated vg/vg$^+$ ♂

Males and virgin females 2–4 days old were irradiated and mated after 3 days with untreated flies; only the eggs (1000 for each dose) laid in the first 2 days after mating were taken. Two independent replicates of treatment were carried out. The flies were maintained at constant temperature of 25 °C.

The induced lethality was assessed by scoring the number of eggs (E), the number of larvae (L) and the number of adults (F).

The L/E ratio is taken as an estimate of embryonic survival; the post-embryonic survival is expressed both as 1-(L-F)/E and as F/L. In fact the usual F/L ratio is affected by embryonic mortality which determines the number of larvae actually present (L); the 1-(L-F)/E ratio expresses the lethality of the post-embryonic stage (L-F is the number of larvae not reaching the adult stage) unaffected by the embryonic lethality. The F/E ratio is an estimate of egg-to-adult survival.

Results. In table 1 the regression coefficients estimating the dose-response relationship at different developmental stages after treatment with X-rays at 30 and 97 effective KeV are reported. At 30 KeV we can observe a similar mortality trend when male and female gametes are treated; particularly, lethal effects are detected in both embryonic (L/E) and post-embryonic (F/L) stages of cross A and cross B progenies.

The data from X-irradiation at 97 KeV show a different picture: when female gametes are treated we can see a dose-response relationship at the post-embryonic stage, while the effects of treatment on males are expressed at the embryonic one. The egg-to-adult survival of cross A and cross B progenies shows a dose-response relationship in both energies. Furthermore, the regression coefficients relative to 1-(L-F)/E, although not statistically significant, are always negative in cross A progenies and positive in cross B progenies of both energies. In table 2 the regression coefficient values relative to treatment with gamma-rays singly as well as in combination are given. We notice that treatment with ^{137}Cs produces a similar mortality trend as the X-rays at 97 KeV; once again, in cross A progenies the lethal effects are detected only in the post-embryonic stage, expressed both as F/L and as 1-(L-F)/E, while in the cross B progenies only at the embryonic one. As far as egg-to-adult survival is concerned, a significant regression is observed in both crosses. Conversely, the induced damage by ^{60}Co is expressed only as embryonic and egg-to-adult mortality in male and female gametes. It is noticeable that the signs of regression coefficients relative to post-embryonic survival, as 1-(L-F)/E, are both positive indicating that whole mortality is expressed at the embryonic stage.

The 2 temporal sequences of gamma-rays produce heterogeneous responses in males and females: when female gametes are treated we observe that mortality is expressed in all developmental stages; conversely, when the treatment is applied to male gametes the lethality is not evident at the post-embryonic stage (F/L). The post-embryonic survival, expressed as 1-(L-F)/E, indicates that embryonic and post-embryonic survival curves are not parallel only in $^{60}Co + ^{137}Cs$ sequence.

Discussion. Our results clearly show that the effects of various radiations appear in terms of differences among developmental stages and/or between treated sexes/genotypes.

Indeed we notice that X-rays at 30 KeV and gamma-rays from ^{60}Co show different and sex-independent lethality patterns, the former inducing lethal effects in all developmental stages, the latter only in the embryonic one. Conversely, when X-rays at 97 KeV and gamma-rays by ^{137}Cs are used, the lethality expression is dependent on sex/genotype treated but independent of energy: in the progenies of treated females (vg/vg) the lethal effects are detected prevalently in the post-embryonic stage, while in those of males (vg$^+$/vg) in the embryonic one.

The different expression of lethality at developmental stages may reasonably be thought to depend upon radiation damage involving different genes or biochemical lesions, implying that electromagnetic ionizing radiations of various energies can induce qualitatively different damages or involve differential repair mechanisms.

For the two sexes, the different responses observed in successive developmental stages might be attributable to differential mechanisms of repair; in fact there is evidence of the existence of a functional relationship between the processes of meiotic recombination and DNA repair in *Drosophila*[10–12]. On the other hand, this interpretation is not suitable to explain how the expression of lethality when the two sexes are separately irradiated is modified by the various radiations.

Our results might be better explained if different gene functions, affected by various energies, are assumed. The data obtained combining gamma radiations seem to confirm this view; in fact only in one of the 2 temporal sequences are differences expressed both between sexes/genotypes and among stages.

The non-additive effects of 2 sequences support, therefore, a model of mutagenic process in which the different gene functions involved are not completely independent.

