

LETHAL EFFECTS OF FAR UV ON PRE-BLASTODERMIC EMBRYOS OF *Sciara ocellaris* (DIPTERA; SCIARIDAE)

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ABSTRACT

The lethal effect of far UV was studied in *Sciara* embryos at four pre-blastodermic stages. In two of them the nuclei were immersed within the endoplasm (EC2 and EC3), and in the other two, the nuclei were at the periplasm (SB4.5 and SB5.5). Embryos at stages SB proved to be more sensitive to radiation. Differences in the survival curves between the two SB stages were due to an increase in the number of targets. No significant differences were observed between the survival curves for EC stages. Analysis of the fluence corrected by the absorbance of the chorion and cytoplasm, at each stage, showed that while at SB stages the nuclei were certainly being affected, at EC2 only peripheral cytoplasmic layers were damaged and, at EC3, cytoplasmic and nuclear targets were reached by UV. Irradiation at SB and EC3 resulted in immediate death, while at EC2 most of the embryos proceeded further in development (late death). Incubation at low temperatures after irradiation accentuated the effect of UV. For SB, this is probably caused by inhibition of dark repair mechanisms, while for EC, interferences in physiological factors other than repair must have occurred. Action spectra for inactivation showed peaks of efficiency at 295 nm and 265 nm respectively, for stages EC and SB. These results indicated that upon irradiation death is due at EC2 to damage imposed on cytoplasmic targets; at EC3, to nuclear and cytoplasmic lesions and at SB, to damages imposed on nuclear targets.

INTRODUCTION

The characteristics of insect early development make their embryos a suitable system for studying the lethal effects of nuclear or cytoplasmic damages induced by UV radiation. It is assumed that when embryos are exposed to UV during the stages of early intravitelline cleavages (EC), only peripheral cytoplasmic targets are affected. Due to the low penetrance of UV in biological tissues, the nuclei are strongly shielded from incident radiation by a thick layer of endoplasm. However, once the nuclei reach the periplasm at the syncytial blastoderm stage (SB), they become the main targets for UV (revision in Kalthoff and Jäckle, 1982).

The lethal effect of UV radiation on insect embryos has been studied in *Drosophila* (Goldman and Setlow, 1956; Levin and Koslova, 1973; Ghelelovitch, 1966), *Bracon* (*Habrobracon*) (von Borstel and Wolff,

1955; von Borstel and Rekemeyr, 1958; Amy, 1964, 1965) and *Smittia* (Kalthoff, 1976). In these three species, embryos at SB stages showed higher sensitivity to UV radiation than embryos at EC. In general, the survival curves are sigmoid, indicating the involvement of multi-target mechanisms (Amy, 1964; Kalthoff, 1971a). Exposure of eggs to germicide UV causes inactivation of the embryos, that is, an arrest in the developmental process, followed by death. The reports in the literature, regarding the stage of development attained by the embryos after irradiation, are rather controversial. It was observed that irradiation of *Drosophila* (Goldman and Setlow, 1956) and *Bracon* embryos (von Borstel and Rekemeyer, 1958) at SB stages, resulted in immediate inhibition of development. However, when the cytoplasm was exposed to UV (EC stages), the embryos could reach more advanced embryonic stages before dying. Quite different results were obtained by Ghelelovitch (1966) and Amy (1964) for embryos of these two species. They observed that both cytoplasmic and nuclear damage allowed the embryos to proceed further in development.

The action spectra for inactivation of *Smittia* embryos, however, displayed different peaks of efficiency:

at 295 nm for EC and between 265 and 280 nm for SB stages (Kalthoff, 1976; Kalthoff *et al.*, 1978). These results indicated that different targets or chromophores might be involved in the process of inactivation, when cytoplasm or nuclei are exposed to UV. Indeed, photoreactivation (PR) treatments on *Drosophila*, *Bracon* and *Smittia* embryos, inactivated by 254 nm wavelength, were effective for nuclear damage but not for lesions imposed to cytoplasmic targets (von Borstel and Wolff, 1955; Amy, 1965; Ghelelovitch, 1966; Levin and Koslova, 1973; Kalthoff, 1976). For *Sciara* embryos, however, it was observed that PR treatment was effective after inactivation at early stages of intravitelline cleavages, as well as at SB stages (Perondini, 1984). This special behavior of *Sciara* embryos raises the possibility that nuclear damage may also be involved in inactivation at EC stages.

We studied the sensitivity of *Sciara* embryos at preblastodermic stages to far UV, in order to find some clues to answer two major questions: (1) Can inactivation of *Sciara* embryos at EC stages be ascribed exclusively to cytoplasmic damage? (2) Is there any correlation between nuclear or cytoplasmic damage and the stage of development attained by embryos after irradiation?

MATERIAL AND METHODS

Lineage and preparation of eggs

In all experiments a strain of *Sciara ocellaris* kept in our laboratory since 1966 (Pavan and Perondini, 1967) was used. To obtain the eggs, mature females were collected from the stock and briefly etherized. The females were induced by decapitation to lay their eggs during a short time interval, so that large numbers of synchronous eggs could be obtained (Carson, 1946).

Stages of development

The choice of the stages of development used in this study was made taking into account the distances of the nuclei to the egg surface, so that varying degrees of nuclear shielding from incident radiation could be examined. Four pre-blastodermic stages were chosen, as follows: early intravitelline cleavages at 2 and 3 hours of development (EC2 and EC3); and syncytial blastoderm at 4.5 and 5.5 hours of development (SB4.5 and SB5.5). This timing was established for embryos kept at 22°C (Perondini *et al.*, 1986). These four stages are easily recognized *in vivo* by simple inspection through a stereomicroscope. Table I contains some information about these stages, that are relevant for the present study. More detailed descriptions can be found in Perondini *et al.* (1986).

Table I - Number of nuclei present in *Sciara ocellaris* embryos and the distances between the nuclei and egg surface, at four preblastodermic stages. Data from Perondini *et al.* (1986).

Stage	Average number of nuclei (min.-max.)	Distance from nuclei to surface (μ m)
EC2	3.4 \pm 2.5 (2 - 12)	60 - 40
EC3	22 \pm 10 (12 - 60)	30 - 20
SB4.5	247 \pm 77 (91 - 426)	< 5
SB5.5	683 \pm 268 (269 - 1350)	< 5

Generation and measurement of UV radiation

UV radiation of 254 nm wavelength was obtained from fluorescent germicidal lamps. Monochromatic radiation was generated by a Xe arc lamp (450 W) coupled with a monochromator (Oriel Co., USA). The band width was fixed at 5 nm. Fluence rates were measured by a thermopile detector (Oriel Co., USA) and a microvoltmeter (2740 Keithley Co., USA).

Irradiation of eggs and analysis of embryony development

Embryos were distributed onto agar plates and irradiated with the longitudinal axis perpendicular to the UV beam. Afterwards the embryos were kept in the dark to avoid uncontrolled photoreactivation for at least 24 hours at 22°C, or at other experimental temperatures. The eggs were then transferred to depression slides and immersed into boiled, filtered tap water. Hatching of normal larvae was used as the criterion for survival.

Measurements of cytoplasm transmittance

Embryos at 1.5 hours of development were burst on a quartz coverslip, and the chorion debris discarded. Slices of paraffin 5 μ m thick were placed around the cytoplasm mass. A second quartz coverslip was put on top of this preparation, and gently pressed, so that a 5 μ m layer of cytoplasm was obtained. This preparation was positioned on top of the thermopile window, and the fluence rate read through the cytoplasmic layer or outside of it, through only the two quartz coverslips. After correction of the measurements, an average transmittance of 25 \pm 6.5% was obtained from three independent

preparations. As an estimate of the chorion transmittance we used the value of 80% obtained for *Drosophila* (Goldman and Setlow, 1956), and *Smittia* (Kalthoff, 1973).

RESULTS

Survival of embryos irradiated with germicide UV at different stages of development

This series of experiments were undertaken to analyse the survival of embryos exposed to UV at stages in which cytoplasmic or nuclear targets were supposed to be preferentially irradiated. Embryos at stages EC2, EC3, SB4.5 and SB5.5 were used in this study. In each experiment, synchronous eggs collected from several females were divided into groups. Once the embryos reached the adequate stage, different groups were exposed to increasing fluences of germicide UV (254 nm wavelength). After irradiation the embryos were kept at 22°C. In each experiment, one group was not irradiated, being used as a control and, for each stage, at least two independent experiments were carried out.

The empiric data obtained for each stage were statistically adjusted according to the equation

characteristic of fluence-effect relationship for a multi-target case:

$$S/S_0 = 1 - (1 - e^{-cF})^n \quad (1)$$

where S = number of individuals surviving the treatment; S_0 = initial number of individuals; c = compound constant, referring to the sensitivity of the biological unit, reflected in the slope of the curve; n = number of targets and F = UV fluence (Harm, 1980).

The survival curves are presented in a semi-logarithmic plot in Figures 1 and 2, together with the values of n and c . Embryos at different stages of development displayed quite distinct responses to UV radiation. To compare the sensitivities of the embryos to UV, the LF_{50} (fluence necessary to give 50% survival) was used as a standard. No significant differences in the sensitivity to UV were observed between EC2 and EC3 (Figure 1), the LF_{50} values being 355 J.m^{-2} and 380 J.m^{-2} , respectively. From these stages on, however, this sensitivity increased as the nuclei reached the periplasm at stage SB4.5 (Figure 2), the highest level being observed at SB4.5 ($LF_{50} = 21 \text{ J.m}^{-2}$). At the next stage, SB5.5, the sensitivity decreased again ($LF_{50} = 44 \text{ J.m}^{-2}$), but was still