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Regulation of testosterone production in fetal testicular cells: effect of androgens

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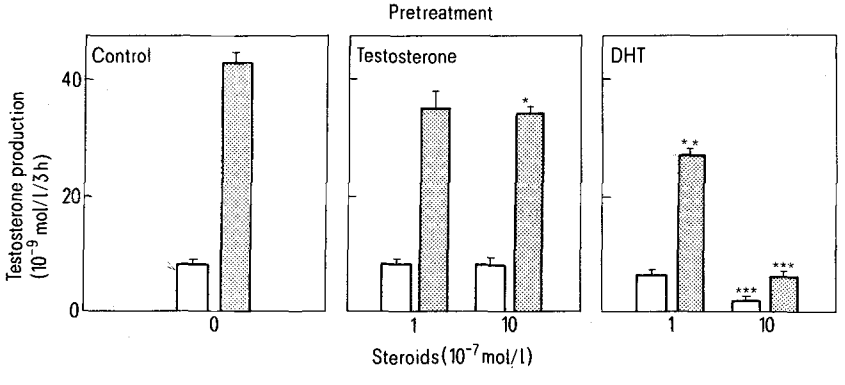
Summary. Pre-incubation of dispersed testicular cells from 18-day-old mouse fetuses in presence of testosterone or dihydrotestosterone resulted in a significant decrease of the hCG-stimulated testosterone production. These results suggest that during late fetal life testosterone production may be inhibited by an ultra-short loop feedback of androgens.

In vivo and in vitro studies have suggested that in immature and adult rats testicular testosterone production may be locally[1-3] controlled by androgens via a short-loop negative feedback mechanism. In the fetus, high concentrations of testosterone have been reported to exert a direct inhibitory influence on testicular testosterone synthesis in organ culture[4]. However, physiological concentrations of this androgen did not produce a negative effect[5]. The present study was undertaken to explore the influence of androgens such as testosterone and dihydrotestosterone (DHT) on basal and hCG-stimulated testosterone production by dispersed fetal testicular cells.

Materials and methods. Testes were excised from 18-day-old mouse fetuses. Fetal testicular cell suspensions were obtained from 150–200 fetal testes by mechanical dissection and collagenase dispersion using the method described earlier[6]. The cell suspension contained about 10% Leydig cells as identified by their bright yellow ring[7]. Aliquots of this cell suspension (10^5 cells/500 µl) were incubated with or without increasing concentrations of testosterone or dihydrotestosterone. After 30 min of incubation at 37 °C, the cellular suspension was centrifuged for 10 min at 100 × g and the supernatant discarded. The cells were then washed twice and incubated for 3 h at 37 °C in the absence or presence of 25 pM hCG (2nd IS for chorionic gonadotrophin bioassay). Since testosterone values obtained using unextracted medium did not differ from those obtained after ether extraction and the chromatographic procedure, testosterone was measured directly in the incubation media by radioimmunoassay as previously reported[6]. Testosterone levels were compared between groups by 1-way analysis of variance.

Results. The effect of pretreatment of fetal testicular cells with testosterone or DHT on basal and hCG-stimulated testosterone production is shown in the figure. The exposure of fetal cells to testosterone had no discernible effect on the quantity of testosterone produced under basal conditions during a 3-h incubation period. Pre-incubation of the cells with 10^{-7} M testosterone did not significantly affect the stimulatory effect of hCG on testosterone production, but the subsequent hCG-response of cells pre-exposed to 10^{-6} M testosterone was reduced ($p < 0.01$). Basal testosterone levels were not modified when the cells were pre-exposed to 10^{-7} M DHT, but were decreased with a higher concentration of DHT (10^{-6} M; $p < 0.001$). DHT had a significant inhibitory effect on hCG-stimulation of testosterone secretion in cells pre-incubated either with 10^{-7} M DHT ($p < 0.005$) or 10^{-6} M DHT ($p < 0.001$).

Discussion. The results of the present study show that both testosterone and DHT can influence the steroidogenic activity of the fetal testis. This effect is mainly characterized by a decrease of the stimulatory influence of hCG. Other authors[5] reported that physiological concentrations of testosterone were ineffective in inducing inhibition of testosterone production by whole testis in organ culture. Our data show that doses in the same range are able to reduce testosterone production by dispersed fetal testicular cells significantly, suggesting that this model is more appropriate for studying the regulation of testosterone synthesis in the fetal testis. Furthermore, it is interesting to note that the lowest concentration of androgen which is effective in our model is only 20 times greater than



Effect of a 30-min pre-exposure without or with T or DHT on T production by fetal testicular cells incubated 3 h in the absence (open bars) or presence of 25 pM hCG (solid bars). The results are the means of triplicate incubations. *$p < 0.05$, **$p < 0.001$, ***$p < 0.001$ as compared with appropriate control group.