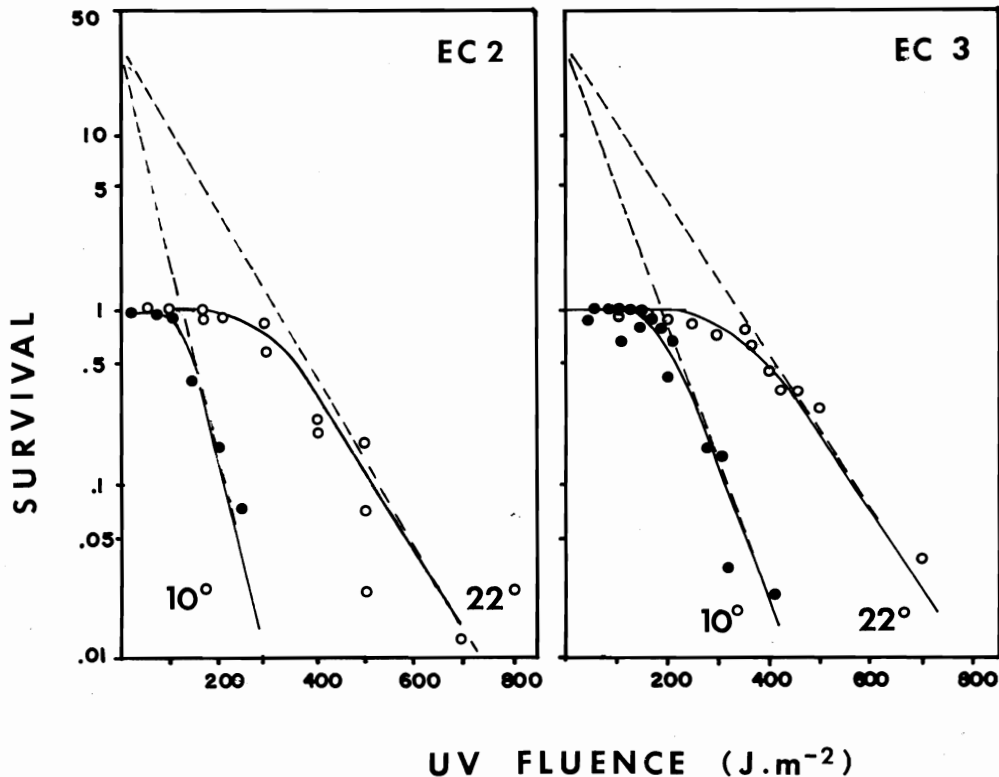


Figure 1 - Survival curves for embryos irradiated at stages EC and incubated at 22°C or at 10°C after irradiation. Extrapolation lines (broken lines) were traced based in the values of n . Each point represents the analysis of 80-100 embryos. EC2 (10°C), $n = 27$, $c = 0.026$; EC2 (22°C), $n = 29$, $c = 0.011$; EC3 (10°C), $n = 30$, $c = 0.018$; EC3 (22°C), $n = 32$, $c = 0.010$.

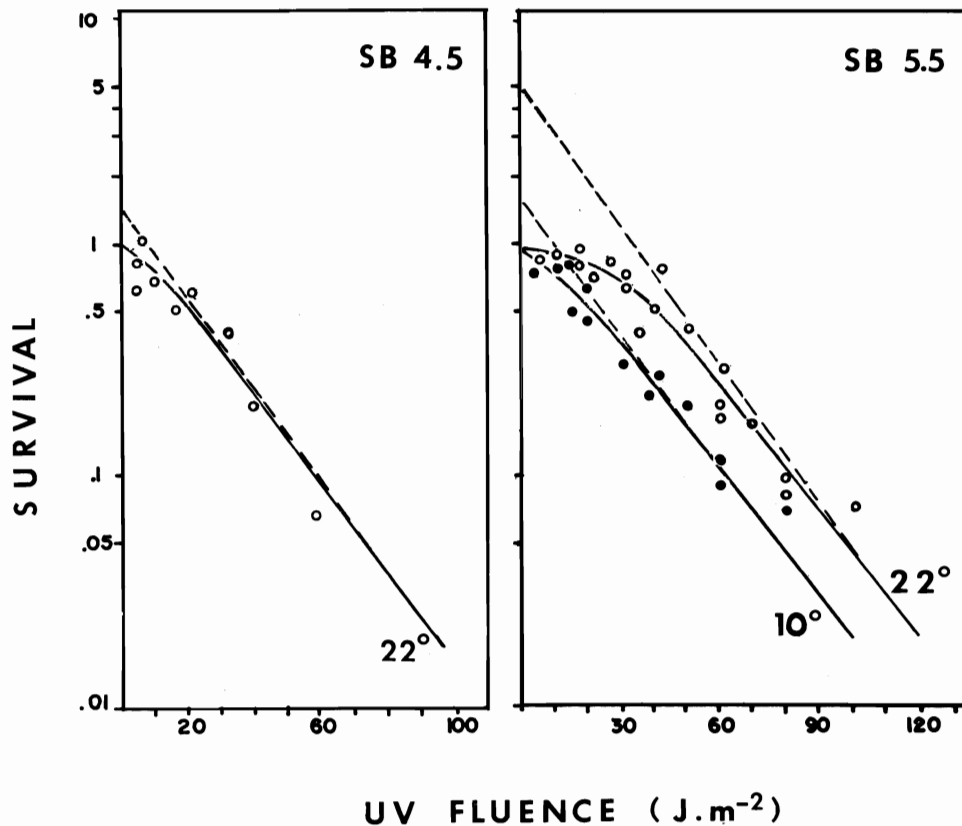


Figure 2 - Survival curves for embryos irradiated at SB stages and incubated at 10°C or 22°C after irradiation. Extrapolation lines (dashed lines) were traced based on the values of n . Each point represents the analysis of 80-100 embryos. SB4.5 (22°C), $n = 1.4$, $c = 0.045$; SB5.5 (10°C), $n = 1.7$, $c = 0.045$; SB5.5 (22°C), $n = 5$, $c = 0.046$.

superior to that observed for EC stages. Stages SB4.5 and EC2 are, therefore, opposite extremes among the stages considered, the sensitivity of the first being almost 20 times higher.

For all stages but SB4.5, the survival curves had the same general shape (i.e. sigmoid in arithmetic plot), displaying shoulders at lower fluences. The presence of shoulders suggests involvement of multi-target mechanisms in the inactivation process. For stage SB4.5, the curve approaches a simple exponential condition, indicating that probably only a few sensitive targets are involved in mortality. Indeed, n is close to the unit. For the other stages, the values of such parameter are much greater, ranging from 4.4 at SB5.5 to 32.0 at EC3. Regarding stages SB, the increase in LF_{50} from SB4.5 to SB5.5 is due only to an increase in n , since the slope of the curves (c) are similar. Between EC stages, no significant differences were observed for n or c . The decrease in LF_{50} from EC stages to SB4.5 was accompanied by changes in both parameters (decrease in n and increase in c).

The stages presenting greater distances between the nuclei and the egg surface (EC), were also those that showed lower sensitivity to UV. This result can be interpreted in two ways. First, considering that UV energy

decreases, as it is absorbed by the egg covers and by layers of cytoplasm, this lower sensitivity could then be explained by the necessity to expose the embryos to higher fluences, to achieve nuclear damage. Alternatively, one could assume that the nuclei are not being directly affected, the lower sensitivity being due to impairment of cytoplasmic targets. To evaluate these possibilities, it was necessary to estimate the fraction of UV energy that would reach the nuclei. The UV fluences applied to the egg surface were thus corrected (Table II), considering the absorbance of the chorion, as well as that of the cytoplasmic layer interposed between the nuclei and the egg covers at each stage. For EC2, such correction resulted in extremely small fluences. On the other hand, for EC3 the values observed were comparable to those obtained for SB. These results indicate that, at EC2, nuclei are not being directly affected by UV, while at stage EC3, there would be enough energy to damage the nuclei.

Effects of low temperature incubation on irradiated embryos

Studies on UV-induced specific malformations in *Smittia* embryos showed that the effects of UV radiation

Table II - UV fluences that would be reaching the nuclei in embryos at different stages, considering the cytoplasm and chorion transmittances.

Stage	Survival level (%)	UV fluences (J.m ⁻²)		Parameters*	
		Incident	Corrected	n	c
EC2	90	240	0.0029	29.5	868.40
	70	300	0.0035		
	50	355	0.0042		
	30	420	0.0049		
	10	560	0.0063		
EC3	90	250	0.78	32.0	3.20
	70	325	1.02		
	50	380	1.19		
	30	455	1.42		
	10	575	1.80		
SB4.5	90	4	0.8	1.4	0.23
	70	12	2.4		
	50	21	4.2		
	30	33	6.6		
	10	58	11.6		
SB5.5	90	23	4.6	5.0	0.23
	70	35	7.0		
	50	44	8.8		
	30	58	14.6		
	10	83	16.6		

*Values calculated using the corrected fluences.

could be enhanced by incubating the irradiated embryos at low temperatures (Kalthoff, 1971b). The following experiments were undertaken to investigate the influence of low temperature incubation on irradiated *Sciara* embryos. An incubation temperature of 10°C was chosen, since this is the lowest temperature that does not impair normal development (Guatimosim, 1989). Embryos at EC2, EC3 and SB5.5, initially kept at 22°C, were exposed to increasing fluences of germicide UV. Immediately after irradiation, the eggs were incubated at 10°C in the dark for the next 48 hours, after which they were transferred back to 22°C. For each stage, at least two independent experiments were performed. The survival curves obtained and the equation parameters, *n* and *c*, are presented in Figures 1 and 2. For the three stages considered, the lethal effect of UV was more accentuated for embryos kept at 10°C; LF₅₀ assuming values considerably smaller than at 22°C: 140 J.m⁻² for EC2, 210 J.m⁻² for EC3 and 25 J.m⁻² for SB5.5. For embryos at EC2 and EC3 (Figure 1), the survival curves at 10°C were steeper than at 22°C, as

shown by the higher values of *c*. For each stage the extrapolation lines of the exponential part of the curves at 10°C and 22°C were convergent, intercepting the log axis at about the same point. At these stages therefore, low temperature treatment affected the slope of the survival curve, and consequently the width of the shoulder, but did not cause any modification of the log S intercept of the extrapolation lines.

On the contrary, for embryos irradiated at SB5.5 (Figure 2), incubation at low temperature caused a diminution in the value of *n*, from 5.0 at 22°C to 1.7 at 10°C, but did not change the slope of the curve (similar *c* = 0.046). Diminution in the width of the shoulder was then caused mainly by the decrease of *n*.

Taking the fluences necessary to result in a similar effect at 10° and 22°C, constant FRF (fluence reduction factors) of 0.39 and 0.55 were observed for stages EC2 and EC3, respectively. On the other hand, for SB5.5 embryos, FRF increased with the increment of the fluence, from a value of 0.3 (survival level of 0.9) to 0.75 (survival level of 0.1). The FRF values this reflect the convergence of the survival curves at 10° and 22°C at both EC stages, and their parallelism at SB5.5.

Developmental arrest of embryos exposed to UV radiation

This series of experiments was performed in order to verify for *Sciara* embryos, possible correlations between the stage of development at which the embryos were irradiated and the moment developmental arrest (death) took place.

In this analysis embryos at stages EC2, EC3, and SB5.5 were exposed to increasing UV fluences, which were chosen based on the survival curves shown in Figures 1 and 2. After irradiation, the embryos were kept at 10°C or at 22°C. Among the embryos that failed to give rise to normal larvae, two categories were distinguished: one comprising those embryos which died before cellular blastoderm formation (early death - ED), and the other, those embryos whose development stopped at stages beyond gastrulation (late death - LD). Embryos which succeeded in hatching but presented morphological aberrations were also included in this last category.

The contribution of each category to the mortality increment at each UV fluence, is shown in Figure 3. For those embryos inactivated at EC2 and maintained at 22°C, the contribution of category LD was prevalent throughout the range of fluence employed, showing that death occurred almost exclusively during late development. An opposite situation was observed for stage SB5.5. Category LD practically did not account for an increase in mortality, only increments in ED frequencies being observed. A different picture emerged for embryos irradiated at EC3.

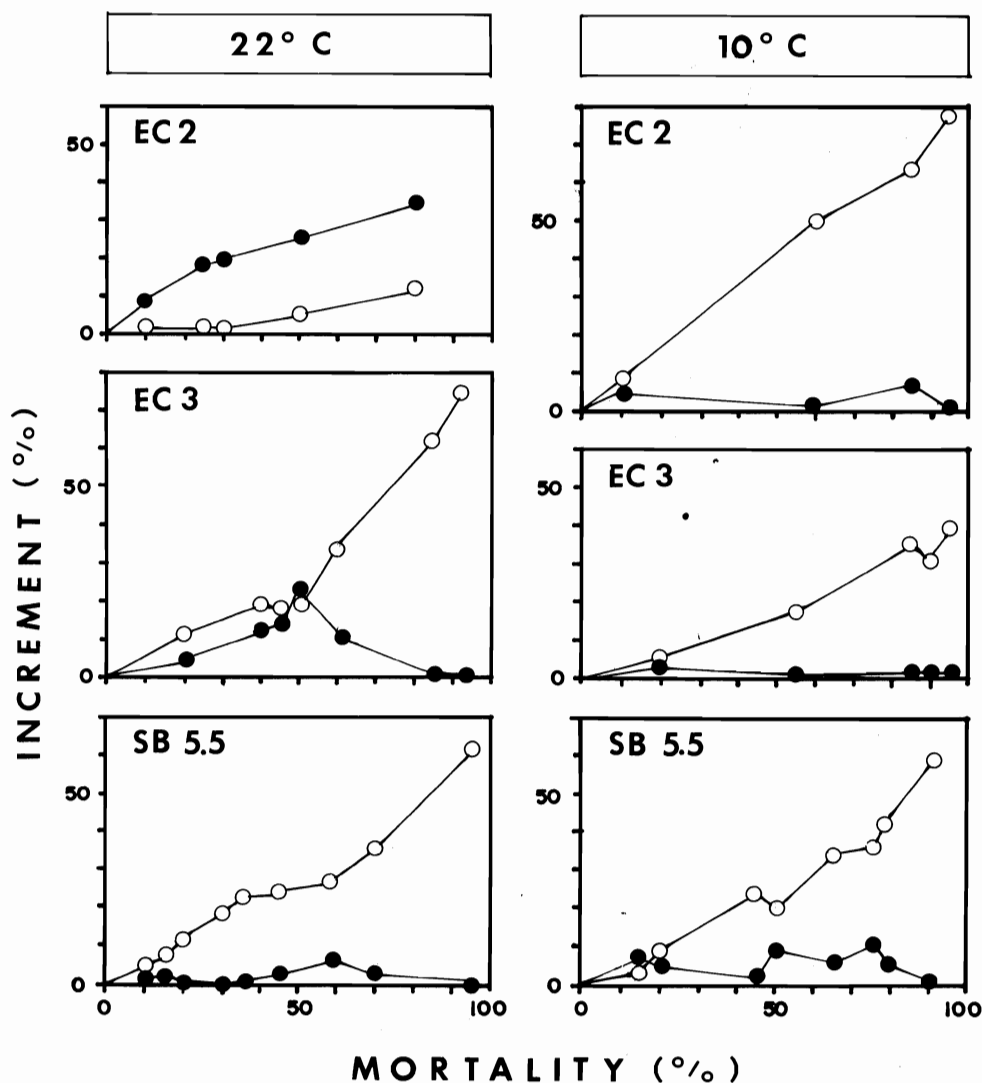


Figure 3 - Contribution of the categories early death (o) and late death (●) to the mortality increment for groups of embryos irradiated at different stages of development and kept at 22°C (right column) or at 10°C (left column) after irradiation.

Up to fluences resulting in mortality levels around 50%, the frequency of ED and LD were similar, but from this level on, the contribution of ED increased to the detriment of LD.

Hence, it can be seen that stages EC2 and SB5.5 behave as opposite extremes, in which one or the other category, ED and LD respectively, contributes significantly to the increase in mortality. EC3 would constitute an intermediate situation where the prevalence of one or the other category depends upon the UV fluence applied.

Incubation of the irradiated embryos at lower temperatures (10°C), however, provoked a rather substantial alteration in this pattern (Figure 3). For embryos at EC2 and EC3, low temperatures abolished the LD category, death occurring immediately after irradiation. For stage SB, the increment of mortality

continued to be due almost exclusively to increases in ED embryos, as observed at 22°C.

Action spectra for inactivation

In order to compare the efficiency of different wavelengths of UV radiation, survival curves for embryos at EC2, EC3 and SB5.5 were generated using wavelengths ranging between 254 and 310 nm. Five to seven different fluences were used at each wavelength, resulting in survival levels from 90 to about 10% (data not shown). For all stages and wavelengths used, there was good adjustment of the empirical data to sigmoid curves defined by the equation (1).

The efficiency of different wavelengths was evaluated based on the LF₅₀ of the corresponding survival

curves. These values were transformed into relative efficiency per incident quantum and plotted against wavelength (Figure 4). Although broad action spectra were obtained, different peaks of efficiency could be observed for stages EC and SB. Interestingly, for embryos at EC2 and EC3, the same general pattern of responses was obtained, with a peak at 295 nm. At SB5.5, the peak of efficiency was observed at 265 nm.

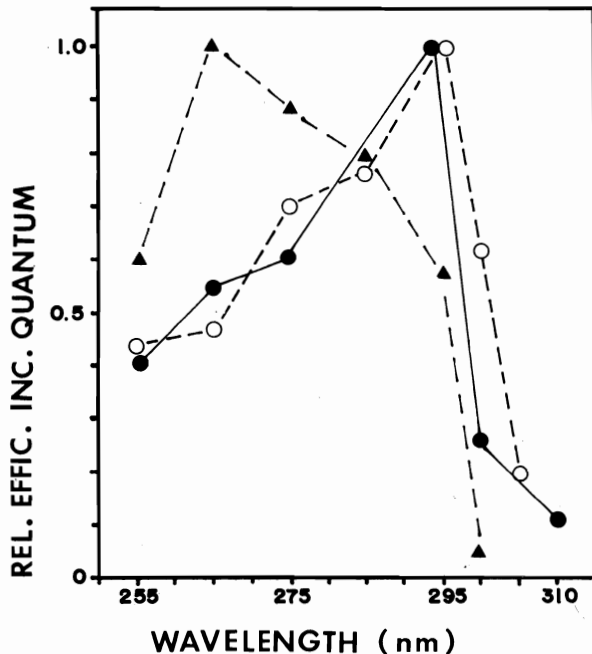


Figure 4 - Action spectra for UV-inactivation of embryos at three stages of development: EC2 (●), EC3 (○) and SB5.5 (▲). Relative efficiency per incident quantum is plotted against UV wavelengths.

DISCUSSION

The results obtained with *Sciara* embryos are, in general, in agreement with the observations of UV-induced lethality in embryos of different insect species. For all stages studied, the survival curves were sigmoid, indicating the involvement of multi-target mechanisms. Variation in UV sensitivity, lower at EC and higher at SB stages, is a pattern of responses observed in embryos of *Bracon* (vonBorstel and Rekemeyer, 1958; Amy, 1964), and there is evidence that the same pattern occurs for *Drosophila* (Ghelelovitch, 1966) and *Smittia* (Kalthoff, 1976).

The results obtained with embryos at SB4.5 and SB5.5 stages are coherent with the assumption that, at both stages, nuclei constitute the major targets for UV. The similar slope of the survival curves are in line with this assumption. The higher resistance at SB5.5 than at SB4.5 can be explained by the increase in the number of nuclei, which occurs during this developmental period. Embryos

at SB4.5 possess 247 nuclei, while for embryos at SB5.5 this number increases to 682, on average (Perondini *et al.*, 1986). Consequently, at SB4.5, each nucleus would assume a greater potential importance for future development. Injury to one nucleus would be more hazardous to normal development, than at SB5.5.

For inactivation at EC2, the estimated fluences that would be reaching the nuclei were so small, that nuclear damage can be practically excluded as the primary cause of inactivation. Indeed, fluences of this magnitude (order of 10^{-3} J.m⁻²) are even below the LF₃₇ for diploid cells of a variety of organisms (Sarachek, 1954; Painter, 1970; Brown *et al.*, 1981; Stacey *et al.*, 1989). Moreover, $n = 29.5$ obtained at EC2 is a value too high to be considered as the number of targets, since only three to four nuclei, on the average, are present at this stage. On the other hand, the fluences estimated for EC3 are comparable to those obtained for stages SB, at which nuclear damage is almost certain to occur. However, the slope of the curves observed after the fluence correction was about two times higher than that at SB stages, suggesting that different targets could be involved in inactivation at these stages. Besides, it seems unlikely that more superficial cytoplasmic structures or components exposed to fluences such as 300 or 400 J.m⁻² would not be affected. Therefore, it is reasonable to suppose that inactivation at EC3 could be a consequence of interacting effects, caused by cytoplasmic as well as nuclear damage.

The analysis of the developmental stage attained by the embryos after irradiation provided further support to the hypothesis that at EC2, only cytoplasmic targets are affected. According to Goldman and Setlow (1956) in *Drosophila* and von Borstel and Rekemeyer (1958) in *Bracon*, nuclear damage caused by UV promotes an immediate interruption of development, while damage introduced in cytoplasmic targets usually does not impede the development of embryos, but induces gross morphological aberrations. Indeed, after irradiation of *Sciara* embryos at EC2, morphological aberrations were prevalent, contributing most to increase in mortality.

UV irradiation of restricted areas of cytoplasm (anterior pole) in eggs of several insect species induces developmental aberrations, giving rise to malformed embryos (Yajima, 1983; Elbetieha and Kalthoff, 1988; Kalthoff, 1971a; Bownes and Kalthoff, 1974), the same being observed for *Sciara* embryos (Perondini *et al.*, 1987). The fact that this UV effect could be reversed by photoreactivation treatments provided strong evidence that mRNA molecules of maternal origin would be the main effective targets for UV radiation (IBID). Indeed, mRNA molecules stored within the egg cytoplasm play a critical role in the determination of the basic body pattern, for their translation products are responsible for the differential regulation of zygotic genes, in such a way that the

embryonic nuclei would become committed to a particular ontogenetic fate (see Kalthoff, 1983; St. Johnston and Nüsslein-Volhard, 1992).

If inactivation at EC2 results from lesions of those mRNA molecules, then how can the value obtained for n be explained? This value is too small if considered as the number of mRNA molecules that must be hit to impair development. However, lesions induced in these molecules would only be effective by the time their translation products are needed, at later stages. Altered products, or a lower concentration, would be expected to cause modifications in the ontogenetic pathway of a small fraction of nuclei. Such deviations, although in a small number at the beginning, could assume great significance, after nuclear multiplication, giving rise to malformed embryos. Therefore, mRNA molecules could be considered as a primary target for UV, but the effect would be observed in a small number of nuclei, the final targets. It is possible then, that n is a measurement of the number of these final targets.

In studies with *Smittia*, Kalthoff (1976) observed that for embryos irradiated at an EC stage, all but a few either stopped developing before cellularization of blastoderm, or developed into apparently normal larvae. In other words, practically no malformed embryos were observed. However, as we have shown in this work, at least for *Sciara* embryos, not in all EC stages inactivation by UV can be ascribed exclusively to cytoplasmic damage. For embryos irradiated at EC3, at mortality levels above 50%, there was a predominance of category ED (early death), again indicating that nuclear targets are also being affected. Perhaps this assumption could also explain the results with *Smittia* embryos.

The results obtained in the present work showed that incubation at low temperatures accentuated the lethal effect of far UV on *Sciara* embryos. At stage SB5.5, in which the nuclei are the main targets, low temperature treatment resulted in a decrease of n , while the slope of the curve remained similar to that observed at 22°C. The survival curves are then parallel. Bos *et al.* (1988), comparing two strains of *Aspergillus*, one of which is defective in dark repair mechanisms, observed that the survival curves had the same slope and that n for the repair defective strain was smaller. Repair inhibition would then be mimicking a reduction in the number of targets. Since repair mechanisms are enzymatic processes and therefore under the influence of temperature, it is possible to suppose that, for embryos at SB, the accentuation of the UV effect at 10°C could be due, among others, to interferences on repair mechanisms. Only future and specific studies will test this hypothesis.

For both EC stages examined, on the contrary, low temperature introduced a constant fluence reduction factor, for the curves were convergent, intercepting the log S axis

at the same point. Results similar to this were obtained by Kalthoff (1971b), in studies with *Smittia* embryos at a stage of development correspondent to EC3 of *Sciara*. If mRNA molecules are effective targets for radiation at this stage, then the synergisms between UV and low temperatures must be ascribed to physiological factors other than dark repair inhibition, since dark repair is not known to occur for RNA. The bases of such synergisms at stages EC are not known. Kalthoff (1971b) has argued that besides the introduction of pyrimidine dimers in RNA molecules, UV radiation may also affect other cytoplasmic structures or metabolic processes. The incubation temperature after irradiation would then interfere with the ability of the embryos to overcome this damage.

The action spectra generated for embryos at SB and EC also indicate that distinct phenomena could be involved in the inactivation process at these stages. For SB5.5, the efficiency peak at 265 nm suggests absorption by nuclei acids, thus being coherent with the hypothesis of nuclear damage. However, the peak at 295 nm observed for embryos at EC2 and EC3 is almost out of the range of absorption of either DNA or RNA. Similar results were obtained by Kalthoff (1976), with *Smittia* embryos. Later studies (Jäckle and Kalthoff, 1980) showed that 295 nm UV radiation resulted in a large yield of pyrimidine dimers in RNA molecules. Since the absorption of UV at 295 nm by nuclei acids is only 10% of that observed at 265 nm, these authors suggested that dimer production would be mediated by a photo-sensitive compound. This sensitizer would absorb the UV energy and transfer it to the RNA molecules. Such an explanation could also be extended to *Sciara* embryos.

In conclusion, the results of the present study suggest: (1) at EC2, the lethal effect of far UV is due to lesions induced in cytoplasmic targets; (2) at EC3, the nuclei are probably being damaged, but cytoplasmic lesions seem to play a major role in inactivation; (3) at SB stages, the nuclei are the main targets for UV radiation; (4) nuclear damage results in immediate death and (5) lesions introduced in cytoplasmic targets allow the embryos to reach more advanced stages of development, depending on the temperature of incubation.

The data obtained in this study raised another question. According to the multi-target function (equation 1), a system is inactivated when all targets are hit at least once. Hence, at stages SB, one would expect n to be close to the number of nuclei at those stages. On the contrary, the values of n were much smaller. Furthermore, the fact that only one hemisphere of the egg is being irradiated means that not all the nuclei must be hit to achieve inactivation. In systems like insect embryos, n could be an estimate of the minimum number of targets that must be hit to result in inactivation. This hypothesis is under investigation.

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RESUMO

Os efeitos da radiação ultravioleta curta foram estudados em quatro estágios preblastodérmicos dos embriões de *Sciara ocellaris*. Em dois deles os núcleos estavam imersos no endoplasma (EC2 and EC3) enquanto nos outros dois, os núcleos estavam já localizados no periplasma (SB4.5 e SB5.5). Embriões nos estágios SB mostraram-se mais sensíveis à UV. As diferenças entre as curvas de sobrevivência para os dois estágios SB foram devidas a um aumento no número de alvos. Nenhuma diferença significativa foi observada entre as curvas dos estágios EC. Análises das fluências corrigidas pela absorvância do córion e do citoplasma mostraram que, em SB, os núcleos certamente foram afetados, em EC2 apenas camadas periféricas do citoplasma puderam ser atingidas e, em EC3, alvos citoplasmáticos e nucleares poderiam ser lesados pela UV. Irradiação em SB e EC3 resulta em morte imediata dos embriões, enquanto que, em EC2 a maioria dos embriões prosseguem o desenvolvimento, morrendo tardiamente. Incubação a baixa temperatura após a irradiação acentuam os efeitos da UV. Nos estágios SB isto deve ser causado provavelmente por inibição de mecanismos de reparo no escuro, enquanto que para EC, interferências em mecanismos fisiológicos diferentes do reparo devem ter ocorrido. Espectros de ação para inativação de embriões mostraram picos de eficiência com os comprimentos de onda de 295 nm e 265 nm, respectivamente para os estágios EC e SB. Estes resultados indicam que a morte dos embriões após irradiação, é devida, no estágio EC2 a danos impostos a alvos citoplasmáticos; em EC3, a lesões nucleares e citoplasmáticas e, em SB, a danos introduzidos em alvos nucleares.

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